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Prognostic Impact of Minimal Residual Disease in AML

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Prognostic impact of minimal residual disease in AML

- Achievement of complete remission (CR) is the most important prerequisite for cure and long-term survival of patients with acute myeloid leukemia (AML)
- The increasing number of new molecular markers and the development of novel technologies [real-time quantitative polymerase chain reaction (RQ-PCR), multi-color flow cytometry, digital polymerase chain reaction (dPCR), next-generation sequencing (NGS)] allow to measure minimal residual disease (MRD) with high sensitivity
- MRD allows to refine our current definition of morphological CR
- New response category proposed by the 2017 ELN recommendations: “Complete remission without MRD” (CR_{MRD-})

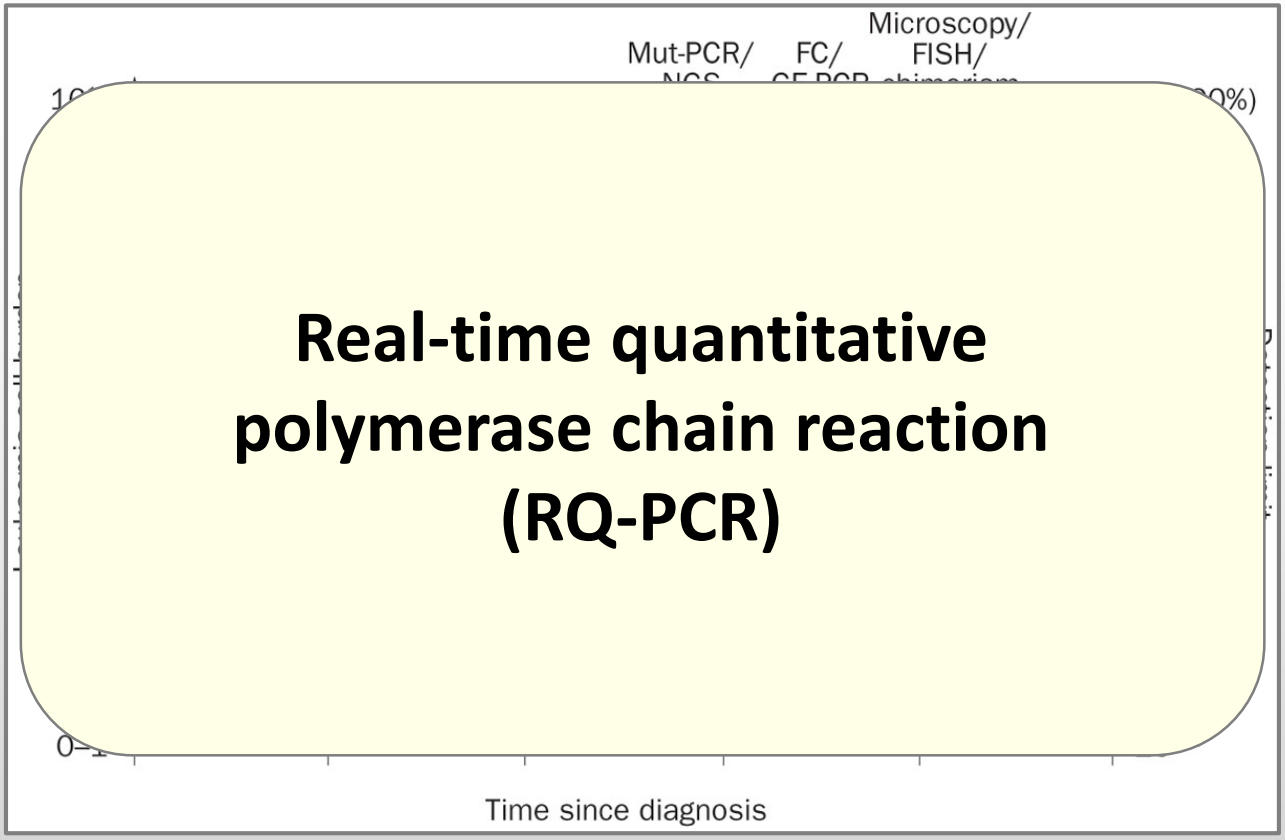
Prognostic impact of minimal residual disease in AML

MRD monitoring: Clinical implications

- Treatment decision making, in particular within the context of allogeneic stem cell transplantation (alloSCT)
- Early detection of relapse
- Guiding pre-emptive therapy
- Monitoring of treatment effects (novel drugs)

Prognostic impact of minimal residual disease in AML

Detection thresholds of various MRD techniques compared to traditional clinical complete remission



Hourigan CS and Karp JE. Nat Rev Clin Oncol. 2013;10:460-471.

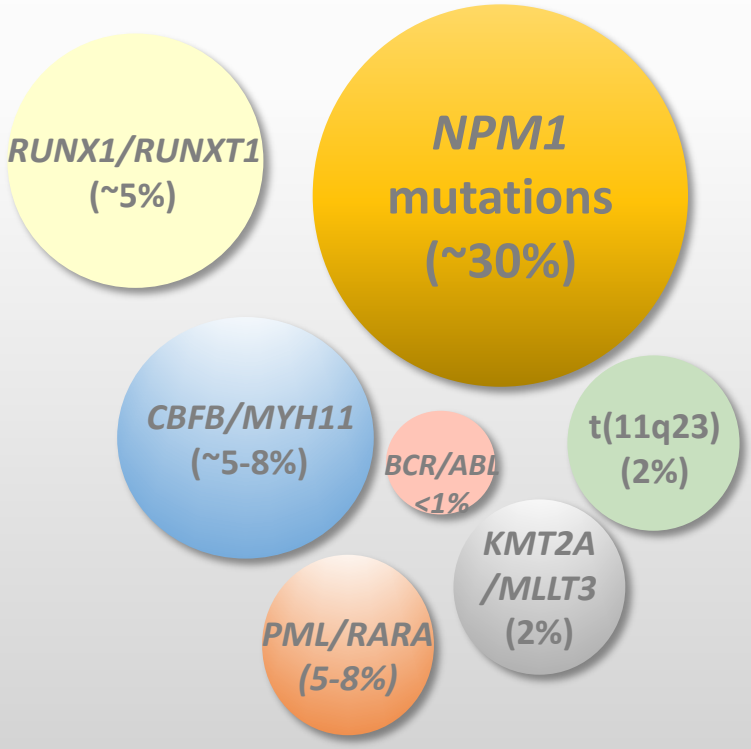
Molecular markers currently used for RQ-PCR based MRD monitoring in AML

- So far, MRD monitoring in AML has been restricted to distinct AML subtypes mainly characterized by gene fusions resulting from translocations/inversions

Molecular targets for MRD monitoring

- *PML/RARA*
- *RUNX1/RUNXT1*
- *CBFB/MYH11*
- *BCR/ABL*
- *(KMT2A/MLLT3)*
- *NPM1*

~ 50% of all AML



Molecular markers not suitable for RQ-PCR based MRD monitoring

Gene mutations being present in pre-leukemic hematopoietic cells and/or persist during clinical remission:

- *DNMT3A*
- *TET2*
- *ASXL1*
- *IDH1/2*

Gene mutations with heterogeneous breakpoints and/or long PCR products (> 150 bp):

Can be monitored by NGS

- *CEBPA*
- *RUNX1*
- *TP53*

Prognostic impact of minimal residual disease in AML : Important Issues

- Most, if not all studies published so far are retrospective and MRD was not included as a primary or secondary endpoint
- Studies were performed on heterogeneous patient populations with respect to age, treatment, cohort size, or type of material
- MRD monitoring has not been standardized yet; existence of different MRD assays with distinct sensitivities and definitions for „MRD negativity“
- Studies are not comparable with regard to cut-off values / values for transcript levels / copy numbers
- In most studies, achievement of MRD-negativity / RQ-PCR-negativity after two cycles of therapy and/or at the end of treatment was significantly associated with outcome

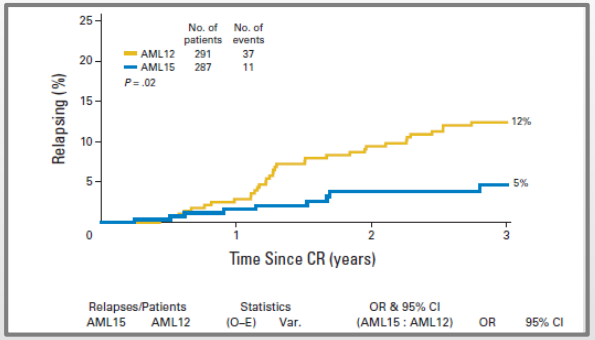
Prognostic impact of minimal residual disease in AML : Current Data

Acute Promyelocytic Leukemia

Grimwade D et al. J Clin Oncol 2009; 27(22): 3650-3658

- Prospective study on 406 newly diagnosed adult APL pts (MRC AML15 trial)
- 6.727 serial BM/PB samples (2.276 paired samples) were analyzed by RQ-PCR
- At the end of treatment achievement of RQ-PCR-negativity was highly predictive for clinical relapse and relapse-free survival (RFS)
- Persistent PCR positivity and molecular relapse were significantly associated with clinical relapse and RFS
- Pre-emptive therapy with arsenic trioxide prevented progression to overt relapse in the majority of the pts

CIR in patients treated with pre-emptive therapy (blue)



Prognostic impact of minimal residual disease in AML : Current Data

Core-binding Factor (CBF) Leukemia

t(8;21)(q22;q22.1); inv(16)(p13.1q22)

- MRD-negativity at end of treatment in PB impacts clinical outcome – French Intergroup

  Willekens et al., Haematologica (2016) [t(8;21), n=94])

- Transcript level reduction (3-log) before consolidation II influences relapse risk – French Intergroup

  Jourdan et al., Blood (2013) [t(8;21), n=96; inv(16), n=102]

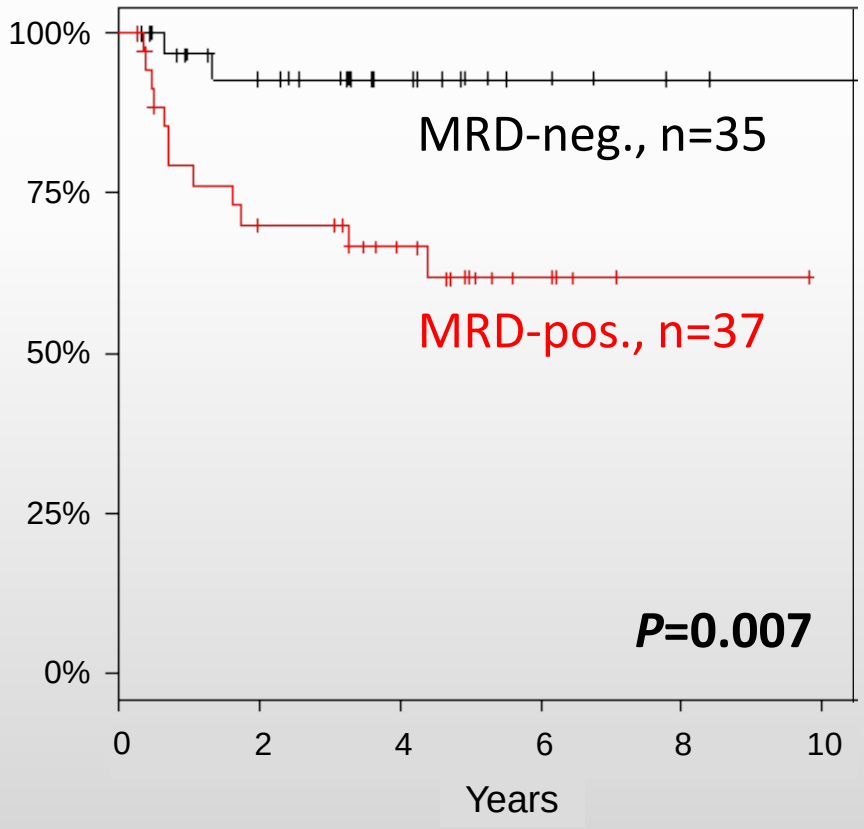
- Distinct absolute transcript levels and log reduction after induction I and during follow-up correlate with clinically relevant endpoints – UK MRC15

  Yin et al., Blood (2012) [t(8;21), n=163; inv(16), n=115]

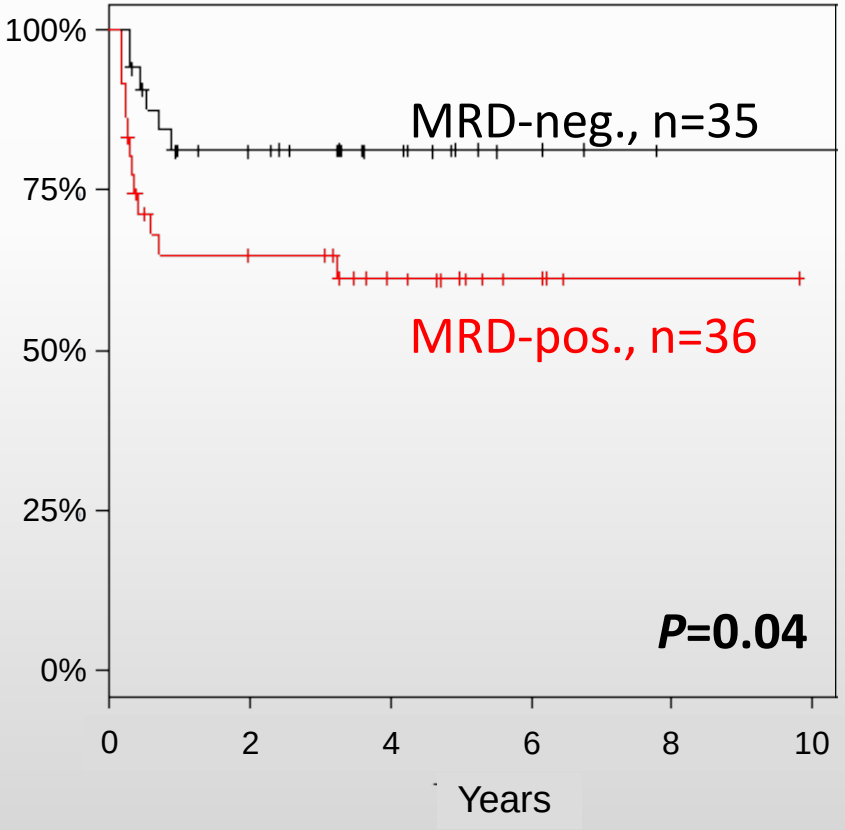
Prognostic Impact of *RUNX1/RUNX1T1* MRD-negativity at the end of treatment

Analysis of n=120 *RUNX1/RUNX1T1* positive pts of the AMLSG

Overall survival

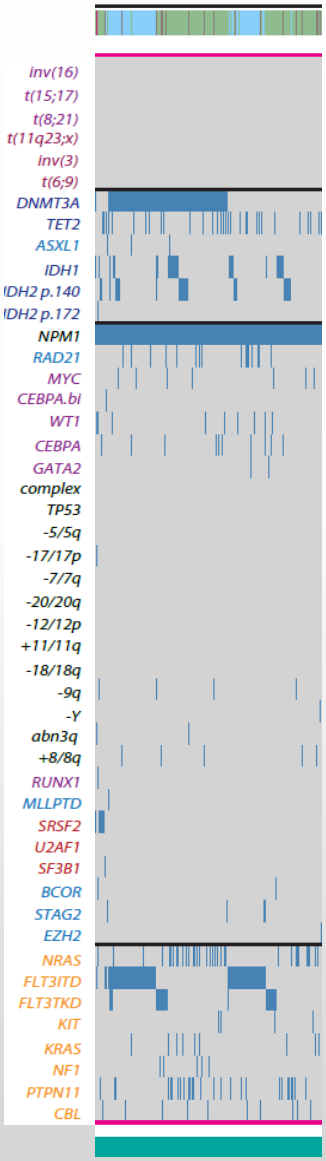


Event-free survival



Agrawal M et al., ASH meeting 2016, abstract #1207

MRD monitoring in *NPM1* mutated AML



NPM1 class

- In 25-35% of AML, particular in CN-AML (45-60%)
- AML with *NPM1*^{mut}/*FLT3*-ITD^{neg} and *NPM1*^{mut}/*FLT3*-ITD$^{low\text{-}ratio}$ is associated with favorable outcome
- Older patients with *NPM1*-mutated AML benefit from intensive chemotherapy

  Becker H, et al. J Clin Oncol 2009; Büchner T, et al. J Clin Oncol 2009; Schlenk RF, Döhner K, et al. Haematologica 2009.
- Mutant *NPM1* is an excellent target for MRD monitoring

  – *MRD levels assessed by NPM1 mutation-specific RQ-PCR provide important prognostic information in AML.* ***Schnittger et al., Blood 2009;114:2220-31;*** [n=252]

  – *MRD monitoring in NPM1 mutated AML: a study from the German-Austrian Acute Myeloid Leukemia Study Group.* ***Krönke et al., JCO 2011;19:2709-2716;*** [n=245]

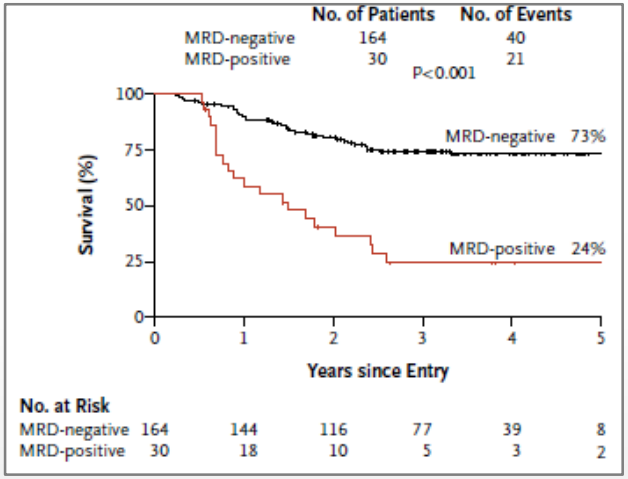
  – *The level of residual disease based on mutant NPM1 is an independent prognostic factor for relapse and survival in AML.* ***Shayegi et al., Blood 2013;122:83-92;*** [n=155]

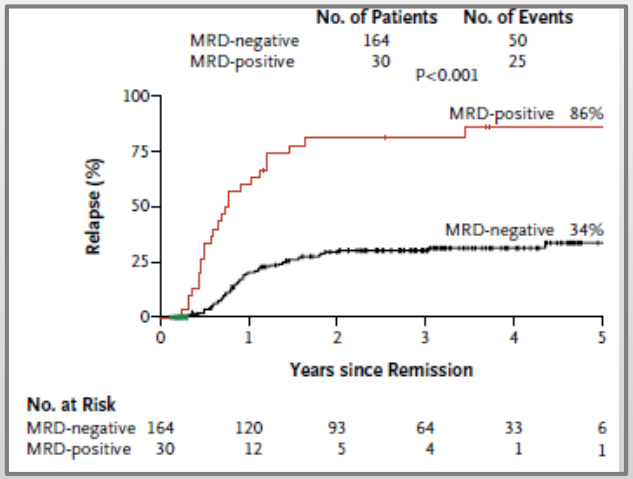
  – *MRD assessed by WT1 and NPM1 transcript levels identifies distinct outcomes in AML patients and is influenced by gemtuzumab ozogamicin.* ***Lambert et al., Oncotarget 2014; 5:6280-8;*** [n=77]

Assessment of minimal residual disease in standard-risk AML

Ivey A et al. N Engl J Med 2016; 374(5):422-33

- Retrospective study on 437 AML pts (pediatric and adults, NCRI AML17 trial)
- 2569 BM/PB (902/1667) samples were analyzed by RQ-PCR after each treatment cycle and during follow-up; sensitivity 10^{-5}
- MRD positivity in PB after 2 cycles of therapy was significantly associated with inferior OS (24% vs 73%) and higher risk of relapse (82% vs 30%) after 3 years
- In multivariate analysis MRD positivity in PB was significantly associated with death (HR 4.38) and relapse (HR 5.09)

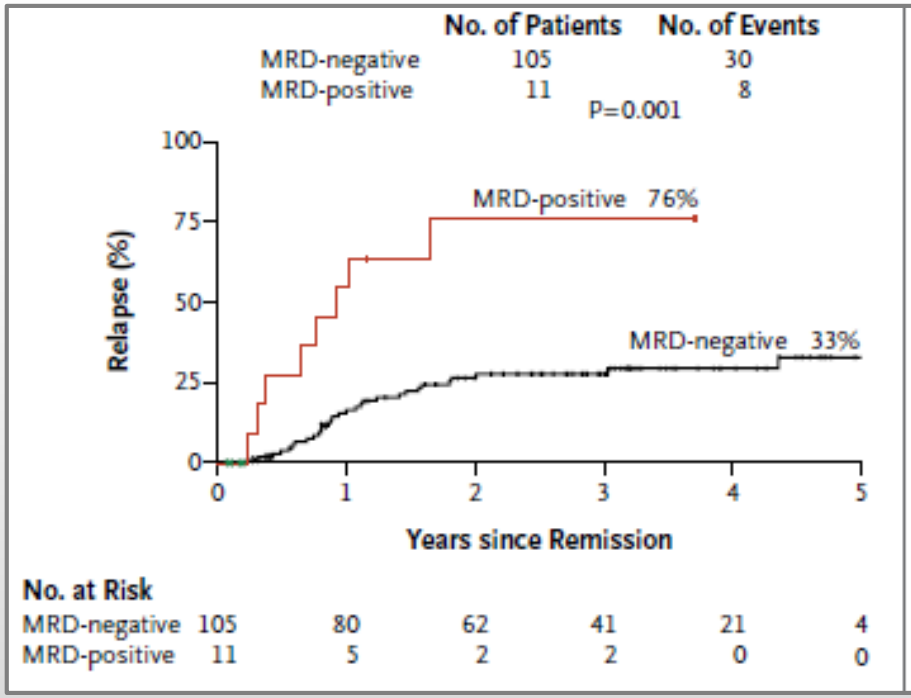




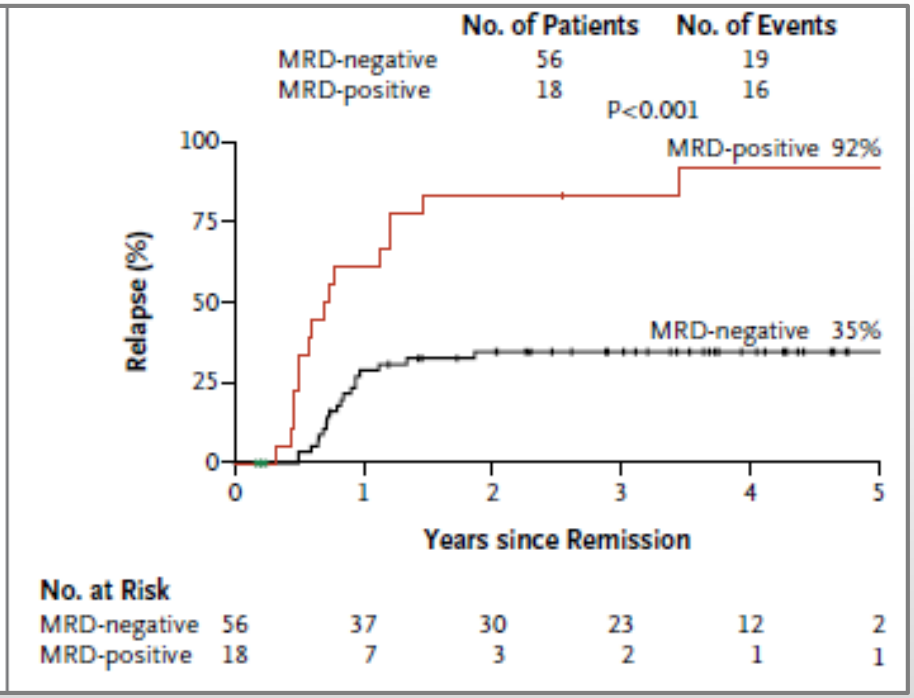
Assessment of minimal residual disease in standard-risk AML

Impact of concurrent *FLT3*-ITD

Relapse in pts without *FLT3*-ITD



Relapse in pts with *FLT3*-ITD

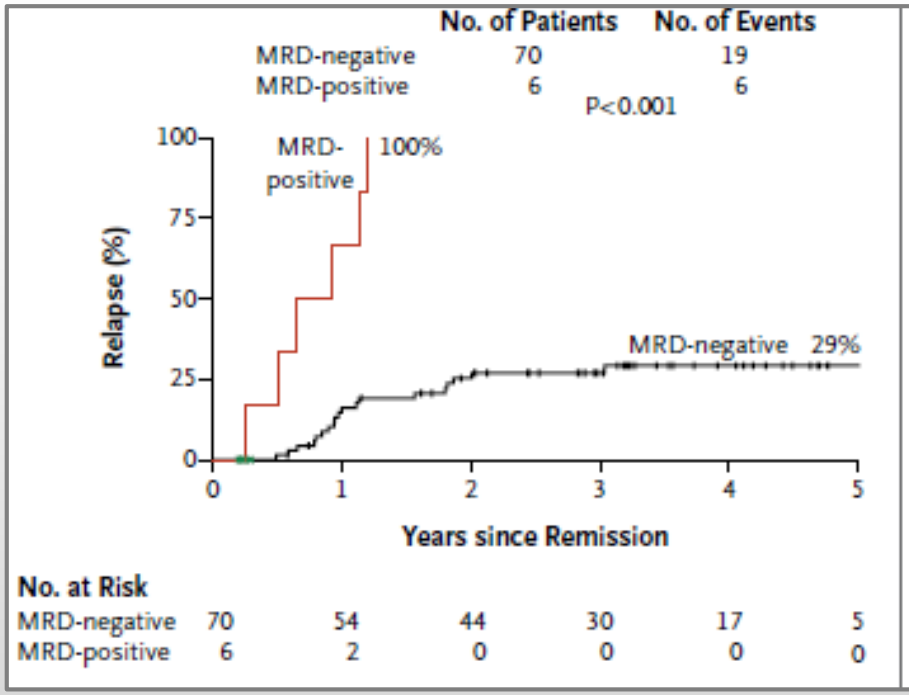


Ivey A et al. N Engl J Med 2016; 374(5):422-33

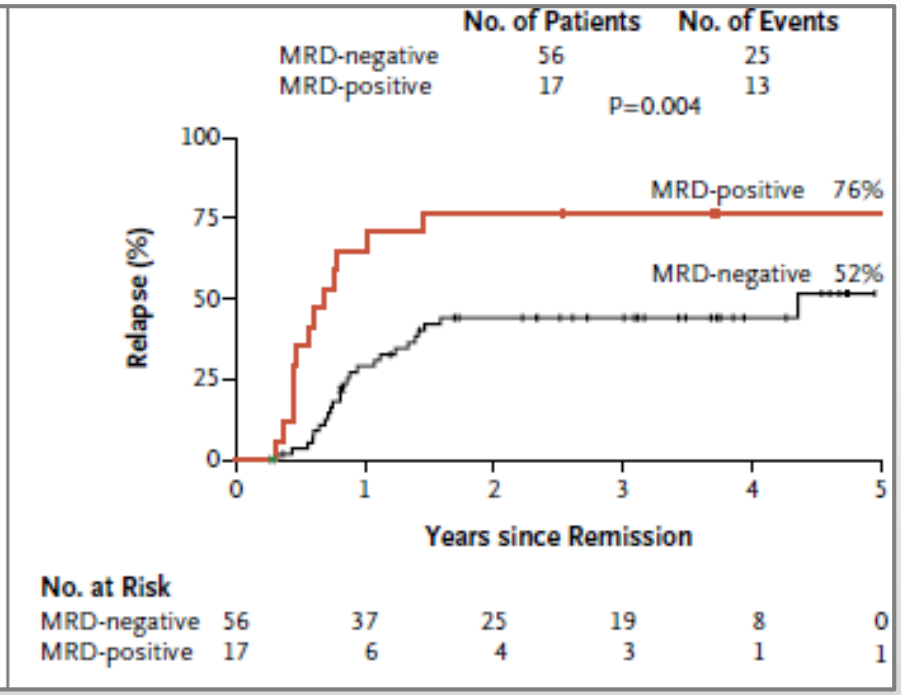
Assessment of minimal residual disease in standard-risk AML

Impact of concurrent *DNMT3A*^{mut}

Relapse in pts without *DNMT3A*^{mut}



Relapse in pts with *DNMT3A*^{mut}



Ivey A et al. N Engl J Med 2016; 374(5):422-33

MRD monitoring in *NPM1* mutated AML:

A Study of the German-Austrian AML Study Group (AMLSG)

Patients

- 611 *NPM1*^{mut} AML patients (age 18 to 60 years) enrolled in one of 4 AMLSG treatment trials [AMLHD98A (NCT00146120) n=46; AMLSG 07-04 (NCT00151242) n=199; AMLSG 09-09 (NCT00893399) n=256; AMLSG 16-10 (NCT01477606) n=110]

Treatment

- Double induction with ICE (idarubicin, cytarabine, etoposide) -/+ ATRA or GO, or 1 induction cycle with daunorubicin and cytarabine followed by 1 to 4 cycles of high-dose cytarabine (n= 363, 59%), or autologous (n=19, 3%) or allogeneic hematopoietic stem cell transplantation (n=162, 27%); 67 (11%) patients did not complete/receive consolidation

- Median follow-up for all patients/trials: 3.2 years

MRD monitoring in *NPM1* mutated AML:

A Study of the German-Austrian AML Study Group (AMLSG)

Methods

- cDNA-based RQ-PCR assays for mutation types A, B, C, D, Jt, 4, Qm, Nm and Km; sensitivity of 10^{-5} (type 4) to 10^{-6} (A, B, C, D, Qm, Nm, Km, Jt) (*Gorello et al., Leukemia 2006*)
- MRD levels were defined as the normalized value of *NPM1*^{mut} transcripts per *ABL1* transcripts x 10^4 (*NPM1*^{mut} transcript levels)

Material

Time point	Bone Marrow	Peripheral Blood
Diagnosis	532	358
Therapy	1790	1264
Follow up	1205	1163
Total	3527	2785

Prognostic impact of *NPM1*^{mut} transcript levels at the time of diagnosis

BM samples n=532

- Median *NPM1*^{mut} transcript levels varied between 7.03 x 10^3 to 1,13 x 10^9 (*NPM1*^{mut}/ *ABL* copies x 10^4); median 6.47 x 10^5
- No correlation with age, sex, WBC, BM blasts, *FLT3*-ITD and *FLT3*-TKD, *DNMT3A*, *IDH1/2*, *NRAS* mutation status, karyotype and *FLT3*-ITD/*DNMT3A* genotypes; except of LDH (*P*=0.004)
- *NPM1*^{mut} transcript levels as $\log_{10}$ transformed continuous variable did not impact RFS, EFS, OS and cumulative incidence of relapse (CIR)

Prognostic impact of *NPM1*^{mut} transcript levels during treatment

	Bone Marrow						Peripheral Blood					
	Transcript level	Pts	Relapse		Death		Transcript level	Pts	Relapse		Death	
Timepoint	Median Range	n	HR	p	HR	p	Median Range	n	HR	p	HR	p
After induction I	1359 0 - 1826000	481	1.45	<0.0001	1.18	0.007	220.5 0 - 936700	348	1.78	<0.0001	1.32	<0.001
After induction II	45 0 - 904500	381	1.89	<0.0001	1.66	<0.0001	3 0 - 598600	270	1.9	<0.0001	1.63	<0.0001
After consolidation I	16 0 - 2183000	342	1.89	<0.0001	1.59	<0.0001	0 0 - 2108000	256	1.99	<0.0001	1.66	<0.0001
After consolidation II	6 0 - 2875000	256	1.92	<0.0001	1.85	<0.0001	0 0 - 717000	176	2.92	<0.0001	2.25	<0.0001
After consolidation III	3 0 - 2368000	209	2.18	<0.0001	1.68	<0.0001	0 0 - 176700	146	2.28	<0.0001	2.13	<0.0001
After allogeneic SCT (as consolidation)	0 0 - 2187000	58	2.55	0.0009	1.55	0.0001	0 0 - 1365000	48	13.8	0.01	1.85	<0.0001
End of treatment (overall)	2 0 - 2368000	290	2.17	<0.0001	1.58	<0.0001	0 0 - 2108000	198	2.00	<0.0001	1.85	<0.0001
End of treatment (according protocol)	1.6 0 - 2368000	268	2.15	<0.0001	1.59	<0.0001	0 0 - 176700	183	2.44	<0.0001	1.83	<0.0001

NOTE: HR for 10-fold increase in *NPM1*^{mut} transcript level

Impact of achievement of RQ-PCR negativity in BM and PB after 2 cycles of therapy

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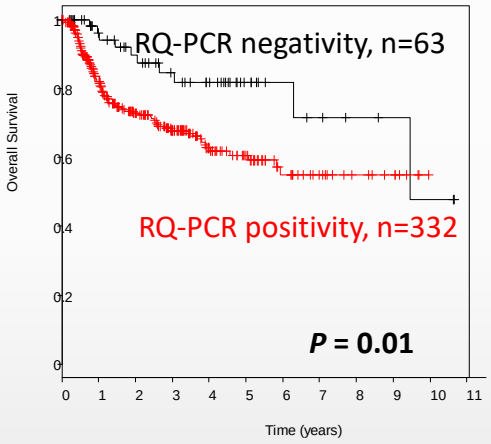
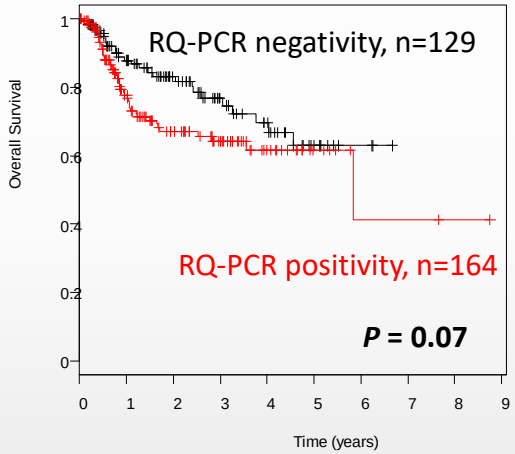
After 2 cycles of therapy in patients in CR

BM, n=395

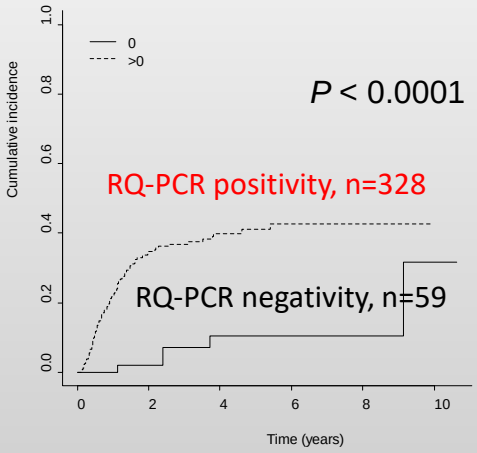
PB, n=293

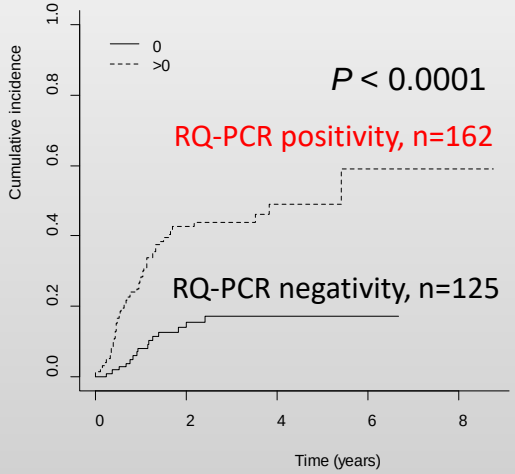
Overall Survival





Cumulative Incidence of Relapse





Prognostic impact of *NPM1*^{mut} transcript levels in BM after 2 cycles of therapy

BM, n=395 in patients in CR

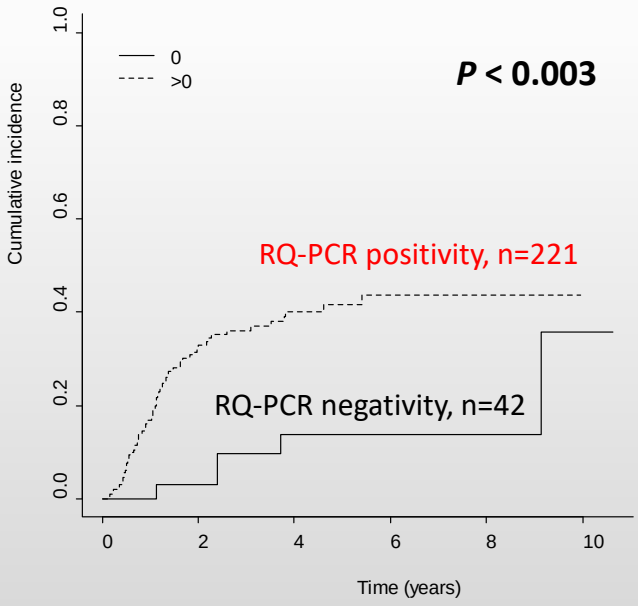
	Relapse			Death		
Variable	**HR**	**95% CI**	***P***	**HR**	**95% CI**	***P***
***NPM1*^{mut} cont. variable**	1.87	1.58-2.21	**<0.001**	1.44	1.24-1.69	**<0.001**
***FLT3*-ITD**	2.32	1.09-4.95	**0.02**	4.94	2.31-10.55	**<0.001**
***FLT3*-TKD**	0.721	0.37-1.37	0.32	1.21	0.65-2.25	0.53
Age	1.28	0.99-1.65	**0.05**	1.28	0.96-1.71	0.08
BM blasts	1.00	0.99-1.01	0.70	1.00	0.99-1.01	0.27
LDH	1.35	0.59-3.05	0.47	0.94	0.42-2.07	0.88
WBC	0.90	0.61-1.33	0.61	0.89	0.58-1.39	0.63
DNMT3A	2.09	1.21-3.59	**0.007**	1.96	0.99-3.86	**0.05**
Allogeneic SCT	0.84	0.37-1.91	0.68	0.74	0.31-1.74	0.49
FLT3*-ITD/*DNMT3A	0.81	0.33-2.01	0.66	0.68	0.27-1.65	0.39

NOTE: HR for 10-fold increase in *NPM1*^{mut} transcript level

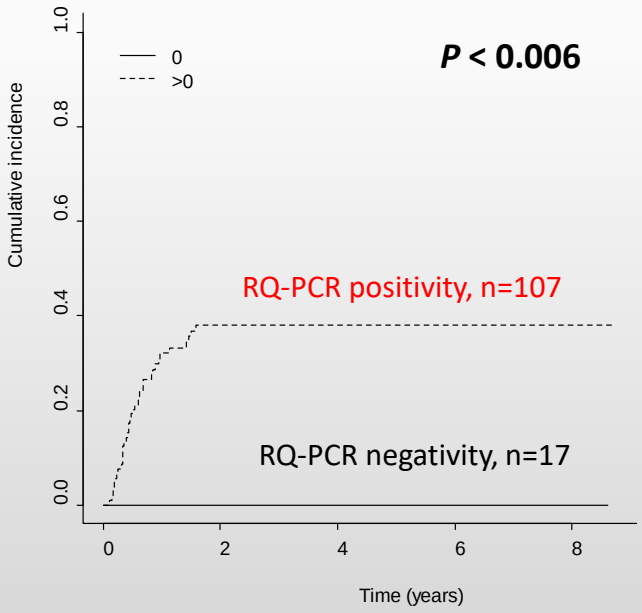
Impact of concurrent *FLT3*-ITD on clinical outcome

After 2 cycles of therapy in patients in CR BM, n=395

CIR in pts without *FLT3*-ITD



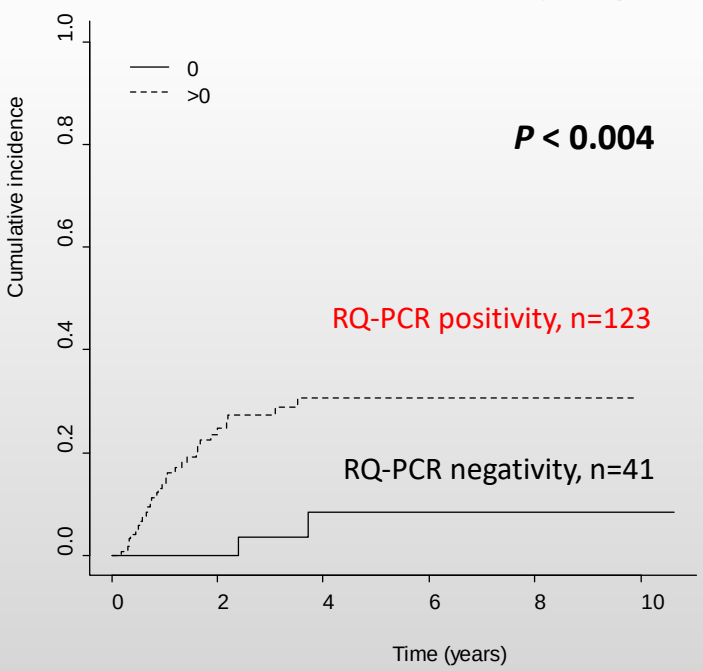
CIR in pts with *FLT3*-ITD



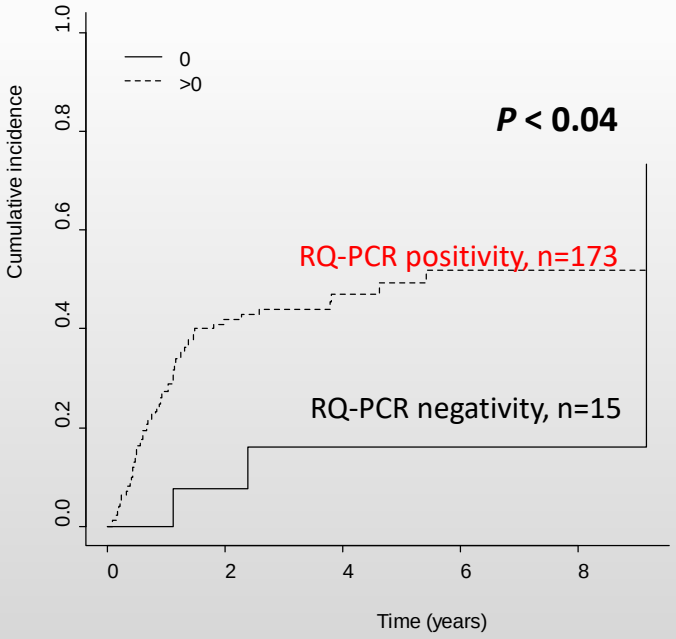
Impact of concurrent *DNMT3A* mutation on clinical outcome

After 2 cycles of therapy in patients in CR BM, n=395

CIR in pts without *DNMT3A*^{mut}

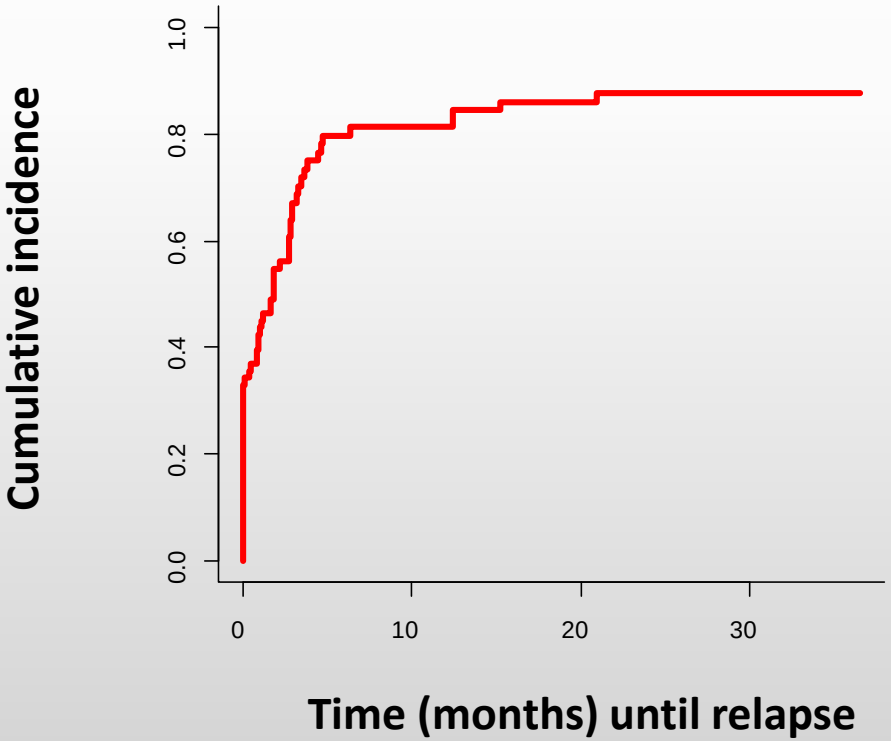


CIR in pts with *DNMT3A*^{mut}



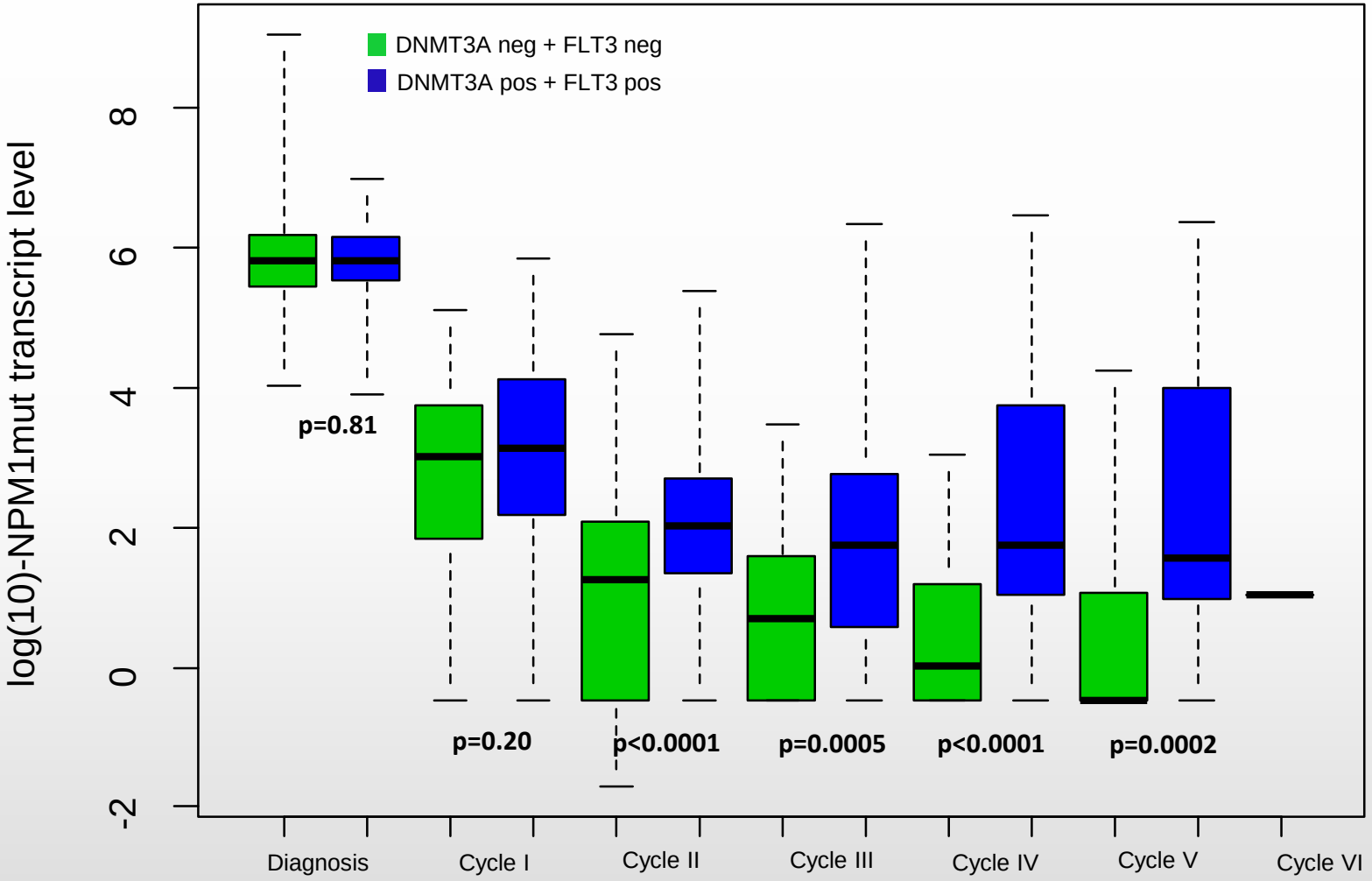
Impact of *NPM1*^{mut} transcript levels during follow-up period

NPM1^{mut} transcript level in BM > 200; n=82



Median time to relapse: 1.7 months after exceeding the cut-off

Impact of concurrent *FLT3*-ITD/*DNMT3A* mutations on kinetics of *NPM1*^{mut} transcript levels



Samples, n	140	111	95	70	114	71	98	43	92	19	83	16	1	0
Median	660547	670832	1022	1330	17	108	5	55	1	54	0	36	11	---
Negative, n	0	0	7	1	30	6	39	10	42	3	43	3	0	---
Negative %	0.0	0.0	7.4	1.4	26.3	8.5	39.8	23.3	45.7	15.8	51.8	18.8	0-0	---

Summary and Conclusions

- In most of the studies achievement of MRD negativity by RQ-PCR is associated with reduced relapse risk and improved survival
- In *NPM1*^{mut} AML the MRD status after two cycles of therapy is clinically relevant and allows the identification of pts at high risk of relapse
- During follow-up period, cut-off value > 200 *NPM1*^{mut}/*ABL* x10^4 copies is highly predictive for relapse
- The *FLT3*-ITD/*DNMT3A* genotype impacts on reduction of *NPM1*^{mut} transcript levels and achievement of RQ-PCR negativity, especially in triple positive patients
- NGS-based MRD monitoring is not established yet; further development of the techniques is ongoing
- Standardization/guidelines for MRD monitoring are needed
- Inclusion of MRD monitoring into clinical trials

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STUDY AML GROUP

SFB 1074

DFG Deutsche Forschungsgemeinschaft

ulm university universität ulm

SFB 1074 Experimental Models and Clinical Translation in Leukemia

Achievement of RQ-PCR negativity in *NPM1*^{mut} patients according to *FLT3*-ITD/*DNMT3A* mutation status in BM

After 2 cycles of therapy

Genotype	*NPM1*^{mut} *FLT3*-ITD WT *DNMT3A* WT	*NPM1*^{mut} *FLT3*-ITD^{mut} *DNMT3A* WT	*NPM1*^{mut} *FLT3*-ITD WT *DNMT3A*^{mut}	*NPM1*^{mut} *FLT3*-ITD^{mut} *DNMT3*^{mut}
RQ-PCR negative (n)	30 (26%)	14 (25%)	10 (8%)	6 (8%)
RQ-PCR positive (n)	84 (74%)	41 (75%)	110 (92%)	65 (92%)
% negative	**26%**	25%	8%	**8%**

P=0.0002

Achievement of RQ-PCR negativity in *NPM1*^{mut} patients according to *FLT3*-ITD/*DNMT3A* mutation status in BM

End of treatment

Genotype	*NPM1*^{mut} *FLT3*-ITD WT *DNMT3A* WT	*NPM1*^{mut} *FLT3*-ITD^{mut} *DNMT3A* WT	*NPM1*^{mut} *FLT3*-ITD WT *DNMT3A*^{mut}	*NPM1*^{mut} *FLT3*-ITD^{mut} *DNMT3*^{mut}
RQ-PCR negative (n)	53 (55%)	21 (60%)	36 (37%)	17 (40%)
RQ-PCR positive (n)	44 (45%)	14 (40%)	61 (63%)	26 (60%)
% negative	55%	60%	37%	40%

P=0.02