Analysis of Patient-Level Data From 3 Cooperative Group Trials Confirms a Survival Advantage for *NPM1*-mutated Patients Achieving MRD-Negative CR After Intensive Induction

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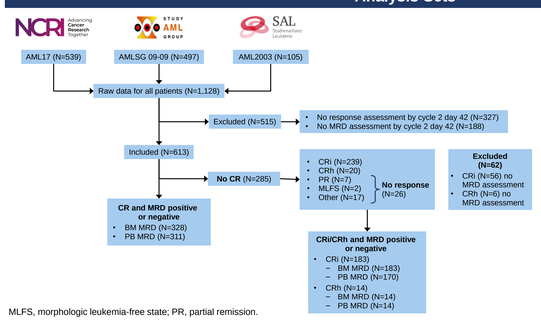
Background

Surrogate endpoints that are reliable predictors of clinical benefit are urgently needed to enable the efficient and timely clinical assessment of novel agents targeting specific genetic drivers of acute myeloid leukemia (AML). *NPM1* mutations (*NPM1*m), predominantly tetra-nucleotide insertions in exon 12, are found in approximately 30% of younger, newly diagnosed AML patients. As clonal leukemogenic driver mutations, *NPM1*m are found exclusively in leukemic blasts and their daughter cells, making them ideal for sensitive detection of measurable residual disease (MRD) using quantitative polymerase chain reaction (qPCR)– or next-generation sequencing (NGS)–based methods. Over 50 studies reported in peer-reviewed publications including several cooperative group trials, have shown that *NPM1*m AML patients in complete remission (CR) have significantly better survival if they are MRD negative compared to those who are MRD positive. The prognostic significance of MRD negativity in patients with CR with incomplete count recovery (CRi) or partial hematologic recovery (CRh) has not yet been established. Based on this body of evidence, the European LeukemiaNet (ELN) recommends monitoring molecular MRD in *NPM1*m AML patients during treatment to help guide treatment decisions. In January 2020, the US FDA issued guidance on the use of MRD as an endpoint in clinical trials in hematologic malignancies, including AML. Recommendations included the use of bone marrow (BM) as the preferred substrate for measuring MRD, as well as the selection of a prespecified post-induction therapy time point, ideally in CR with recovery of peripheral blood (PB) counts. Ivey and colleagues investigated the prognostic value of post-remission MRD status in *NPM1*m patients treated in the AML17 trial.[1] This study showed that MRD detected after 2 cycles of intensive chemotherapy in PB was more strongly associated with relapse-free and overall survival than MRD after 1, 3, or 4 cycles.

Purpose

To build on this foundation and to provide further evidence for the value of MRD as a surrogate endpoint for prospective, randomized trials in patients with newly diagnosed *NPM1*m AML receiving novel agents in combination with intensive chemotherapy, we have evaluated the relationship between MRD status in patients who have achieved CR, CRi, or CRh after 2 cycles of chemotherapy and event-free survival (EFS) and overall survival (OS) in an analysis of patient-level data pooled from 3 large cooperative group trials. MRD in all 3 studies was assessed by quantitation of NPM1m transcripts using reverse transcriptase-mediated qPCR (RT-qPCR) normalized to ABL transcripts. To account for timing consistency of MRD detection across studies, we have limited this analysis to patients who had available data to assign response and MRD status by day 42 from the start of chemotherapy cycle 2.

Analysis Sets



MLFS, morphologic leukemia-free state; PR, partial remission.

Figure 1: Deidentified data for 1,128 patients who achieved CR, CRi, or CRh and had MRD data after 2 cycles of chemotherapy were provided by the UK National Cancer Research Institute (NCRI) for the AML17 trial[1] (N=539), the German AML Study Group (AMLSG) for the AMLSG 09-09 trial[2] (N=497), and the Study Alliance Leukemia (SAL) for the AML2003 trial[3] (N=105). These data were standardized using Standard Data Tabulation Models. Data included demographic information, disease history, induction and consolidation treatments received, morphologic response, MRD quantitation after 2 chemotherapy cycles, relapse and survival status. A total of 515 patients were excluded from the analysis due to lack of data to support a morphologic response assessment and/or MRD status within 42 days of the start of cycle 2 of chemotherapy.

Baseline Characteristics for Patients Included or Excluded From Analysis

	Included (N=613)	Excluded (N=515)
Age (years)		
<60	405 (66.1%)	385 (74.8%)
≥60	208 (33.9%)	130 (25.2%)
Sex		
Male	272 (44.4%)	225 (43.7%)
Female	341 (55.6%)	290 (56.3%)
Race		
Asian	3 (0.5%)	14 (2.7%)
Black or African American	5 (0.8%)	3 (0.6%)
White	519 (84.7%)	451 (87.6%)
Other	14 (2.3%)	10 (1.9%)
Not reported	72 (11.7%)	37 (7.2%)

	Included (N=613)	Excluded (N=515)
AML subtype		
De novo	576 (94.0%)	485 (94.2%)
Treatment related	37 (6.0%)	30 (5.8%)
ECOG/WHO PS		
0	269 (43.9%)	283 (55.0%)
1	287 (46.8%)	192 (37.3%)
2	51 (8.3%)	30 (5.8%)
3	3 (0.5%)	8 (1.6%)
Missing/Unknown	3 (0.5%)	2 (0.4%)
ELN risk category*		
Favorable	438 (71.5%)	371 (72.0%)
Intermediate	121 (19.7%)	96 (18.6%)
Adverse	5 (0.8%)	5 (1.0%)
Missing/Unknown	49 (8.0%)	43 (8.3%)

ECOG, Eastern Cooperative Oncology Group; PS, performance status; WHO, World Health Organization.

Table 1: Included and excluded patient subgroups appear to be well balanced with respect to baseline demographic characteristics and risk factors. There were slightly more younger patients in the excluded patient group, which is not reflected in the ELN risk stratification.

*ELN risk stratification is based on the version employed in each study.

Induction Regimens

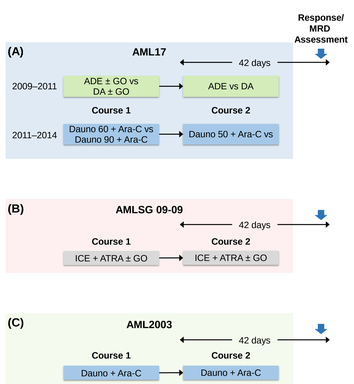


Figure 2: Patients in all 3 trials underwent double induction with a variety of intensive regimens. **(A)** The induction chemotherapy randomization prior to October 2011 was between cytarabine (Ara-C), daunorubicin (Dauno), and etoposide (ADE; course 1 [ie, cycle 1]: Dauno 50 mg/m^2 days 1, 3, and 5; Ara-C 100 mg/m^2 every 12 hours days 1–10, and etoposide 100 mg/m^2 days 1–5; course 2: Dauno 50 mg/m^2 days 1, 3, and 5; Ara-C 100 mg/m^2 every 12 hours days 1–8, and etoposide 100 mg/m^2 days 1–5) and Dauno and Ara-C (DA; course 1: Dauno 50 mg/m^2 days 1, 3, and 5; Ara-C, 100 mg/m^2 every 12 hours days 1–10; course 2: Dauno 50 mg/m^2 days 1, 3, and 5; Ara-C, 100 mg/m^2 every 12 hours days 1–8) combined with gemtuzumab ozogamicin (GO) as a single dose of 3 mg/m^2 or 6 mg/m^2 in course 1. Neither the addition of etoposide nor GO at 6 mg/m^2 improved outcomes thus, from October 2011 onward all patients received DA with either 60 mg/m^2 or 90 mg/m^2 of Dauno in course 1, and for all patients, 50 mg/m^2 in course 2. After the first course of induction treatment, patients were designated as high, intermediate, or low risk based on a validated weighted risk score. High-risk patients were subjected to a separate random assignment of fludarabine, Ara-C, granulocyte colony-stimulating factor, and idarubicin (FLAG-Ida) vs Dauno and clofarabine with the intention to proceed to transplantation. Low- and intermediate-risk patients received a second course of induction as described above, with or without a targeted agent (CEP-701; lestaurtinib). After the 2 induction courses, all intermediate- and good-risk patients were eligible to be randomly assigned to have 1 or 2 consolidation courses following the confirmation of CR. **(B)** Patients were randomized to 2 cycles idarubicin (induction cycle 1: 12 mg/m^2 intravenously [IV] on days 1, 3, and 5 [for patients >60 years old, reduced to days 1 and 3]; induction cycle 2: 10 mg/m^2 IV on days 1 and 3 for all patients), Ara-C (100 mg/m^2 continuously IV on days 1 to 7 [for induction cycle 2 reduced to days 1 to 5]), and etoposide (100 mg/m^2 IV on days 1 to 3 [in induction cycle 2 and for patients >60 years old, reduced to days 1 and 3]; ICE) plus all-*trans* retinoic acid (ATRA; 45 mg/m^2) on days 6–8, 15 mg/m^2 on days 9–21 with or without GO 3 mg/m^2 IV on day 1. **(C)** Induction consisted of 2 cycles of standard dose Dauno and Ara-C.

A Higher Proportion of Patients Have Detectable MRD in BM Than in PB

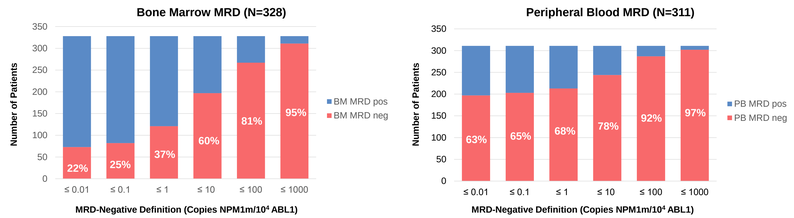


Figure 3: Numbers of patients in CR after cycle 2 classified as MRD negative or positive based on various copy number thresholds in BM or PB.

Patients With MRD-Negative CR Have Better EFS Than Patients With MRD-Positive CR

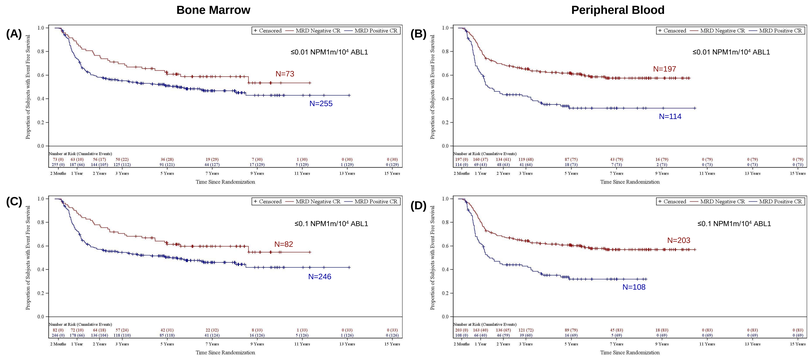


Table 2: EFS Hazard Ratios (HRs) for MRD-Negative CR vs MRD-Positive CR	Bone Marrow	Peripheral Blood
MRD-Negative Definition	EFS HR (95% CI)	EFS HR (95% CI)
≤0.01 NPM1m/10⁴ ABL1	0.63 (0.43–1)	0.38 (0.28–0.56)
≤0.1 NPM1m/10⁴ ABL1	0.59 (0.4–0.91)	0.4 (0.29–0.56)
≤1 NPM1m/10⁴ ABL1	0.48 (0.33–0.71)	0.37 (0.26–0.53)
≤10 NPM1m/10⁴ ABL1	0.43 (0.31–0.62)	0.38 (0.26–0.56)
≤100 NPM1m/10⁴ ABL1	0.38 (0.26–0.56)	0.31 (0.19–0.53)
≤1,000 NPM1m/10⁴ ABL1	0.27 (0.16–0.48)	0.21 (0.10–0.43)

Figure 4: Kaplan-Meier estimates of EFS for patients who achieved or remained in morphologic CR and underwent molecular MRD assessment in BM **(A, C)** or PB **(B, D)** by RT-qPCR within 42 days of the start of chemotherapy cycle 2. Patients are classified as being MRD positive or negative based on the normalized copies of NPM1m transcripts per 10^4 ABL1 transcripts as shown in each plot. EFS HRs at varying thresholds for defining MRD negativity are summarized in **Table 2**.

Patients With MRD-Negative CR Have Better OS Than Patients With MRD-Positive CR

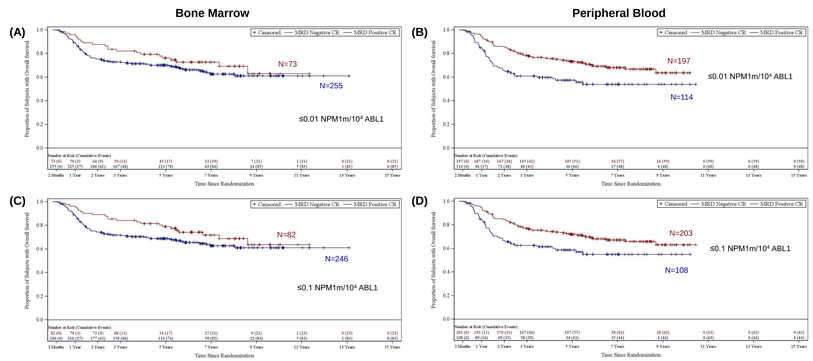


Table 3: OS Hazard Ratios (HRs) for MRD-Negative CR vs MRD-Positive CR	Bone Marrow	Peripheral Blood
MRD-Negative Definition	OS HR (95% CI)	OS HR (95% CI)
≤0.01 NPM1m/10⁴ ABL1	0.71 (0.43–1.11)	0.45 (0.29–0.67)
≤0.1 NPM1m/10⁴ ABL1	0.67 (0.42–1.1)	0.48 (0.31–0.77)
≤1 NPM1m/10⁴ ABL1	0.53 (0.33–0.83)	0.45 (0.29– 0.71)
≤10 NPM1m/10⁴ ABL1	0.48 (0.31–0.77)	0.42 (0.26–0.67)
≤100 NPM1m/10⁴ ABL1	0.56 (0.34–0.91)	0.33 (0.18–0.62)
≤1,000 NPM1m/10⁴ ABL1	0.27 (0.14–0.5)	0.16 (0.08–0.36)

Figure 5: Kaplan-Meier estimates of OS for patients who achieved or remained in morphologic CR and underwent molecular MRD assessment in BM **(A, C)** or PB **(B, D)** by RT-qPCR within 42 days of the start of chemotherapy cycle 2. OS HRs at varying thresholds for defining MRD negativity are summarized in **Table 3**.

MRD-Negative CR and CRi/CRh Patients Have Better EFS Than MRD-Positive CR and CRi/CRh Patients

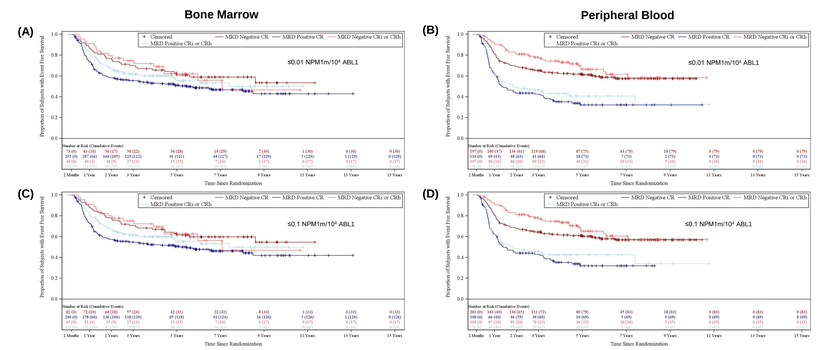


Figure 6: Kaplan-Meier estimates of EFS for patients who achieved or remained in morphologic CR, CRi, or CRh and underwent molecular MRD assessment by RT-qPCR within 42 days of the start of chemotherapy cycle 2. EFS HRs at varying thresholds for defining MRD-negative CR, CRi, or CRh are summarized in **Table 4**.

Table 4: EFS Hazard Ratios (HRs): MRD-Negative CR vs MRD-Positive CR, MRD-Negative CRi/CRh, and MRD-Positive CRi/CRh	Bone Marrow EFS HR (95% CI) MRD-Negative CR vs			Peripheral Blood EFS HR (95% CI) MRD-Negative CR vs		
MRD-Negative Definition	MRD-Positive CR	MRD-Negative CRi/CRh	MRD-Positive CRi/CRh	MRD-Positive CR	MRD-Negative CRi/CRh	MRD-Positive CRi/CRh
≤0.01 NPM1m/10⁴ ABL1	0.63 (0.42–0.91)	1.0 (0.5–2.0)	0.77 (0.5–1.25)	0.36 (0.25–0.5)	1.11 (0.71–1.67)	0.38 (0.25–0.59)
≤0.1 NPM1m/10⁴ ABL1	0.59 (0.38–0.91)	1.0 (0.53–1.67)	0.77 (0.48–1.25)	0.34 (0.24–0.5)	1.11 (0.71–1.67)	0.37 (0.24–0.59)
≤1 NPM1m/10⁴ ABL1	0.45 (0.31–0.67)	0.83 (0.48–1.43)	0.63 (0.4–1)	0.33 (0.23–0.48)	1 (0.67–1.43)	0.38 (0.24–0.59)
≤10 NPM1m/10⁴ ABL1	0.40 (0.28–0.59)	0.77 (0.5–1.25)	0.67 (0.42–1)	0.36 (0.24–0.53)	1 (0.71–1.43)	0.4 (0.25–0.67)
≤100 NPM1m/10⁴ ABL1	0.36 (0.24–0.53)	1.0 (0.67–1.43)	0.71 (0.42–1.11)	0.3 (0.24–0.5)	1.11 (0.83–1.43)	0.31 (0.16–0.59)
≤1,000 NPM1m/10⁴ ABL1	0.26 (0.15–0.45)	1.1 (0.83–1.67)	0.53 (0.23–1.25)	0.19 (0.1–0.42)	1.11 (0.83–1.67)	0.06 (0.02–0.15)

MRD-Negative CR and CRi/CRh Patients Have Better OS Than MRD-Positive CR and CRi/CRh Patients

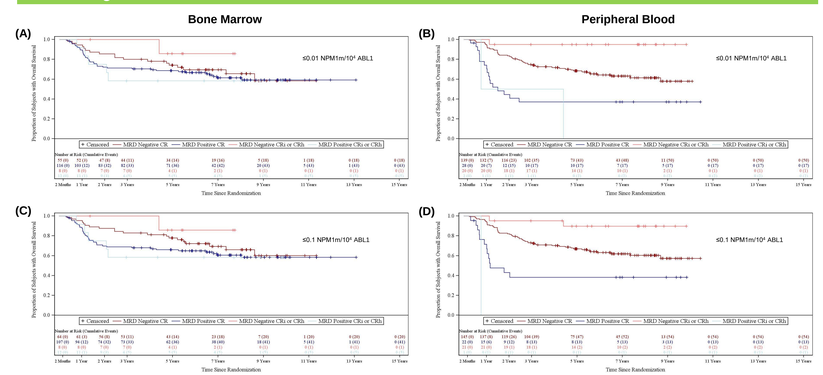


Figure 7: Kaplan-Meier estimates of OS for patients who achieved or remained in morphologic CR, CRi, or CRh and underwent molecular MRD assessment by RT-qPCR within 42 days of the start of chemotherapy cycle 2. OS HRs at varying thresholds for defining MRD-negative CR, CRi, or CRh are summarized in **Table 5**.

Table 5: OS Hazard Ratios (HRs): MRD-Negative CR vs MRD-Positive CR, MRD-Negative CRi/CRh, and MRD-Positive CRi/CRh	Bone Marrow OS HR (95% CI) MRD-Negative CR vs			Peripheral Blood OS HR (95% CI) MRD-Negative CR vs		
MRD-Negative Definition	MRD-Positive CR	MRD-Negative CRi/CRh	MRD-Positive CRi/CRh	MRD-Positive CR	MRD-Negative CRi/CRh	MRD-Positive CRi/CRh
≤0.01 NPM1m/10⁴ ABL1	0.71 (0.43–1.25)	1.11 (0.53–2.5)	0.77 (0.43–1.43)	0.45 (0.29–0.71)	1.43 (0.77–2.5)	0.34 (0.21–0.56)
≤0.1 NPM1m/10⁴ ABL1	0.67 (0.42–1.11)	1.11 (0.5–2.5)	0.71 (0.4–1.25)	0.48 (0.31–0.77)	1.43 (0.77–2.5)	0.36 (0.21–0.59)
≤1 NPM1m/10⁴ ABL1	0.53 (0.34–0.83)	1 (0.45–2)	0.59 (0.34–1)	0.48 (0.3–0.71)	1.25 (0.71–2)	0.37 (0.23–0.63)
≤10 NPM1m/10⁴ ABL1	0.5 (0.32–0.83)	0.83 (0.48–1.43)	0.63 (0.38–1.11)	0.43 (0.28–0.71)	1 (0.67–1.67)	0.37 (0.21–0.63)
≤100 NPM1m/10⁴ ABL1	0.59 (0.36–1)	1 (0.67–1.67)	0.67 (0.37–1.11)	0.37 (0.2–0.71)	1.11 (0.71–1.67)	0.25 (0.13–0.5)
≤1,000 NPM1m/10⁴ ABL1	0.28 (0.14–0.53)	1 (0.71–1.43)	0.43 (0.17–1.11)	0.18 (0.08–0.38)	1 (0.71–1.43)	0.04 (0.02–0.11)

Conclusions

- Assessment of MRD using RT-qPCR in PB or BM after 2 cycles of intensive chemotherapy provides valuable prognostic information for patients with *NPM1*m AML.
- A range of normalized copy number (NCN) cutoff definitions for MRD negativity revealed good discriminatory power for EFS and OS in BM and PB.
- MRD is more frequently detected at lower NCN thresholds in BM than PB but has less negative prognostic implications than similar levels of MRD detected in PB; applying a higher threshold of MRD positivity for BM relative to PB can identify a population at comparable risk of relapse and death.
- MRD negativity is associated with better EFS and OS regardless of whether peripheral count recovery is complete or incomplete at the time of BM CR determination (ie, CRi/CRh); patients with MRD-negative CRi/CRh show similar outcomes to patients with MRD-negative CR.

References

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