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Independent Prognostic Significance of Monosomy 17 and Impact of Karyotype Complexity in Monosomal Karyotype / Complex Karyotype Acute Myeloid Leukemia: Results from Four ECOG-ACRIN Prospective Therapeutic Trials

Running head: Significance of Monosomy 17 and Karyotype Complexity in MK+ and CK+ AML

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Highlights

- Prognostic impact of monosomal karyotype appears dependent on karyotype complexity
- Monosomy 5 lacks prognostic impact in context of AML with monosomal karyotype
- Monosomy 17 independently predicts for inferior survival among AML patients

Abstract

The presence of a monosomal karyotype (MK+) and/or a complex karyotype (CK+) identifies subcategories of AML with poor prognosis. The prognostic significance of the most common monosomies (monosomy 5, monosomy 7, and monosomy 17) within MK+/CK+ AML is not well defined. We analyzed data from 1,592 AML patients age 17-93 years enrolled on ECOG-ACRIN therapeutic trials. The majority of MK+ patients (182/195; 93%) were MK+/CK+ with 87% (158/182) having ≥5 clonal abnormalities (CK≥5). MK+ patients with karyotype complexity ≤4 had a median overall survival (OS) of 0.4y compared to 1.0y for MK- with complexity ≤4 ($p<0.001$), whereas no OS difference was seen in MK+ vs. MK- patients with CK≥5 ($p=0.82$). Monosomy 5 (93%; 50/54) typically occurred within a highly complex karyotype and had no impact on OS (0.4y; $p=0.95$). Monosomy 7 demonstrated no impact on OS in patients with CK≥5 ($p=0.39$) or CK≤4 ($p=0.44$). Monosomy 17 appeared in 43% (68/158) of CK≥5 patients and demonstrated statistically significant worse OS (0.4y) compared to CK≥5 patients without monosomy 17 (0.5y; $p=0.012$). Our data suggest that the prognostic impact of MK+ is limited to those with less complex karyotypes and that monosomy 17 may independently predict for worse survival in patients with AML.

Keywords: Acute Myeloid Leukemia; Monosomal Karyotype; Complex Karyotype; Cytogenetics; Prognosis; Monosomy 17; Monosomy 5; Monosomy 7

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Introduction

Despite the increasing use of molecular characterization of mutations in the prognostic risk classification of acute myeloid leukemia (AML), conventional cytogenetic studies at diagnosis remain a highly influential risk factor. The presence of a complex karyotype (CK+)[1,2] or monosomy of either chromosome 5[3] or chromosome 7[4] has been universally associated with unfavorable prognosis. Recently the UK MRC added monosomy 17 to its list of independent predictors of poor outcome with their refinement of the AML cytogenetic classification categories.[5] Although there has been no change in induction therapy for nearly 4 decades[6], subgroups that respond particularly well or poorly have been identified based on their genetic features.[1,4,5,7-9] Careful examination of disease characteristics at diagnosis is imperative to identify high-risk patients and to appropriately apply risk-adapted molecularly targeted therapies or intensified treatment strategies including hematopoietic stem cell transplantation (HSCT).

The Dutch-Belgian Hemato-Oncology Cooperative Group and the Swiss Group for Clinical Cancer Research (HOVON/SAKK) group was the first to demonstrate the potential of monosomal karyotypes (MK) to delineate a subgroup with very poor risk and thus refined the cytogenetic classification of AML patients < 60 years.[8] MK+ was defined as either the presence of two or more autosomal monosomies or one monosomy plus at least one structural abnormality and was associated with a 4% 4-year overall survival (OS) compared to 26% for MK- patients. Particularly unfavorable outcomes in MK+ patients > 60 years were reported by the Southwest Oncology Group (SWOG) with a 4-year OS of 1% and less than one third of MK+ patients between ages 31 to 60 years achieved complete remission (CR).[10]

Haferlach and colleagues in the Munich Leukemia Laboratory Group reported that complex karyotype defined as ≥4 unrelated abnormalities identified the largest proportion of very poor risk AML patients and suggested that the combination of CK≥4 and MK+ status would be the most sensitive metric in identifying those with unfavorable prognosis.[11] Additionally, degree of cytogenetic complexity has been shown to identify the subgroups of MDS patients with the worst prognosis, independent of MK+ status.[12,13] In fact, when accounting for karyotype complexity MK was not an independent prognostic factor in patients with myelodysplastic syndrome.[12,13]

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Whether the prognostic value of specific monosomies such as monosomy 5, monosomy 7, or monosomy 17 is preserved in the context of MK+ AML is uncertain. Therefore, we conducted a retrospective analysis of AML patients enrolled in four Eastern Cooperative Oncology Group and the American College of Radiology Imaging Network Cancer Research Group (ECOG-ACRIN) therapeutic clinical trials between 1990 and 2008 to determine whether individual monosomies, karyotype complexity, age, or baseline disease characteristics impact outcome in MK+ disease.

Methods

Patients

Eligible patients for this study were defined as previously untreated AML patients enrolled in one of four prospective ECOG-ACRIN therapeutic clinical trials (E1490, E1900, E3993, and E3999) between 1990 and 2008. [14-17]

Newly diagnosed AML patients (excluding acute promyelocytic leukemia) were eligible for enrollment onto the therapeutic protocols. Except for those enrolled onto E1900, patients were required to be at least 55 years of age. All patients received induction chemotherapy containing cytarabine combined with daunorubicin, idarubicin, or mitoxantrone +/- additional investigational agents as defined by the respective protocol. Post-remission therapeutic strategies were defined per individual protocol and not influenced by the identification of MK+ disease. All patients signed informed consent prior to enrollment. The studies were approved by ethics committees of all participating institutions and conducted in accordance with the Declaration of Helsinki. A total of 1,592 AML patients ranging 17-93 years of age were enrolled on the aforementioned trials.

Evaluable cytogenetics data are available on 1,188 patients included in this analysis. Patients enrolled on E1900 accounted for the largest number of the 1,188 patients (45%; 535), followed by those on E3999 (29%; 345), E3993 (22%; 266), and E1490 (4%; 42).

Cytogenetic evaluation

Diagnostic bone marrow aspirate or heparinized peripheral blood was examined for cytogenetic abnormalities by unstimulated standard culturing and banding techniques by individual institutional or referral cytogenetic laboratories. Results and karyotypes were centrally reviewed by the ECOG-ACRIN Cytogenetics Committee

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and designated in accordance with the International System for Human Cytogenetic Nomenclature (ISCN). Karyotypic analysis was based on minimum review of 10 available metaphases. Normal karyotype required a minimum of 20 normal diploid metaphases. Abnormalities were considered clonal when at least 2 metaphases had the same structural abnormality or the same trisomy or when at least 3 metaphases displayed the same monosomy. Structural abnormalities were defined as deletions, translocations, inversions, and additions for the purposes of this study and in accordance with the definition used by HOVON/SAKK[8] The following clonal abnormalities were scored for each chromosome: monosomies, extra copies, structural abnormalities, ring chromosomes as well as the frequency of the individual abnormalities. Marker chromosomes and double minutes were also documented. Complex karyotype was defined as ≥ 3 clonal abnormalities in accordance with the National Comprehensive Cancer Network[18] and the European Leukemia Net recommendations[19]. Degree of cytogenetic complexity was also recorded for CK+ patients having 3, 4, or ≥5 clonal abnormalities. Patients with core-binding factor (CBF) leukemia [t(8;21), inv(16) or t(16;16)] were excluded from the MK+ cohorts regardless of the presence of additional clonal abnormalities.

Statistical Analysis

Patient baseline characteristics were compared using Fisher's exact test if they were categorical and Wilcoxon rank sum tests if they were continuous. OS was defined as time from study randomization/registration to death from any cause, with follow-up censored at the date of last contact. Kaplan-Meier estimates were used to estimate the event-time distributions for OS. Log-rank tests stratified on studies and induction treatments were used to examine the effects of MK or other chromosomal abnormalities on OS. Multivariate Cox model stratified on studies and induction treatments was performed on OS to examine the prognostic effect of MK or other chromosomal abnormalities while controlling for potential risk factors {Multivariate analysis included the factors of MK+ status, karyotype complexity, gender, age, WBC count, hemoglobin, platelet count, marrow and blood blast %, secondary vs de novo AML, and the occurrence of independent high-risk cytogenetic abnormalities [del5q, del7q, del17p, inv3, t(6;9), 11q23, and t(9;22)] where appropriate}. All P values were based on 2-sided tests.

Results

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Cytogenetic Abnormalities

Cytogenetics were evaluable in 1188 / 1592 (75%) of AML patients enrolled onto the four protocols. An overview of the frequency of normal as well as clonal cytogenetic abnormalities is seen in Table 1. Normal karyotype AML occurred in 502 (42%) patients whereas 686 (58%) had clonal cytogenetic abnormalities. CBF leukemia was identified in 108 (9%) patients. Single monosomies were found in 97 (8%) and multiple monosomies in 132 (11%), while 959 (81%) of evaluable patients had no monosomies. Concurrent structural abnormalities were present in 64/97 (66%) of those with a single monosomy with one patient also possessing a CBF abnormality [t(8;21)]. CK was present in 251 (23%) and MK was identified in 195 (18%) of non-CBF patients. Multiple monosomies were identified in the majority of MK+ patients (132/195; 68%) compared to those with a single monosomy and a structural abnormality (63/195; 32%). All patients with multiple monosomies had CK and only 6/132 (5%) patients with multiple monosomies lacked concurrent structural abnormalities.

Baseline Characteristics

Significant differences were observed in age, WBC count, hemoglobin, platelet count, peripheral and bone marrow blast percentages at diagnosis and percentages of secondary AML between MK+ and MK- patients (Table 2). MK+ patients tended to be older and have a lower presenting WBC count, lower percentage of circulating or bone marrow blasts, lower hemoglobin, and a lower platelet count at presentation. MK occurred in 7% (25 / 383) patients <55 years compared to 21% (170 / 805) patients ≥55 years ($p<0.001$).

Monosomies were detected for each chromosome at least once, but occurred least frequently with chromosomes 1, 2, 8, and 15. Monosomy 7 accounted for 53% (51/97) of the single monosomies and was present in 47% (107/229) of all patients in whom monosomies were identified. Monosomy 17 was the most frequent monosomy occurring in patients containing multiple monosomies (48%; 64/132), followed by monosomy 7 (42%; 56/132) and monosomy 5 (39%; 52/132) (Table 3). Double monosomies of individual chromosomes were uncommon (12%; 16/132) and affected chromosomes 3, 5, 9, 12, 13, 14, 15, 16, 17, 18, 20, and 21.

Prognostic Associations

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Median follow-up of the patients included in this analysis was 5.2 years. CR rate amongst all 1,188 evaluable patients was 53.4%. Outcome measures based on MK+/- status of the 578 patients with non-CBF clonal abnormalities are reported in Table 4. CR was achieved in 31% (61/195) of MK+ non-CBF patients compared to 47% (179/383) of MK- non-CBF patients with a 4-year OS of 3% and 14% (P<0.001), respectively. Among patients ≥ 55 years, CR occurred in 30% (51/170) of MK+ non-CBF patients compared to 42% (116/276) of MK- non-CBF patients and was associated with a 4-year OS of 1% versus 10%, respectively (p<0.001). MK+ non-CBF patients <55 years achieved CR at a rate of 40% (10/25) compared to 59% (63/107) in MK- non-CBF patients and was related to a 4-year OS of 16% versus 27%, respectively (p=0.02).

CR was achieved in 22% (14/63) of patients with a single monosomy and structural abnormalities compared to 33% (11/33) with an isolated monosomy (p=0.33, with 4-year OS of 5% and 9%, respectively (p=0.03).

Monosomal Karyotype

MK+ patients demonstrated an inferior median OS of 0.4 years compared to 1.2 years of the collective group of MK- patients (p<0.001). Evaluation of the impact of the combination of CK+/- and MK+/- status among patients with non-core-binding factor (non-CBF) abnormalities demonstrated that the CK-/MK- AML patients (n=314) had the best median OS (1.0 years). MK+ patients fared poorly regardless of the presence or absence of CK+ disease with a median OS of only 0.4 years and 0.2 years, respectively (Supplemental Figure 1). When accounting for karyotype complexity among patients with at least one clonal abnormality, 22% MK+ patients with karyotype complexity ≤4 clonal abnormalities achieved CR compared to 47% of those non-CBF MK- AML and the same degree of complexity (p=0.003) (Table 5). Median OS for MK+ patients with ≤4 clonal abnormalities was 0.3 years compared to 1.0 years for those that were MK- with non-CBF clonal abnormalities (p<0.001; HR: 1.98; 95%CI 1.40, 2.81) (Figure 1A). Of note, median OS was essentially unchanged when limiting the analysis to those MK+ patients with karyotype complexities of only 3 or 4 abnormalities (OS 0.3 years) compared their MK- counterparts (1.1 years; p=0.039;)(Supplemental Figure 2). MK+ status in patients

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with karyotype complexity ≤4 remained significant (p=0.01, HR 1.78; 95%CI 1.13, 2.79) by multivariate (Table 6).

No difference was seen in CR (38% vs 34%; p=0.82; Table 5) or OS (median 0.5y vs 0.4y; p=0.82;) in patients with karyotype complexity ≥5 (CK≥5) and differing MK+/- status (Figure 1B). MK+ status also failed to demonstrate significance within the CK≥5 population (p=0.47) by multivariate (Table 6).

Monosomy 5

Monosomy 5 was present in 27% (53/195) of all patients with MK+ and its presence failed to have prognostic impact when compared to MK+ patients without monosomy 5 (log rank test p=0.65). The majority of the occurrences of monosomy 5 (98%, 53/54) carried the MK+ designation with 94% (50/53) of monosomy 5 also having CK≥5. Survival of MK+/CK≥5 was not impacted by monosomy 5 status (median OS: 0.4y vs. 0.4y; Figure 2B). Monosomy 5 status also failed to demonstrate significance within the MK+/CK≥5 population (p=0.94;) by multivariate analysis (Supplemental Table 1).

Monosomy 5 rarely occurred in patients with non-CBF clonal abnormalities with a karyotype complexity ≤4 (8%; 3/37), but its presence in this population appeared to be associated with a poor overall survival and a HR of 4.13 (95%CI 0.82, 20.8) (Figure 2A) although the small number of occurrences (n=3) in this context precludes any definitive statement of significance.

Monosomy 7

Monosomy 7 (+/- monosomy 5 +/- monosomy 17) was identified in 107 patients and the majority (74%; 79/107) met the criteria for MK+ disease with 63% (59/79) also possessing CK≥5. CR rates were not different for monosomy 7 patients with or without MK+ (32% v. 33%) and were similar (30%) for MK+ patients lacking monosomy 7 (MK+/monosomy 7-). Monosomy 7 status demonstrated no impact on OS of MK+ patients (p=0.20). Univariate analysis of MK+ patients with monosomy 7 and karyotype complexity ≤4 revealed median OS of 0.3 years compared to 0.2 years for those without monosomy 7 (p=0.44; HR 0.76; 95%CI 0.37, 1,53) (Figure 2C). Similarly, MK+ patients with monosomy 7 and CK≥5 had an OS of 0.5 years versus 0.4 years for those without this monosomy (p=0.39; HR 0.86; 95%CI 0.61, 1.21) (Figure 2D). Interestingly, multivariate analysis suggested improved survival among MK+ patients with monosomy 7 compared to those without

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monosomy 7 in both the CK≤4 (p=0.0005; HR: 0.03; 95%CI 0.004, 0.21) and CK≥5 cohorts (p=0.03; HR 0.62; 95%CI 0.40, 0.94) (Supplemental Table 2).

Monosomy 17

Loss of chromosome 17 occurred with the highest frequency (48%; 64/132) of monosomies detected in MK+ patients with multiple monosomies and only 39% (25/64) achieved CR in this context. All cases of monosomy 17 were observed within the context of MK+ disease and none survived beyond 4 years. The presence of monosomy 17 was associated with a median OS of 0.3y compared to 0.5y for MK+ patients without monosomy 17 (p=0.004; Supplemental Figure 3.). Accounting for karyotype complexity, MK+ patients with monosomy 17 and complexity ≤4 demonstrated an OS of 0.1 years versus 0.4 years for those without monosomy 17 (p=0.06; HR:3.11; 95%CI 0.90, 10.7) (Figure 2E). Similarly, the HR for this group (Monosomy17+/ MK+/ CK ≤4) was 12.67 (95%CI 1.30, 123.30) when multivariate analysis was performed, although the small number of occurrences (n=4) again precludes any definitive statements regarding significance. The majority of the occurrences of monosomy 17 (94%, 68/72) were among patients with a highly complex karyotype (CK≥5). The presence of monosomy 17 in this context had an OS of 0.4 years compared to 0.5 years in those without this monosomy (p=0.01; HR: 1.53; 95%CI 1.09, 2.13) (Figure 2F). Similarly, the HR for this group (Monosomy17+/ MK+/ CK≥5) was 1.54 (95%CI 1.02, 2.33, p=0.04) when multivariate analysis was performed among MK+ patients (Supplemental Table 3).

Contribution of Concurrent Monosomy 5, 7, and/or 17

Outcomes of patients with monosomy 5 and/or monosomy 7 and/or monosomy 17 relative to MK+ status are presented in Table 4. In the absence of monosomy 7 and monosomy 17, monosomy 5 occurred in 13 patients with non-CBF cytogenetic abnormalities with 12/13 being MK+ (CR 25%, 4yOS= 0%). In the absence of monosomy 5 and monosomy 17, monosomy 7 occurred in 74 patients with 28/74 being MK- (CR 32.1%, 4yOS=11%) and 46/74 being MK+ (CR 30.4%, 4yOS 7%; log-rank test for OS p=0.10). In the absence of monosomy 5 and monosomy 7, monosomy 17 occurred in 33 patients with all being MK+ (CR 36.4%, 4yOS = 0%). Of 172 patients possessing karyotypes with monosomy 5 and/or monosomy 7 and/or monosomy 17, only 4% (7/172) were known to be alive at 4 years and each were observed to possess monosomy 7.

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Karyotypes lacking monosomy 5, monosomy 7, and monosomy 17 occurred in 406/686 (59%) of patients with non-CBF clonal abnormalities with 87% (354/406) having MK- and 13% (52/406) having MK+ AML. Those patients with MK- disease lacking monosomy 5 /monosomy 7 /monosomy 17 had a CR of 47.7% and a 4yOS of 14% compared to a CR of 26.9% and a 4yOS of 4% for those with MK+ disease also lacking monosomy 5 /monosomy 7/ monosomy 17 (log-rank test for OS p <0.001).

In a multivariate model including Monosomy 5 status, Monosomy 7 status, Monosomy 17 status, and other risk factors, those with monosomy 7 still showed improved OS compared to those without monosomy 7 in MK+/ CK ≤4 patients (p=0.0012; HR: 0.02; 95%CI 0.001, 0.19). In MK+/ CK≥5 patients, monosomy 7 and monosomy 17 are still independent prognostic factors. Patients with monosomy 7 (p=0.003; HR: 0.62; 95%CI 0.40, 0.95) or patients without monosomy 17 (p=0.03; HR: 1.63; 95%CI 1.05, 2.56) had improved OS (Supplemental Table 4).

Discussion

Through the collective analysis of the data and outcomes of 1,592 patients enrolled onto four prospective ECOG-ACRIN AML therapeutic trials, we now contribute a number of new observations relevant to the cytogenetic categories of MK+ and CK+ AML. Monosomy 5 appeared to have no independent prognostic value and the negative prognostic value of monosomy 7 was lost in the context of MK+ AML. Additionally, the prognostic value of the MK+ designation appeared to be restricted to those patients with karyotype complexity ≤4 clonal abnormalities. However, monosomy 17 was found to occur exclusively within monosomal karyotype patients and to independently predict for worse survival among MK+ patients as well as those with highly complex karyotypes (MK+/CK≥5).

Poor prognosis associated with chromosomal aberrations involving the long arm of chromosomes 5 or 7 [add/del (5q) or add/del (7q)] as well as complete loss of chromosome 5 or 7 (monosomy 5 or monosomy 7) has been previously described.[1,5] Our data demonstrate that monosomy 5 occurred almost exclusively in the setting of MK+ (53/54) and highly complex (50/54 with CK≥5) karyotypes, and that monosomy 5 contributed no prognostic value within the CK≥5 patients. This data suggests that the negative impact previously attributed to monosomy 5 is actually attributable to karyotype complexity. Review of the HOVON/SAKK, SWOG, and

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Groupe Ouest-Est d'Etude des Leucémies et Autres Maladies du Sang (GOELAMS) reports of their individual monosomal karyotype experiences also failed to reveal a single case of monosomy 5 occurring outside of MK+ AML.[8,10,20] While these reports do not directly describe the frequency of monosomy 5 within their CK+ AML population, it should be noted that 89% (417/470) of the collective MK+ patients described by HOVON/SAKK, SWOG, GOELAMS carried the CK+ designation with CK+ defined as ≥ 3 clonal abnormalities in each analysis.

Although our data confirm that the prognosis for AML patients with monosomy 7 (in the absence of monosomal karyotype) is poor (Table 4), it should be noted that the negative prognostic impact of monosomy 7 was lost in the context of MK+ AML (Figures 2C, 2D; Supplemental Table 2). Although no difference in survival was seen by univariate analysis amongst MK+ / CK≥5+ / Monosomy 7+ patients compared to those without monosomy 7 (Figure 1D), the presence of monosomy 7 was actually observed to confer a statistically significant improvement in survival for MK+ patients regardless of karyotype complexity ≤4 or ≥5; Supplemental Table 2).

While the poor prognostic impact of 17p aberrations[21,22] and the collective group of chromosome 17 abnormalities[23,24] in AML have been demonstrated, the significance of monosomy 17 itself has not been well described. In our experience monosomy 17 occurred exclusively (72/72) within the setting of MK+ disease and predominantly within the CK≥5+ population (68/72). The presence of monosomy 17 identified a distinct subgroup of MK+ patients with an inferior OS (Figure 2F and Supplemental Figure 2).

The strong association between monosomies and their occurrence in complex karyotypes illustrates the significant shortcomings of the current ISCN nomenclature when defining a "monosomy". Nearly all complex karyotypes with apparent monosomies have multiple concurrent unclassifiable portions of chromosomal material, i.e., "add" chromosomes, marker chromosomes or ring chromosomes, which likely represent portions of the missing monosomies listed in the ISCN. In should also be noted that, the identification of double monosomies (loss of *both* copies of the same chromosome) is always accompanied by structural abnormalities, presumably representing critical portions of one of the missing chromosomes since true double monosomy is not compatible with cell viability. Thus, evaluating the whole genome of leukemia cells via the emerging technologies of whole genome microarrays and next generation sequencing techniques[25] will provide

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more accurate characterization as to the extent of true chromosomal gains and losses associated with complex karyotypes with apparent monosomies.

Although numerous reports have demonstrated the association of dismal outcomes with AML patients of advanced age and the increased incidence of high-risk cytogenetic abnormalities among this population[26-28], it should not be assumed that the prognostic impact of MK is simply a reflection of the advanced age of the population in which is more commonly occurs. The data reported by HOVON/SAKK, SWOG, and GOELAMS has consistently demonstrated inferior outcomes among MK+ AML patients compared to their age-matched MK- counterparts.[8,10,20] While the ECOG-ACRIN experience reported here also demonstrated an increased incidence of MK+ among older patients (≥55y) compared to their younger counterparts, the prognostic significance of MK+ occurring within less complex karyotypes (≤4 clonal abnormalities) was retained when controlling for age via multivariate analysis (Table 6). It should also be noted that the prognostic significance of monosomy 17 (occurring exclusively within the MK+ population) was also retained when controlling for age by multivariate analysis regardless of karyotype complexity (Supplemental Table 3). Our data further support the delineation of the risk group characterized by the presence of MK+ with subgroups possessing similar median OS regardless of karyotype complexity (Supplemental Figure 1). Median OS of CK+/MK- disease was twice that of CK+/MK+ patients and was best among those with CK-/MK- AML.

The ECOG-ACRIN experience demonstrates that CK≥5 and MK+ (with karyotype complexity ≤4) designations reliably predict poor outcomes for which no consistently effective therapeutic strategy has yet been identified. Allogeneic HSCT and possibly the incorporation of high-dose cytarabine[29] into a patient's therapeutic regimen may improve outcomes. However, the benefit of these treatments appears marginal and advanced age and resultant comorbidities limit the feasibility of these therapies for many CK≥5 and MK+ patients. While allogeneic HSCT appears to be the only potentially curative option available, overall outcomes remain poor with HSCT of MK+ patients yielding a 4-year OS of 25% compared to 0% without transplant as reported by the Fred Hutchinson Cancer Research Center/Seattle Cancer Care Alliance.[30] Similarly, the Center for International Blood and Marrow Transplant Research has reported a 3-year OS of 29% and that MK+ AML patients was associated with higher mortality ($p<0.001$; RR 1.67; 95%CI 1.38, 2.01) than that of

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normal karyotype AML patients undergoing HSCT.[31] Cumulative relapse incidences of MK+ patients at 3-years have been reported at 52-62% for those transplanted in CR1[31,32] and 67% for those transplanted beyond CR1.[32]

Although AML is often treated uniformly, it is increasingly evident that this disease is largely heterogeneous with genetically distinct subgroups. The possibility exists that prior lack of awareness of disease subgroups, such as MK+ disease, has negatively impacted outcomes and inhibited the advancement of therapeutic options for better risk subgroups. Inclusion of the MK+ patient population and its dismal prognosis on clinical trials along with relatively better risk AML patients may obscure the recognition of therapeutic benefits for patients with better risk disease. Accounting for these subgroups via mechanisms such as the hierarchical classification according to specific chromosomal lesions as proposed by Middeke et al[22] may enable more appropriate selection of patients for particular therapeutic modalities such as allogeneic HSCT. They describe the ability to select MK+ patients with better HSCT outcomes based on the presence or absence of abnl(17p) or monosomy 5 / del(5q) lesions. Given the association of TP53 abnormalities with MK+ AML[33], the authors allude to the possibility that TP53 abnormalities may play a role in resistance of this MK+ subset against the graft-versus-leukemia effect of allogeneic HSCT.[22] Of note, TP53 mutation status was known on six of the monosomy 17 patients included in our analysis and, interestingly, none of these six were found to possess TP53 mutations. As such, the true degree to which TP53 abnormalities or possible aberrations associated with the loss of genes on 17q such as *ERBB2, NF1, RARA, BRCA1, or STAT3* contribute to resistance within monosomy 17 patients is unknown and warrants further investigation.

With the potential promise of molecular targeting it is imperative that we decipher the pathophysiologic mechanisms associated with the poor outcome of MK+ AML patients. Thoughtful design of future AML clinical trials, accurate identification of a patient's cytogenetic/molecular risk profile prior to trial enrollment, and investigation of more homogenous AML subgroups may open the door to therapeutic advances and improved outcomes that have been lacking in this disease. The feasibility of such an approach is currently under investigation [NCT01684150] based on the discovery that DOT1L inhibition is able to induce selective cytotoxicity in poor risk *MLL*-rearranged leukemic cells while sparing non-rearranged cells.[34,35] Similarly, the

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prevalence of TP53 alterations within CK+ AML[33] makes p53 a potentially attractive target to explore in this population. Although targeting p53 has presented significant challenges in the past, agents are now available which restore wild-type activity to mutant p53 proteins (APR-246[36,37]) and agents which disrupt MDM/p53 interactions (RG7112 [38,39], JNJ-26854165 [40]) and subsequently lead to apoptosis.[41]

In conclusion, while MK is an independent predictor of outcomes in adult AML patients, our data suggest the prognostic utility of the MK+ designation appeared to be limited to those with less complex karyotypes (complexity ≤4). Our study draws into question the independent prognostic value of monosomy 5 as this monosomy occurred almost exclusively in MK+ and CK≥5 patients. The majority of monosomy 7 occurrences appeared in the context of MK+ disease where its prognostic value was lost regardless of karyotype complexity. Although the independent significance of monosomy 5 and monosomy 7 were lacking within the context of MK+ AML, monosomy 17 defined a distinct subcategory of MK+ AML. The very poor prognosis associated with MK+/CK≤4 AML, monosomy 17+ AML, and highly complex (CK≥5) AML underscores the need for better understanding of the genetic aberrations and potential targets present within these karyotypes to guide investigation of novel therapeutic strategies in this group of patients.

Conflict of Interest

The authors declare no conflicts of interest pertaining to the content of this manuscript.

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References

1. Grimwade D, Walker H, Oliver F, et al. The importance of diagnostic cytogenetics on outcome in AML: analysis of 1,612 patients entered into the MRC AML 10 trial. The Medical Research Council Adult and Children's Leukaemia Working Parties. *Blood* 1998; **92**(7): 2322-33.
2. Mrozek K, Heinonen K, de la Chapelle A, Bloomfield CD. Clinical significance of cytogenetics in acute myeloid leukemia. *Semin Oncol* 1997; **24**(1): 17-31.
3. Byrd JC, Mrozek K, Dodge RK, et al. Pretreatment cytogenetic abnormalities are predictive of induction success, cumulative incidence of relapse, and overall survival in adult patients with de novo acute myeloid leukemia: results from Cancer and Leukemia Group B (CALGB 8461). *Blood* 2002; **100**(13): 4325-36.
4. Johnson E, Cotter FE. Monosomy 7 and 7q--associated with myeloid malignancy. *Blood Rev* 1997; **11**(1): 46-55.
5. Grimwade D, Hills RK, Moorman AV, et al. Refinement of cytogenetic classification in acute myeloid leukemia: determination of prognostic significance of rare recurring chromosomal abnormalities among 5876 younger adult patients treated in the United Kingdom Medical Research Council trials. *Blood* 2010; **116**(3): 354-65.
6. Yates JW, Wallace HJ, Jr., Ellison RR, Holland JF. Cytosine arabinoside (NSC-63878) and daunorubicin (NSC-83142) therapy in acute nonlymphocytic leukemia. *Cancer Chemother Rep* 1973; **57**(4): 485-8.
7. Bloomfield CD, Goldman A, Hassfeld D, de la Chapelle A. Fourth International Workshop on Chromosomes in Leukemia 1982: Clinical significance of chromosomal abnormalities in acute nonlymphoblastic leukemia. *Cancer Genet Cytogenet* 1984; **11**(3): 332-50.
8. Breems DA, Van Putten WL, De Greef GE, et al. Monosomal karyotype in acute myeloid leukemia: a better indicator of poor prognosis than a complex karyotype. *J Clin Oncol* 2008; **26**(29): 4791-7.
9. Rollig C, Bornhauser M, Thiede C, et al. Long-term prognosis of acute myeloid leukemia according to the new genetic risk classification of the European LeukemiaNet recommendations: evaluation of the proposed reporting system. *J Clin Oncol* 2011; **29**(20): 2758-65.
10. Medeiros BC, Othus M, Fang M, Roulston D, Appelbaum FR. Prognostic impact of monosomal karyotype in young adult and elderly acute myeloid leukemia: the Southwest Oncology Group (SWOG) experience. *Blood* 2010; **116**(13): 2224-8.
11. Haferlach C, Alpermann T, Schnittger S, et al. Prognostic value of monosomal karyotype in comparison to complex aberrant karyotype in acute myeloid leukemia: a study on 824 cases with aberrant karyotype. *Blood* 2012; **119**(9): 2122-5.
12. Schanz J, Tuchler H, Sole F, et al. Monosomal karyotype in MDS: explaining the poor prognosis? *Leukemia : official journal of the Leukemia Society of America, Leukemia Research Fund, UK* 2013; **27**: 1988-95.
13. Valcarcel D, Adema V, Sole F, et al. Complex, not monosomal, karyotype is the cytogenetic marker of poorest prognosis in patients with primary myelodysplastic syndrome. *J Clin Oncol* 2013; **31**(7): 916-22.
14. Cripe LD, Uno H, Paietta EM, et al. Zosuquidar, a novel modulator of P-glycoprotein, does not improve the outcome of older patients with newly diagnosed acute myeloid leukemia: a randomized, placebo-controlled trial of the Eastern Cooperative Oncology Group 3999. *Blood* 2010; **116**(20): 4077-85.
15. Fernandez HF, Sun Z, Yao X, et al. Anthracycline dose intensification in acute myeloid leukemia. *N Engl J Med* 2009; **361**(13): 1249-59.
16. Rowe JM, Andersen JW, Mazza JJ, et al. A randomized placebo-controlled phase III study of granulocyte-macrophage colony-stimulating factor in adult patients (> 55 to 70 years of age) with acute myelogenous leukemia: a study of the Eastern Cooperative Oncology Group (E1490). *Blood* 1995; **86**(2): 457-62.
17. Rowe JM, Neuberg D, Friedenberg W, et al. A phase 3 study of three induction regimens and of priming with GM-CSF in older adults with acute myeloid leukemia: a trial by the Eastern Cooperative Oncology Group. *Blood* 2004; **103**(2): 479-85.
18. O'Donnell MR, Tallman MS, Abboud CN, et al. Acute myeloid leukemia, version 2.2013. *J Natl Compr Canc Netw* 2013; **11**(9): 1047-55.

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19. Dohner H, Estey EH, Amadori S, et al. Diagnosis and management of acute myeloid leukemia in adults: recommendations from an international expert panel, on behalf of the European LeukemiaNet. *Blood* 2010; **115**(3): 453-74.
20. Perrot A, Luquet I, Pigneux A, et al. Dismal prognostic value of monosomal karyotype in elderly patients with acute myeloid leukemia: a GOELAMS study of 186 patients with unfavorable cytogenetic abnormalities. *Blood* 2011; **118**(3): 679-85.
21. Seifert H, Mohr B, Thiede C, et al. The prognostic impact of 17p (p53) deletion in 2272 adults with acute myeloid leukemia. *Leukemia : official journal of the Leukemia Society of America, Leukemia Research Fund, UK* 2009; **23**(4): 656-63.
22. Middeke JM, Beelen D, Stadler M, et al. Outcome of high-risk acute myeloid leukemia after allogeneic hematopoietic cell transplantation: negative impact of abnl(17p) and -5/5q. *Blood* 2012; **120**(12): 2521-8.
23. Lai JL, Preudhomme C, Zandecki M, et al. Myelodysplastic syndromes and acute myeloid leukemia with 17p deletion. An entity characterized by specific dysgranulopoiesis and a high incidence of P53 mutations. *Leukemia : official journal of the Leukemia Society of America, Leukemia Research Fund, UK* 1995; **9**(3): 370-81.
24. Nazha A, Kantarjian HM, Bhatt VR, et al. Prognostic implications of chromosome 17 abnormalities in the context of monosomal karyotype in patients with acute myeloid leukemia and complex cytogenetics. *Clinical lymphoma, myeloma & leukemia* 2014; **14**(2): 163-71.
25. Timothy J. Ley CM, Li Ding, et al. . Genomic and Epigenomic Landscapes of Adult De Novo Acute Myeloid Leukemia. *N Engl J Med* 2013; **368**(22): 16.
26. Appelbaum FR, Rowe JM, Radich J, Dick JE. Acute Myeloid Leukemia. *ASH Education Program Book* 2001; **2001**(1): 62-86.
27. Leith CP, Kopecky KJ, Godwin J, et al. Acute Myeloid Leukemia in the Elderly: Assessment of Multidrug Resistance (MDR1) and Cytogenetics Distinguishes Biologic Subgroups With Remarkably Distinct Responses to Standard Chemotherapy. A Southwest Oncology Group Study. *Blood* 1997; **89**(9): 3323-9.
28. Schoch C, Kern W, Krawitz P, et al. Dependence of age-specific incidence of acute myeloid leukemia on karyotype. *Blood* 2001; **98**(12): 3500-.
29. Lowenberg B, Pabst T, Vellenga E, et al. Cytarabine dose for acute myeloid leukemia. *N Engl J Med* 2011; **364**(11): 1027-36.
30. Fang M, Storer B, Estey E, et al. Outcome of acute myeloid leukemia patients with monosomal karyotype who undergo hematopoietic cell transplantation. *Blood* 2011; **118**(6): 1490-4.
31. Pasquini MC, Zhang MJ, Medeiros BC, et al. Hematopoietic Cell Transplantation Outcomes in Monosomal Karyotype Myeloid Malignancies. *Biol Blood Marrow Transplant* 2016; **22**(2): 248-57.
32. Oran B, Dolan M, Cao Q, Brunstein C, Warlick E, Weisdorf D. Monosomal karyotype provides better prognostic prediction after allogeneic stem cell transplantation in patients with acute myelogenous leukemia. *Biol Blood Marrow Transplant* 2011; **17**(3): 356-64.
33. Rucker FG, Schlenk RF, Bullinger L, et al. TP53 alterations in acute myeloid leukemia with complex karyotype correlate with specific copy number alterations, monosomal karyotype, and dismal outcome. *Blood* 2012; **119**(9): 2114-21.
34. Daigle SR, Olhava EJ, Therkelsen CA, et al. Selective killing of mixed lineage leukemia cells by a potent small-molecule DOT1L inhibitor. *Cancer cell* 2011; **20**(1): 53-65.
35. Stein EM, Garcia-Manero G, Rizzieri DA, et al. The DOT1L Inhibitor EPZ-5676: Safety and Activity in Relapsed/Refractory Patients with MLL-Rearranged Leukemia. *Blood* 2014; **124**(21): 387.
36. Ali D, Mohammad DK, Mujahed H, et al. Anti-leukaemic effects induced by APR-246 are dependent on induction of oxidative stress and the NFE2L2/HMOX1 axis that can be targeted by PI3K and mTOR inhibitors in acute myeloid leukaemia cells. *British Journal of Haematology* 2016: n/a-n/a.
37. Lehmann S, Bykov VJ, Ali D, et al. Targeting p53 in vivo: a first-in-human study with p53-targeting compound APR-246 in refractory hematologic malignancies and prostate cancer. *J Clin Oncol* 2012; **30**(29): 3633-9.
38. Vassilev LT, Vu BT, Graves B, et al. In vivo activation of the p53 pathway by small-molecule antagonists of MDM2. *Science* 2004; **303**(5659): 844-8.

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39. Andreeff M, Kelly KR, Yee K, et al. Results of the Phase I Trial of RG7112, a Small-Molecule MDM2 Antagonist in Leukemia. *Clinical Cancer Research* 2016; **22**(4): 868-76.
40. Kojima K, Burks JK, Arts J, Andreeff M. The novel tryptamine derivative JNJ-26854165 induces wild-type p53- and E2F1-mediated apoptosis in acute myeloid and lymphoid leukemias. *Molecular cancer therapeutics* 2010; **9**(9): 2545-57.
41. Lehmann BD, Pietenpol JA. Targeting mutant p53 in human tumors. *J Clin Oncol* 2012; **30**(29): 3648-50.

Figure Legend:

Figure 1. Overall Survival according to Monosomal Karyotype status and Karyotype Complexity among patients with Non-CBF clonal cytogenetic abnormalities

- **A.** Overall Survival of patients with or without MK and karyotype complexity ≤ 4
- **B.** Overall Survival of patients with or without MK and karyotype complexity ≥ 5

Figure 2. Overall Survival of MK patients according to Monosomy 5, Monosomy 7, or Monosomy 17 status and Karyotype Complexity

- **A.** Overall Survival of MK+ patients with karyotype complexity ≤ 4 with or without Monosomy 5
- **B.** Overall Survival of MK+ patients with karyotype complexity ≥ 5 with or without Monosomy 5
- **C.** Overall Survival of MK+ patients with karyotype complexity ≤ 4 with or without Monosomy 7
- **D.** Overall Survival of MK+ patients with karyotype complexity ≥ 5 with or without Monosomy 7
- **E.** Overall Survival of MK+ patients with karyotype complexity ≤ 4 with or without Monosomy 17
- **F.** Overall Survival of MK+ patients with karyotype complexity ≥ 5 with or without Monosomy 17

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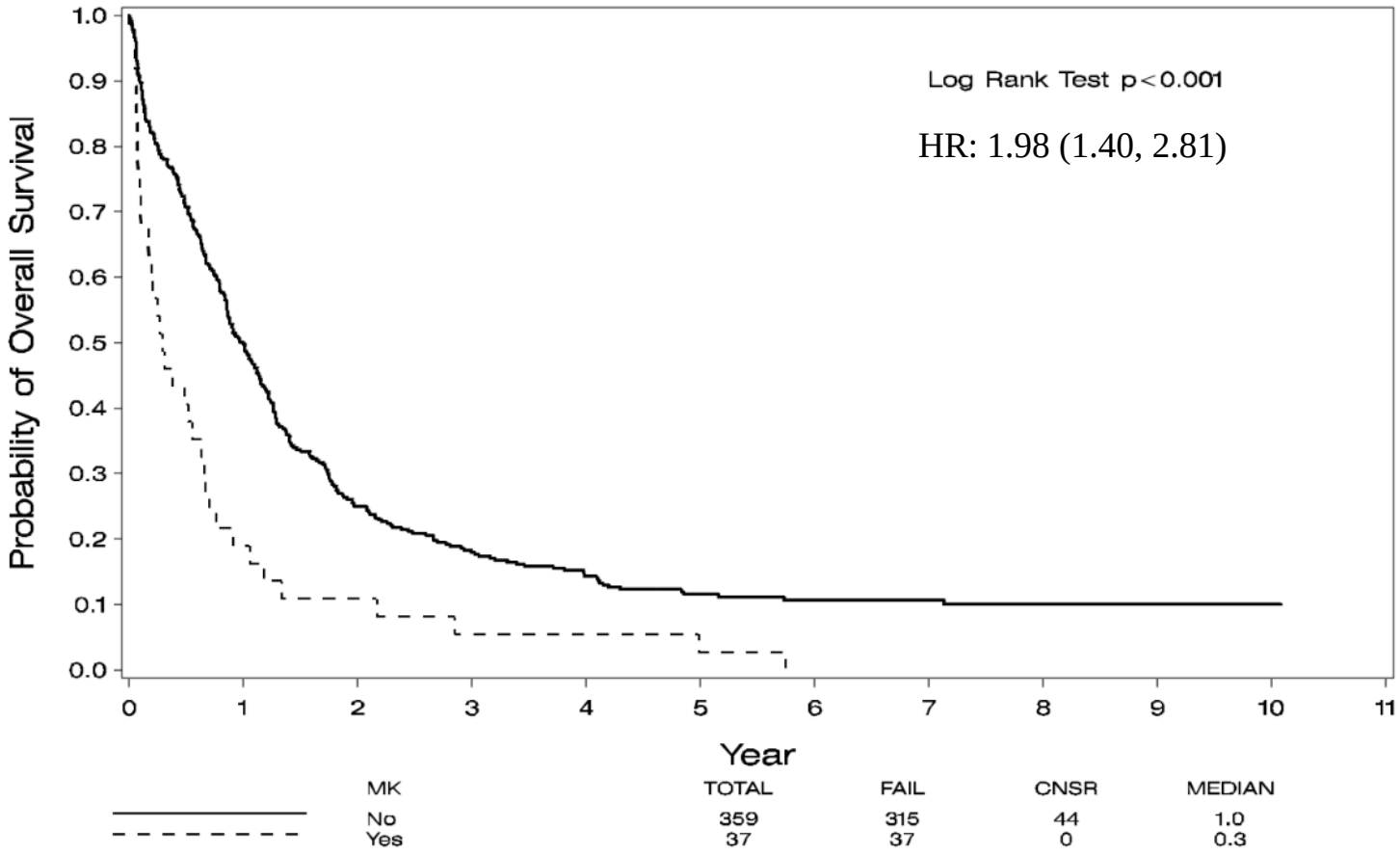
Figure 1. Overall Survival according to Monosomal Karyotype status and Karyotype Complexity among Patients with Non-CBF Clonal Cytogenetic Abnormalities

A.



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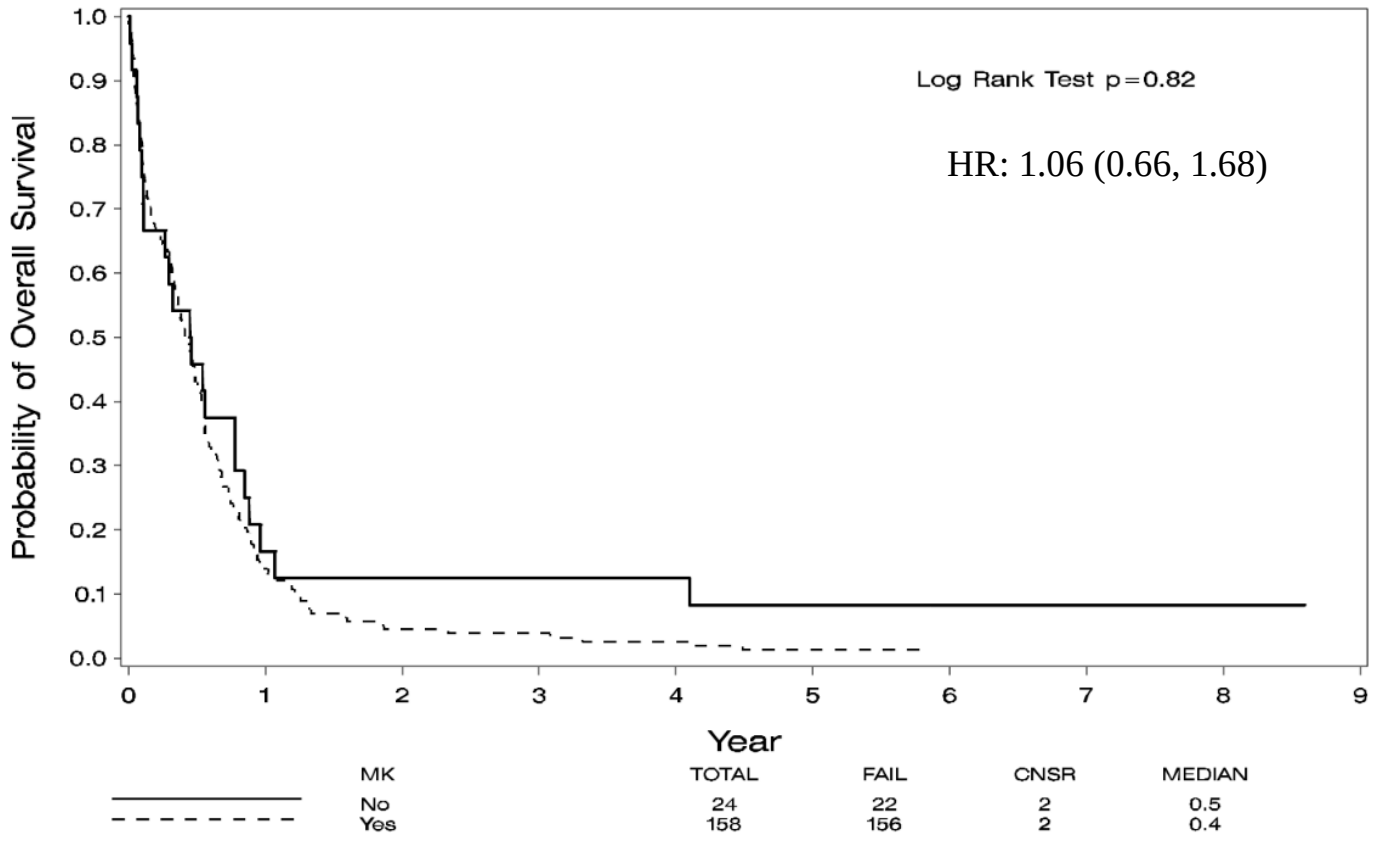

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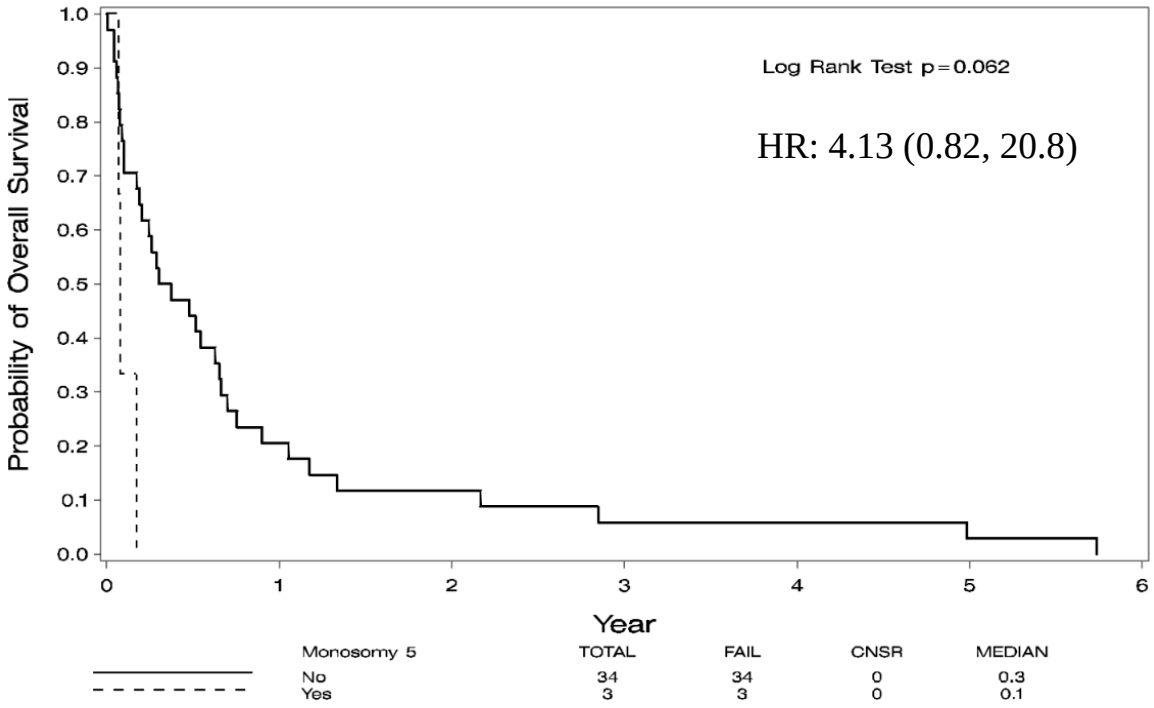
Figure 2. Overall Survival of MK patients according to Monosomy 5, Monosomy 7, or Monosomy 17 status and Karyotype Complexity

A.



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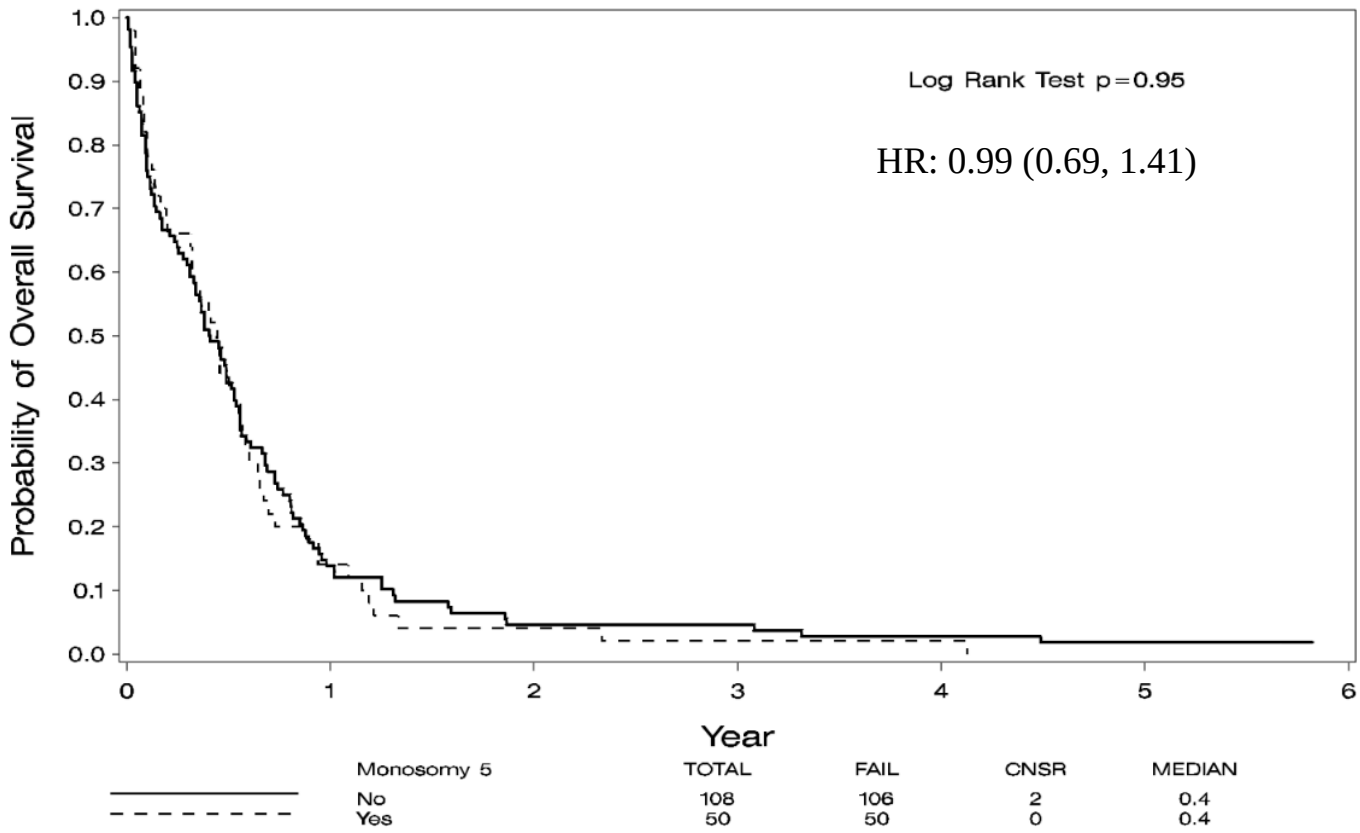

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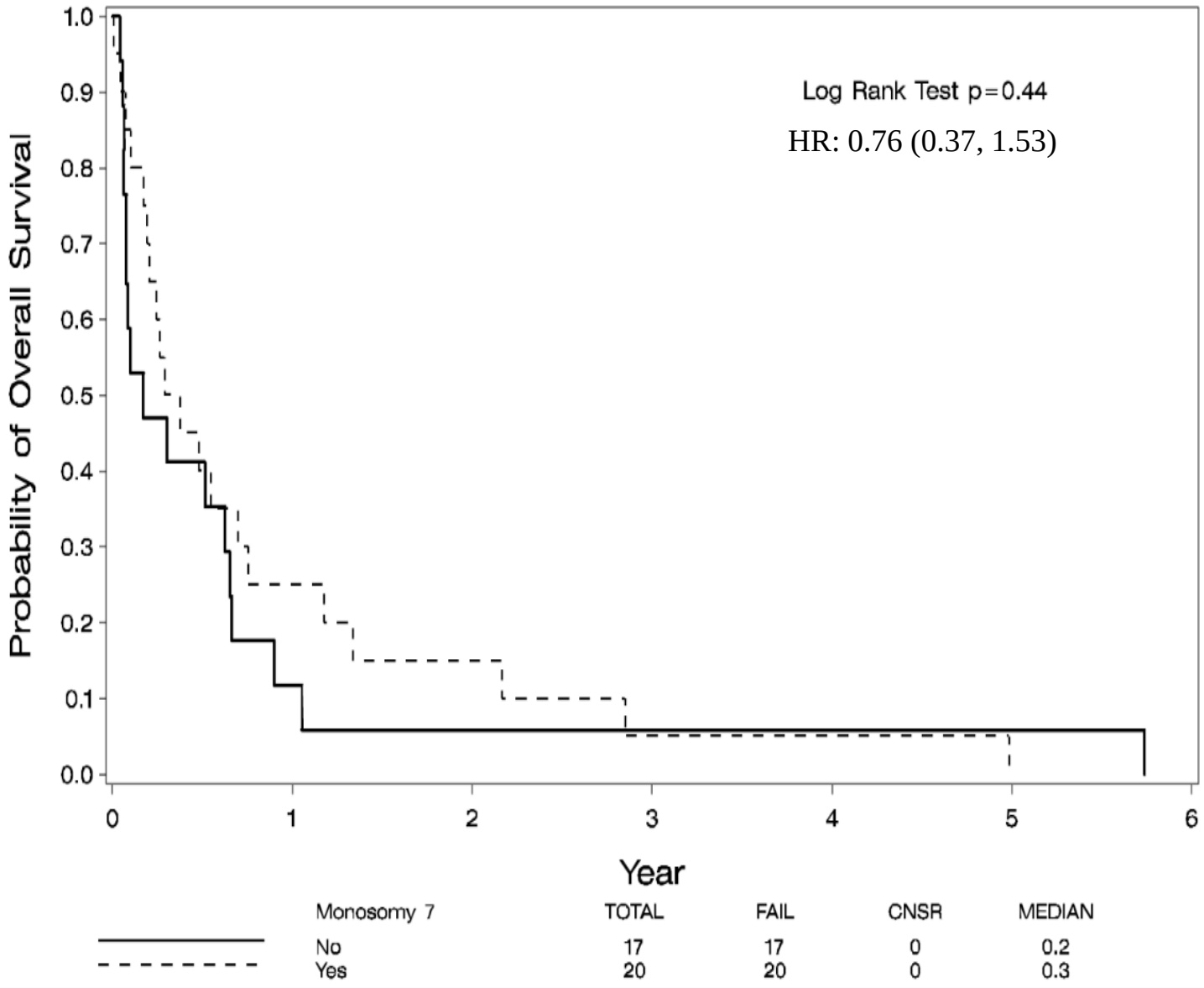
C.



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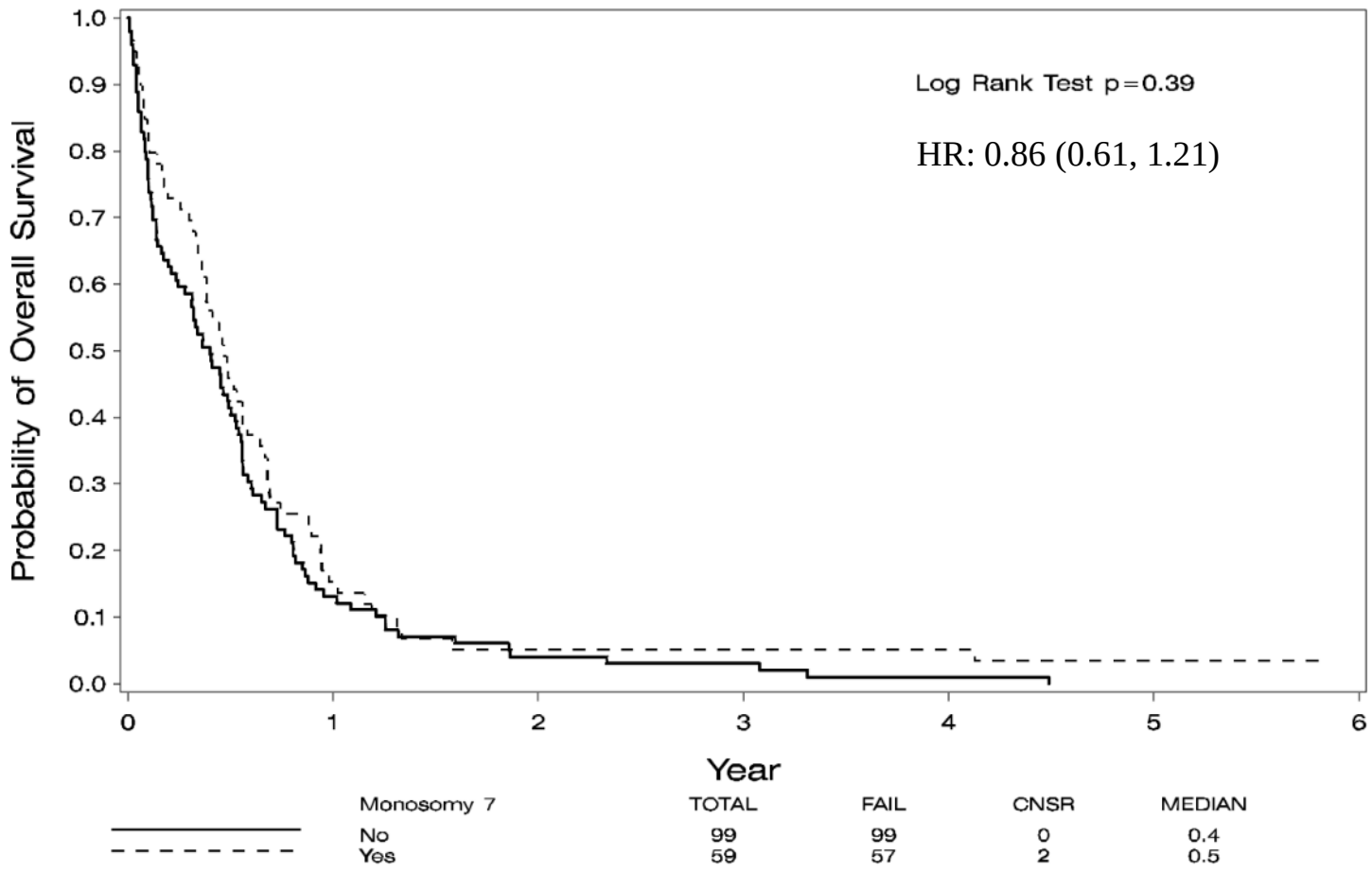

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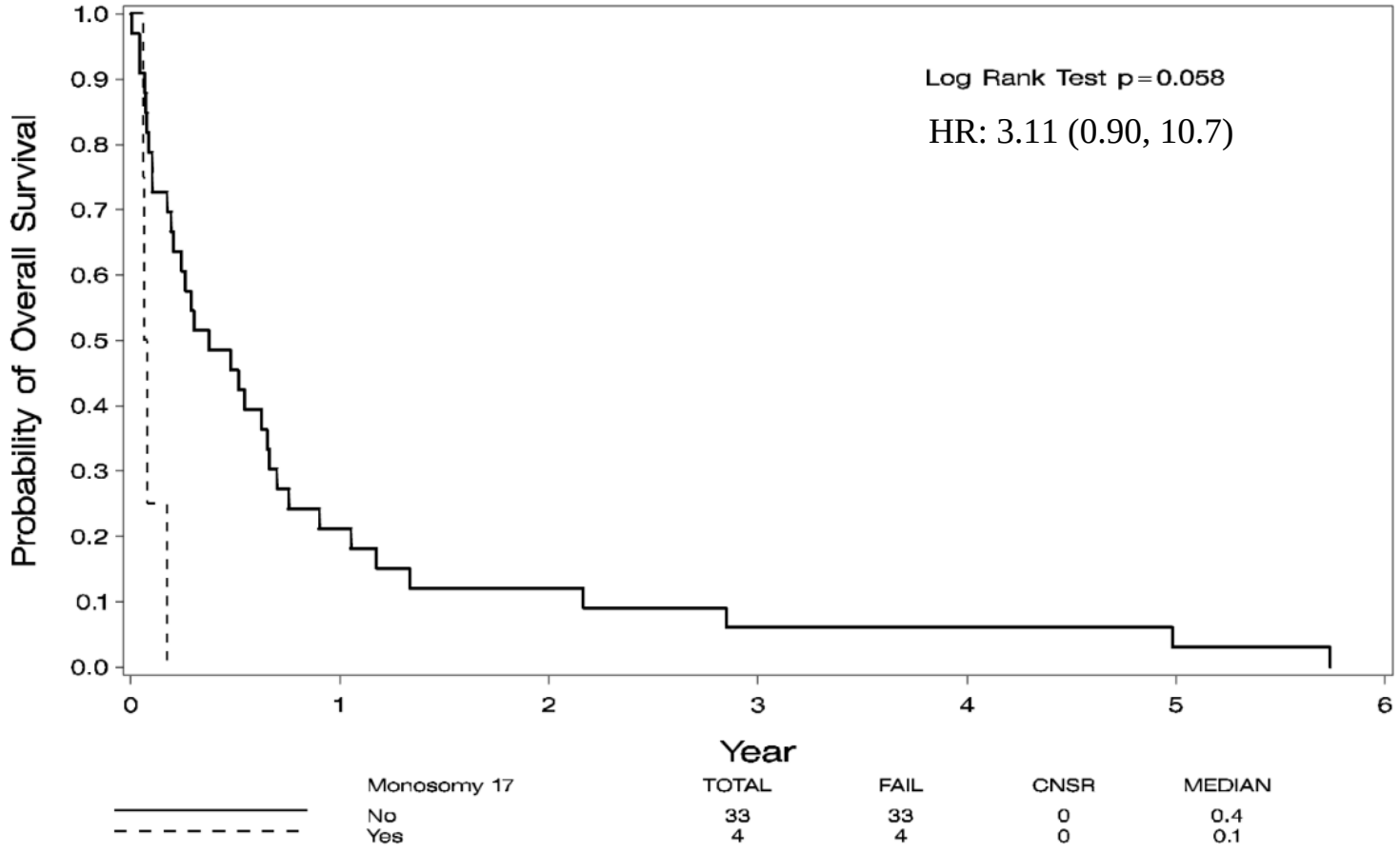

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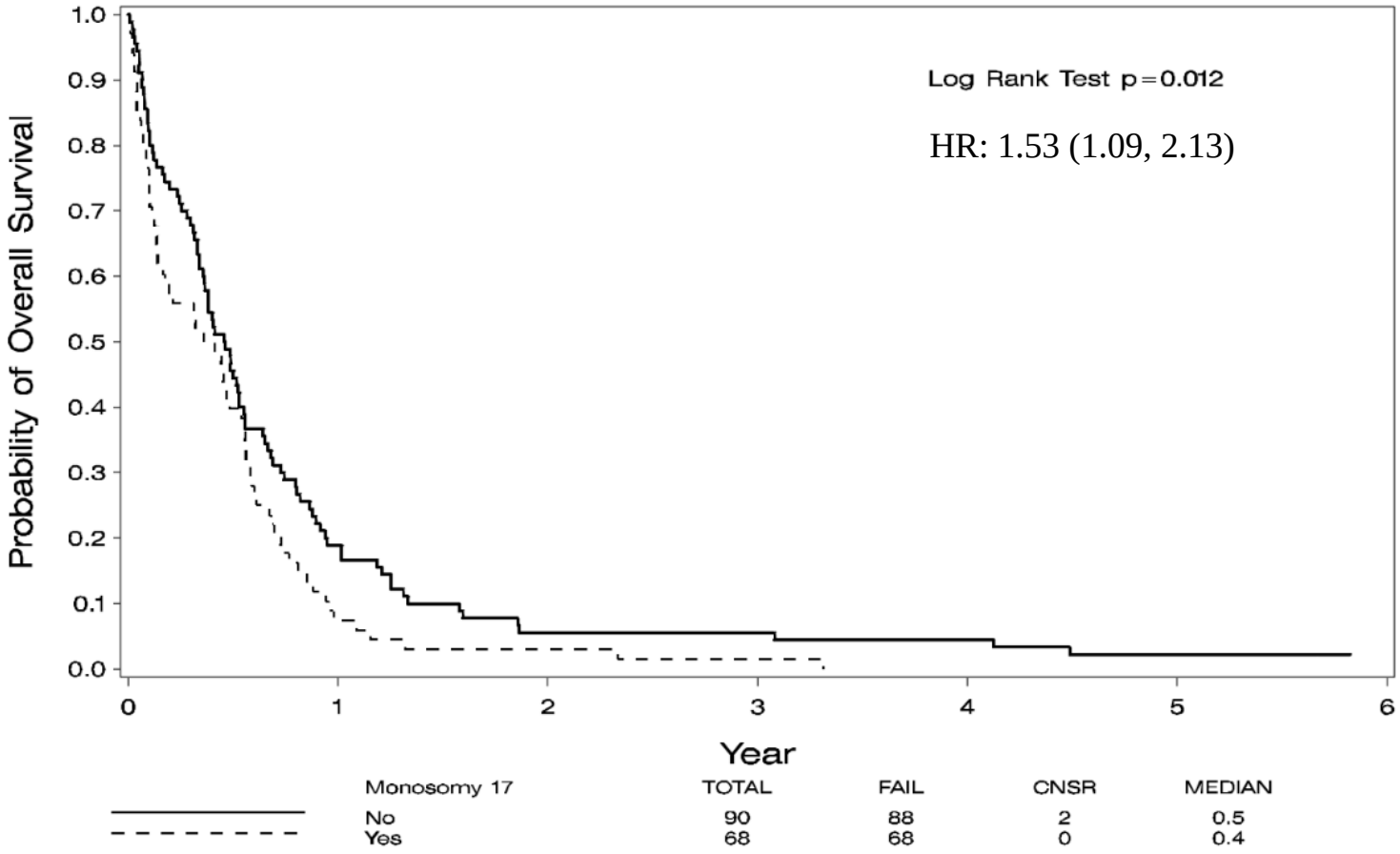

Table Legend:

Table 1. Cytogenetic categorization of 1188 Acute Myeloid Leukemia Patients on ECOG-ACRIN Therapeutic Trials (E1490, E1900,E3993,E3999)

Table 2. Baseline Characteristics by Monosomal Karyotype Status

Table 3. Distribution of Chromosomal Monosomies and Associated Outcomes

Table 4. Outcome Measures by Monosomal Karyotype Status Among Patients with Non-CBF Clonal Cytogenetic Abnormalities

Table 5. Outcome Measures by Karyotype Complexity and Monosomal Karyotype Status Among Patients with Non-CBF Clonal Cytogenetic Abnormalities

Table 6. Multivariate Analysis of Monosomal Karyotype, Karyotype Complexity, and Independent High-Risk Cytogenetic Abnormalities Among Patients with Non-CBF Clonal Cytogenetic Abnormalities

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Table 1. Cytogenetic categorization of 1188 Acute Myeloid Leukemia Patients on ECOG-ACRIN Therapeutic Trials (E1490,E1900,E3993,E3999)		
Karyotype	**N**	**%**
Normal	**502**	**42**
Clonal Abnormalities	**686**	**58**
- **CBF**	**108**	**9**
- **Single Monosomy**	**97**	**8**
- **Multiple Monosomies**	**132**	**11**
- **Complex**	**251**	**21**
- **Monosomal**	**195**	**16**

Table 2. Baseline Characteristics by Monosomal Karyotype Status										
	MK-			**MK+**			**Total**			**P-value**
	Median	**Min**	**Max**	**Median**	**Min**	**Max**	**Median**	**Min**	**Max**	
AGE	**59.0**	**17**	**93**	**65.0**	**20**	**86**	**61.0**	**17**	**93**	**<0.001**
Peripheral WBC count (/mm3)(x1000)	**10.0**	**1**	**213**	**3.5**	**0**	**200**	**8.6**	**0**	**213**	**<0.001**
Hemoglobin	**9.2**	**4**	**30**	**8.9**	**5**	**13**	**9.1**	**4**	**30**	**0.009**
Peripheral Blasts (%)	**26.0**	**0**	**98**	**9.0**	**0**	**98**	**23.0**	**0**	**98**	**<0.001**
Peripheral platelet count (/mm3)(x1000)	**55.0**	**1**	**650**	**43.0**	**5**	**995**	**53.0**	**1**	**995**	**0.002**
Marrow Blasts (%)	**62.0**	**1**	**100**	**48.5**	**0**	**100**	**60.0**	**0**	**100**	**<0.001**
	N	**%**		**N**	**%**		**N**	**%**		
Secondary AML	**126**	**12.7**		**45**	**23.1**		**171**	**14.4**		**<0.001**

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Table 3. Distribution of Chromosomal Monosomies and Associated Outcomes

	Single Monosomy					*≥ 2 Monosomies*				
	N	**N of CR**	**CR%**	**4-Year OS %***	**SE**	**N**	**N of CR**	**CR%**	**4-Year OS %***	**SE**
All patients**	97	25	25.8	7	3	132	47	35.6	2	1
Non-CBF	96	25	26.0	6	2	132	47	35.6	2	1
Mono 1	1	1	100.0	.	.	7	2	28.6	.	.
Mono 2	.	.	.	.	.	7	4	57.1	.	.
Mono 3	1	1	100.0	.	.	24	13	54.2	0	.
Mono 4	.	.	.	.	.	14	7	50.0	7	7
Mono 5	2	1	50.0	.	.	52	18	34.6	2	2
Mono 6	1	0	0	.	.	14	7	50.0	0	.
Mono 7	51	15	29.4	10	4	56	20	35.7	4	2
Mono 8	2	0	0	.	.	6	2	33.3	.	.
Mono 9	.	.	.	.	.	15	5	33.3	7	6
Mono 10	1	1	100.0	.	.	15	7	46.7	0	.
Mono 11	2	1	50.0	.	.	12	5	41.7	0	.
Mono 12	2	1	50.0	.	.	24	9	37.5	8	6
Mono 13	5	2	40.0	.	.	20	11	55.0	5	5
Mono 14	4	1	25.0	.	.	12	7	41.7	0	.
Mono 15	1	0	0	.	.	6	0	0	.	.
Mono 16	3	1	33.3	.	.	22	6	27.3	0	.
Mono 17	8	0	0	.	.	64	25	39.1	0	.
Mono 18	2	0	0	.	.	40	18	45.0	2	2
Mono 19	4	1	25.0	.	.	9	5	55.6	.	.
Mono 20	4	1	25.0	.	.	28	7	25.0	0	.
Mono 21	3	.	.	.	.	30	12	40.0	3	3
Mono 22	.	.	.	.	.	14	5	35.7	0	.

***OS was estimated only for subgroups with ≥ 10 patients**

****One patient demonstrated a single monosomy in association with a CBF abnormality**

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Table 4. Outcome Measures by Monosomal Karyotype Status Among Patients with Non-CBF Clonal Cytogenetic Abnormalities

	MK-					MK+					P-value**
	N	N of CR	CR%	4-Year OS %	SE	N	N of CR	CR%	4-Year OS %	SE#	
Age (yrs)											
Age < 55	107	63	58.9	27	4	25	10	40.0	16	7	0.02
Age ≥ 55	276	116	42.0	10	2	170	51	30.0	1	1	<0.001
CK*											
No	314	153	48.7	15	2	13	2	15.4	8	7	0.04
Yes	69	26	37.7	12	4	182	59	32.4	3	1	<0.001
Neither Monosomy 5, nor Monosomy 7, nor Monsomy 17	354	169	47.7	14	2	52	14	26.9	4	3	<0.001
Monosomy 5, w/o Monosomy 7, w/o Monosomy 17	1	1	100.0	0	.	12	3	25.0	0	.	0.14
Monosomy 7, w/o Monosomy 5, w/o monosomy 17	28	9	32.1	11	6	46	14	30.4	7	4	0.10
Monosomy 5, Monosomy 7, w/o monosomy 17	0	0	.	.	.	13	5	38.5	8	7	.
Monosomy 17, w/o Monosomy 5, w/o Monosomy 7	0	0	.	.	.	33	12	36.4	0	.	.
Monosomy 5, Monosomy 17, w/o Monosomy 7	0	0	.	.	.	19	6	31.6	0	.	.
Monosomy 7, Monosomy 17, w/o Monosomy 5	0	0	.	.	.	11	3	27.3	0	.	.
Monosomy 5, Monosomy 7, Monosomy 17	0	0	.	.	.	9	4	44.4	0	.	.
Total	383	179	46.7	14	2	195	61	31.3	3	1	<0.001

*** CK: defined as patients with ≥ 3 clonal abnormalities**
**** p-values are based on log-rank test of OS**
 # SE represents standard error

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Table 5. Outcome Measures by Karyotype Complexity and Monosomal Karyotype Status Among Patients with Non-CBF Clonal Cytogenetic Abnormalities

	MK-					MK+					P-value**
	N	N of CR	CR%	4-Year OS %	SE†	N	N of CR	CR%	4-Year OS %	SE†	
# of abnormalities											
≤ 4	359	170	47.4	14	2	37	8	21.6	5	4	<0.001
≥ 5	24	9	37.5	13	7	158	53	33.5	3	1	0.82

** p-values are based on log-rank test of OS

† SE represents standard error

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Table 6. Multivariate Analysis of Monosomal Karyotype, Karyotype Complexity, and Independent High-Risk Cytogenetic Abnormalities Among Patients with Non-CBF Clonal Cytogenetic Abnormalities

	Karyotype Complexity ≤ 4 Clonal Abnormalities				Karyotype Complexity ≥ 5 Clonal Abnormalities			
Parameter	**P-value**	**Hazard Ratio**	**95% Hazard Ratio Confidence Limits**		**P-value**	**Hazard Ratio**	**95% Hazard Ratio Confidence Limits**	
MK Yes vs no	**0.01**	**1.78**	**1.13**	**2.79**	**0.47**	**1.22**	**0.72**	**2.06**
# of abnormality 3 vs. <3	**0.77**	**1.06**	**0.71**	**1.58**	-	-	-	-
# of abnormality 4 vs. <3	**0.08**	**1.55**	**0.95**	**2.54**	-	-	-	-
Gender (Male vs. female)	**0.63**	**1.06**	**0.83**	**1.36**	**0.79**	**1.05**	**0.73**	**1.52**
Age (yrs) (Age >=55 vs. <55)	**0.08**	**1.47**	**0.95**	**2.25**	**0.05**	**1.99**	**1.01**	**3.91**
WBC (cells/mm3) >=10,000 vs. <10,000	**0.001**	**1.57**	**1.20**	**2.06**	**0.79**	**1.07**	**0.66**	**1.73**
Hemoglobin (g/dL) hg>=10 vs. <10	**0.92**	**1.02**	**0.77**	**1.33**	**0.97**	**0.99**	**0.65**	**1.51**
Platelets (cells/mm3) >=10,000 vs. <10,000	**0.19**	**0.64**	**0.33**	**1.24**	**0.30**	**0.66**	**0.30**	**1.45**
Marrow: %BLAST	**0.52**	**1.00**	**0.99**	**1.00**	**0.90**	**1.00**	**0.99**	**1.01**
BLOOD:%BLASTS,	**0.06**	**1.00**	**1.00**	**1.01**	**0.002**	**1.01**	**1.01**	**1.02**
Secondary AML (Yes vs. no)	**0.003**	**1.58**	**1.17**	**2.15**	**0.98**	**1.01**	**0.63**	**1.60**
del(5q)	**0.16**	**1.38**	**0.88**	**2.16**	**0.87**	**0.97**	**0.68**	**1.40**
del(7q)	**0.10**	**0.53**	**0.24**	**1.14**	**0.25**	**0.75**	**0.46**	**1.23**
del(17p)	**0.12**	**2.28**	**0.81**	**6.38**	**0.94**	**1.03**	**0.42**	**2.52**
inv(3)	**0.01**	**2.08**	**1.19**	**3.65**	**0.97**	**1.05**	**0.12**	**9.36**
t(6;9)	**0.61**	**1.36**	**0.42**	**4.43**	**.**	**.**	**.**	**.**

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Table 6. Multivariate Analysis of Monosomal Karyotype, Karyotype Complexity, and Independent High-Risk Cytogenetic Abnormalities Among Patients with Non-CBF Clonal Cytogenetic Abnormalities

	Karyotype Complexity ≤ 4 Clonal Abnormalities				Karyotype Complexity ≥ 5 Clonal Abnormalities			
Parameter	**P-value**	**Hazard Ratio**	**95% Hazard Ratio Confidence Limits**		**P-value**	**Hazard Ratio**	**95% Hazard Ratio Confidence Limits**	
11q23	**0.09**	**1.60**	**0.92**	**2.77**	**0.49**	**1.52**	**0.47**	**4.88**
t(9;22)	**0.07**	**2.33**	**0.92**	**5.88**	**.**	**.**	**.**	**.**