Molecular response assessment by quantitative real-time polymerase chain reaction after induction therapy in *NPM1*-mutated patients identifies those at high risk of relapse

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Supplemental materials

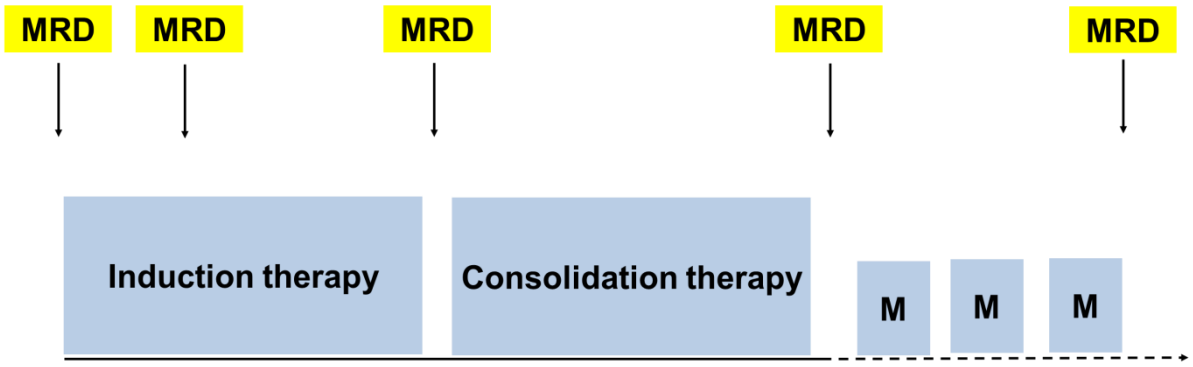
Additional statistical information

Analyses of differences were calculated by the Mann–Whitney U-test, the Kruskal-Wallis-test, or Student's t-test for unpaired data and with Wilcoxon's signed rank test or paired Student's t-test for paired data. Spearman's rank correlation was used to determine the coefficient of correlation as well as the corresponding p value.
To analyze the diagnostic power of the investigated different MRD cut-off values at the different MRD checkpoints, we used Cox's proportional hazards regression and calculate univariate as well as multivariate analyses to analyze the influence of additional baseline factors on the end points: (1) relapse and (2) overall survival. For the prediction of relapse within an observation time of 100 days during the follow-up period, we considered the absolute values before relapse or the peak value of measurements for patients without relapse during the follow-up, respectively. With the help of ROC we selected a cut-off for the prediction of relapse within 100 days in the follow-up period. Characteristics of all selected cut-offs were determined by the analysis of corresponding 2x2 contingency tables of test-positive and -negative cases (with relapse) and controls (without relapse).

RT-PCR negativity versus MRD cut-off ratio

We compared the results of the analyses on relapse of our estimated MRD cut-off after induction and consolidation therapy with the results of RT-PCR negativity at the specific time points. After induction therapy and after consolidation therapy MRD negativity showed inferior results with lower hazard ratios (Supplement Table 1). Likewise, the estimated cut-off of NPM1mut ratio of 0.01 showed a better separation of the cohort in CIR analysis (Supplement Figure 5).

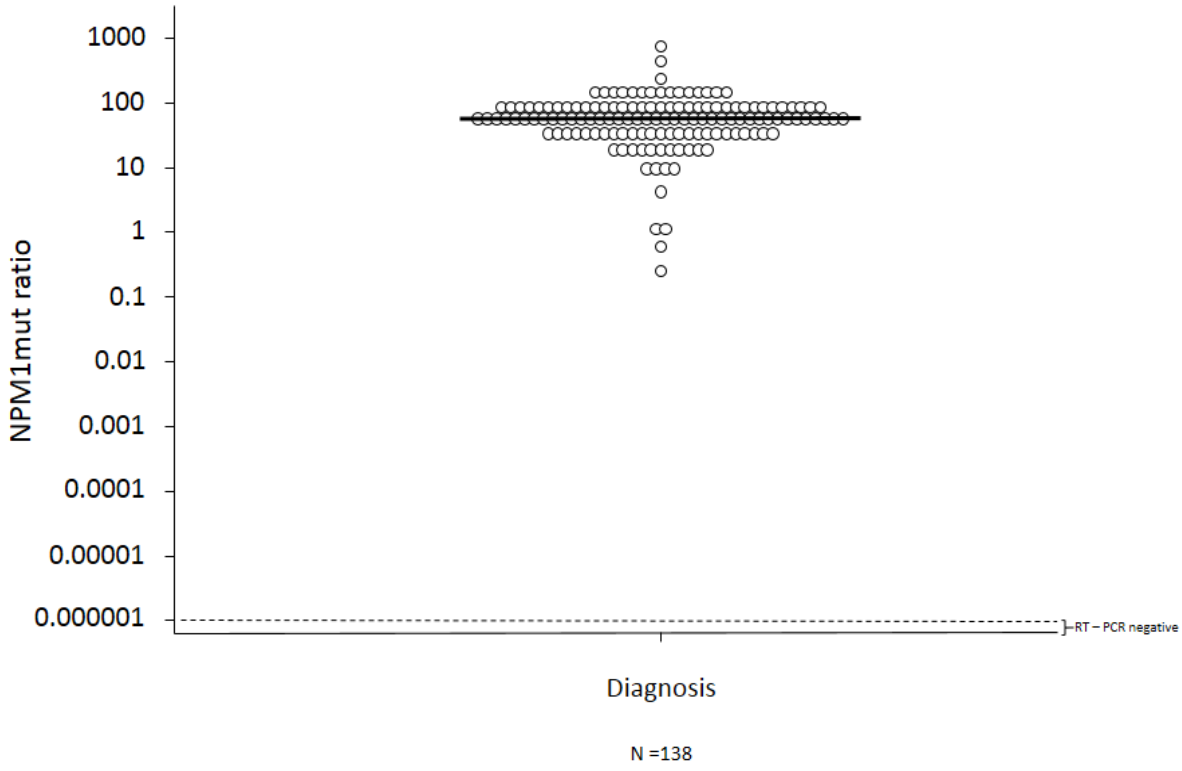
Supplement Figure 1: MRD sampling intervals



Recommended MRD sampling intervals within the AMLCG trials.

Abbreviations: MRD – minimal residual disease; M – three years maintenance therapy of monthly alternating chemotherapy regimens

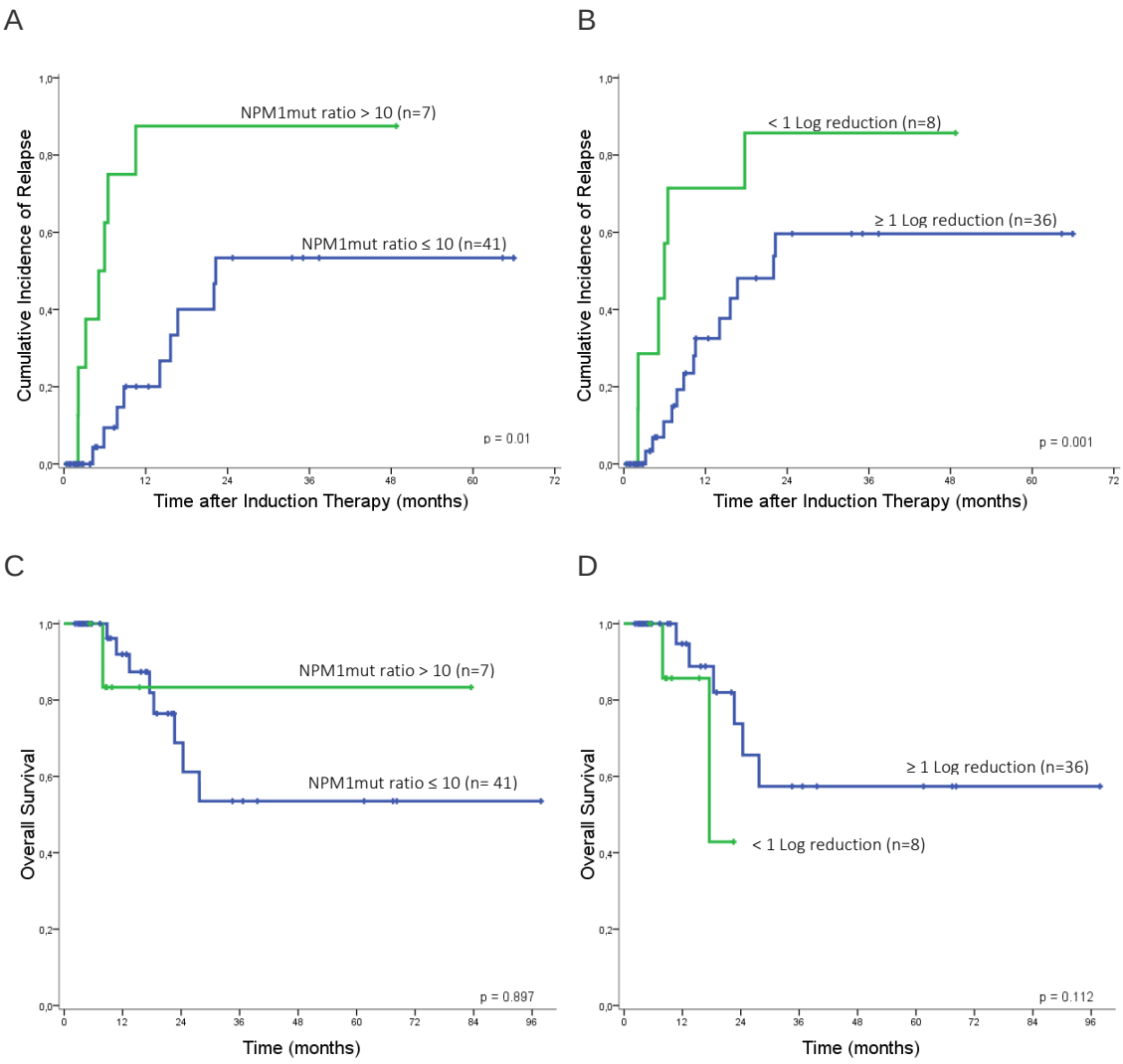
Supplement Figure 2: NPM1mut ratios of all patients at diagnosis



NPM1mut ratios of all patients at diagnosis.

Abbreviations: NPM1mut – NPM1 mutation; RT - PCR – quantitative real-time polymerase chain reaction;

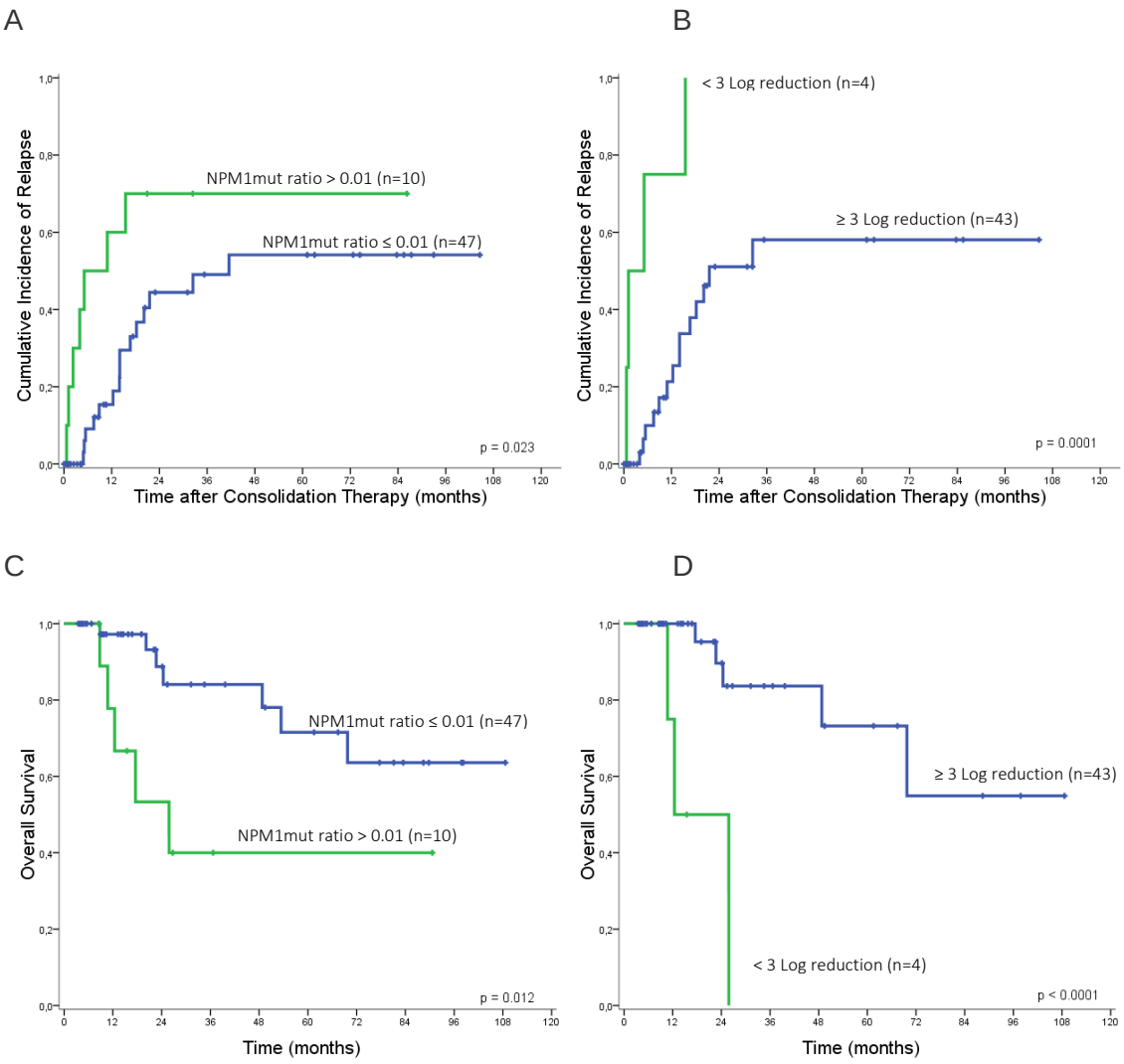
Supplement Figure 3: CIR and OS of patients according to the MRD status in aplasia during induction therapy



(A) + (C) NPM1mut ratios in aplasia with NPM1mut cut-off ratio of 10; (B) + (D) NPM1mut kinetics in aplasia with a cut-off of – 1 Log.

Abbreviations: NPM1mut – NPM1 mutation

Supplement Figure 4: CIR and OS of patients according to the MRD status after consolidation therapy

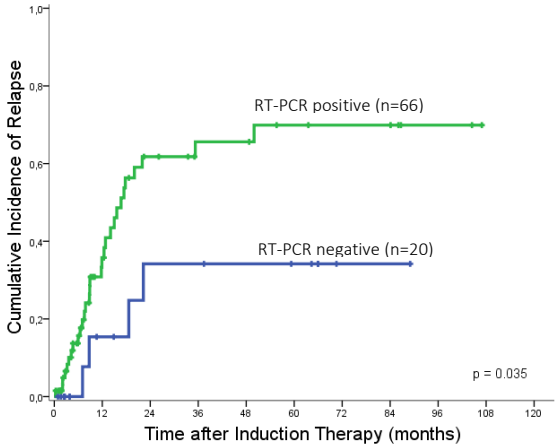


(A) + (C) NPM1mut ratios after consolidation therapy with NPM1mut cut-off ratio of 0.01; (B) + (D) NPM1mut kinetics after consolidation therapy with a cut-off of – 3 Log.

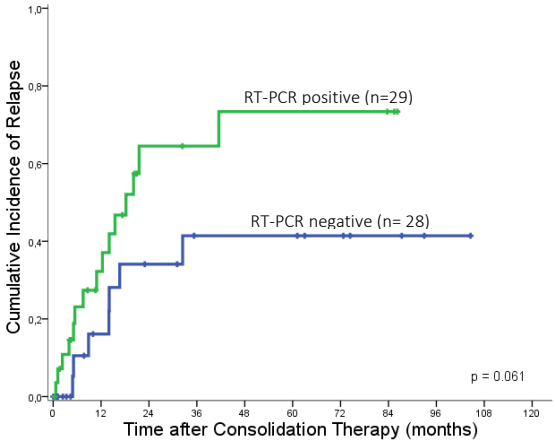
Abbreviations: NPM1mut – NPM1 mutation

Supplement Figure 5: CIR of patients according to RT-PCR negativity after induction (A) and consolidation therapy (B)

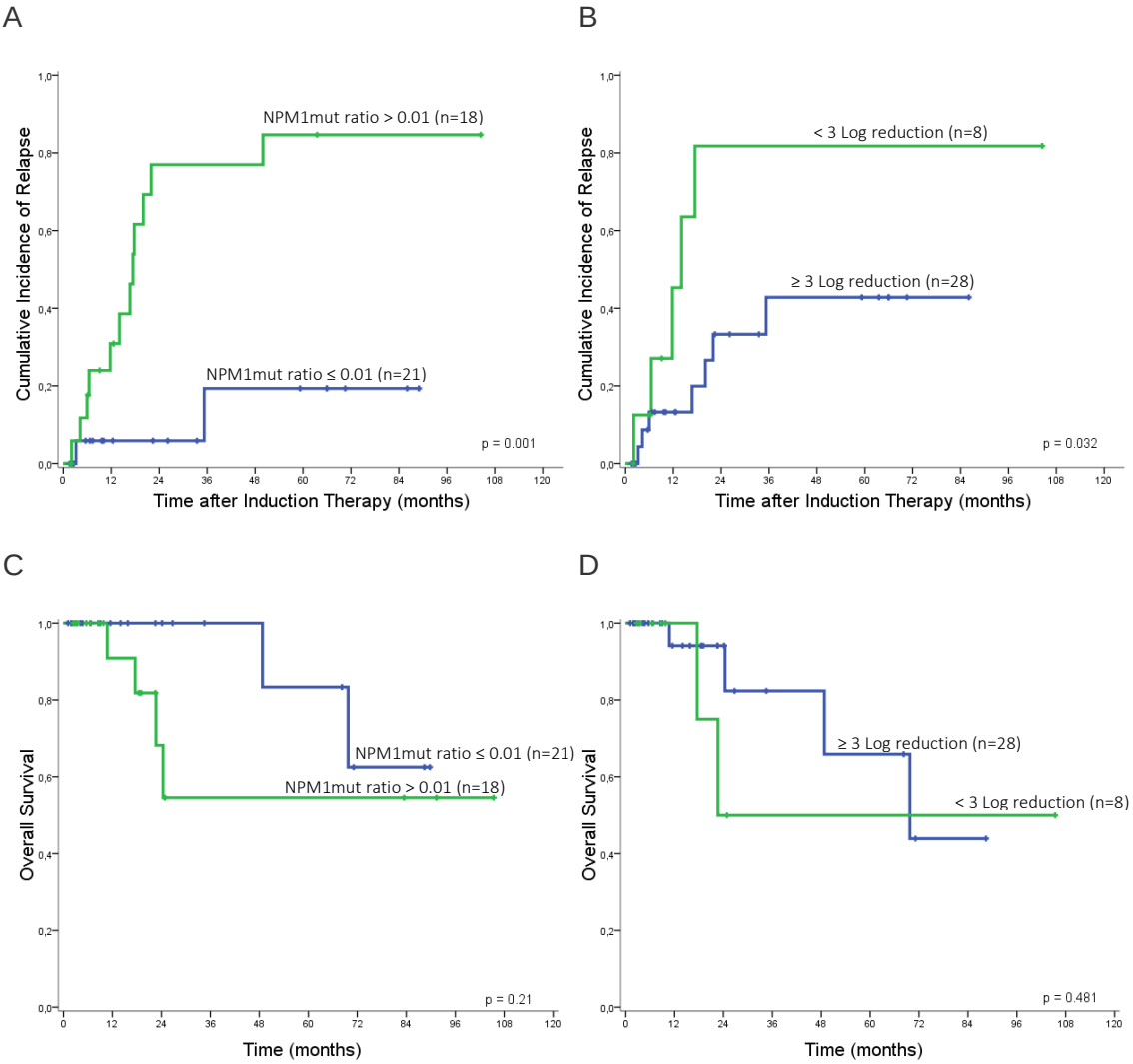
A



B



Supplement Figure 6: CIR and OS of patients within the ELN favorable risk group according to the MRD status after induction therapy.



(A) + (C) NPM1mut ratios after induction therapy with NPM1mut cut-off ratio of 0.01; (B) + (D) NPM1mut kinetics after induction therapy with a cut-off of – 3 Log.

Abbreviations: NPM1mut – NPM1 mutation

Supplement Table 1: Comparison of results of relapse analyses of estimated MRD cut-off with RT-PCR negativity

MRD checkpoint	Cut-off*	HR Cut-off (95% CI)**	P **	Sensitivity	Specificity	PPV	NPV	P ***
After Induction Therapy	0.01	4.26 (1.93 – 9.45)	<0.0001	76% (26/34)	74% (40/54)	65% (26/40)	83% (40/48)	<0.0001
	RT-PCR negative	2.93 (1.03 – 8.35)	0.045	88% (30/34)	33% (18/54)	45% (30/66)	82% (18/22)	0.041
After Consolidation Therapy	0.01	2.72 (1.10 – 6.69)	0.03	32% (7/22)	92% (33/36)	70% (7/10)	69% (33/48)	0.03
	RT-PCR negative	2.31 (0.94 – 5.70)	0.07	68% (15/22)	61% (22/36)	52% (15/29)	76% (22/29)	0.057

Abbreviations: HR – hazard ratio; CI – Confidence Interval; PPV – positive predictive value; NPV – negative predictive value;

* Cut-offs determined by Cox regression models

** Cox regression model of NPM1mut Cut-off values for the occurrence of relapse

*** Chi square test of 2 × 2 Contingency Tables

Supplement Table 2. Patients characteristics and laboratory findings of patients who lost NPM1 mutation at relapse (n=3)

Patient-ID	99185	99074	45019*
Study	**AMLCG99**	**AMLCG99**	**AMLCG99**
Age in years	**42**	**64**	**60**
Time to relapse in months	**5**	**6**	**38**
NPM1mut	**A**	**A**	**A**
Karyotype at diagnosis	**NK**	**NK**	**NK**
Karyotype at relapse	**NK**	**NK**	**Translocation (1;7)**
BM blast at diagnosis in %	**79**	**82**	**95**
BM blast at relapse in %	**30**	**18**	**unknown**
LAIP at diagnosis	**HLA-DR/CD33/CD34**	**CD65/CD87/CD34**	**CD34/CD56/CD33**
LAIP at relapse	**initial LAIP undectable**	**CD65/CD87/CD34**	**CD15/CD13/CD33**
Additional molecular findings at diagnosis	**--**	**--**	**FLT3-ITD, DNMT3A mutation**
Additional molecular findings at relapse	**--**	**JAK2 mutation**	**DNMT3A mutation**
NPM1mut ratio at diagnosis	**40.9**	**47.5**	**5.1**
NPM1mut ratio at relapse	**0.000001**	**0.000001**	**0.000001**

Abbreviations: NPM1mut – NPM1 mutation; NK – normal karyotype; BM – bone marrow; LAIP – leukemia-associated immunophenotype;

* this patient was already published by Papadaki et al.[18]