Molecular MRD monitoring and its role in AML

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DISCLOSURES OF COMMERCIAL SUPPORT

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Name of Company	Research support	Employee	Honoraria	Stockholder	Speaker's Bureau	Advisory Board
Novartis	√		√			√
Janssen			√			√
Celgene			√			√
Daiichi Sankyo			√			√
CTI BioPharma			√			√
Roche			√			√

Measurable 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 determine measurable 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-})

Measurable residual disease in AML

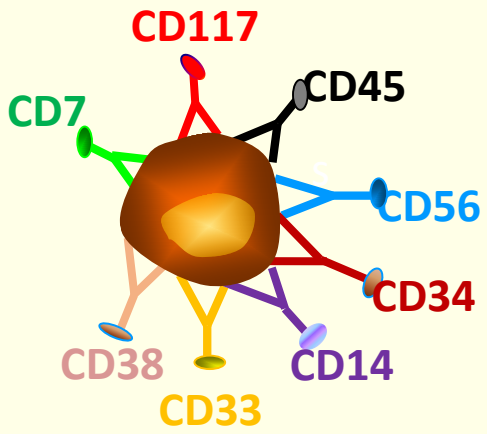
MRD monitoring: clinical implications

- Impact on prognosis
- Response assessment
- Early detection of relapse
- Guiding pre-emptive therapy
- Treatment decision making, in particular within the context of post-remission therapy [e.g.allogeneic stem cell transplantation (alloSCT]
- Monitoring of treatment effects (novel drugs)
- MRD as a surrogate endpoint in clinical trials > rapid approval of novel drugs

Methods for MRD monitoring

Multicolor flow cytometry (MCF)

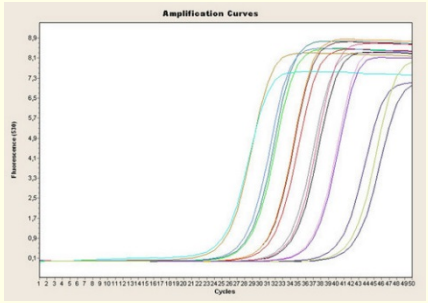
- Leukemia associated immunophenotype (LAIP)



Sensitivity 10^{-3} to 10^{-4}

PCR-based techniques

- Quantitative RT-PCR (RQ-PCR)
- Digital PCR (dPCR), droplet digital PCR (ddPCR)



Sensitivity 10^{-5} to 10^{-6}

NGS-based techniques

- Mainly targeted approaches
- Quantification / identification of multiple gene mutations

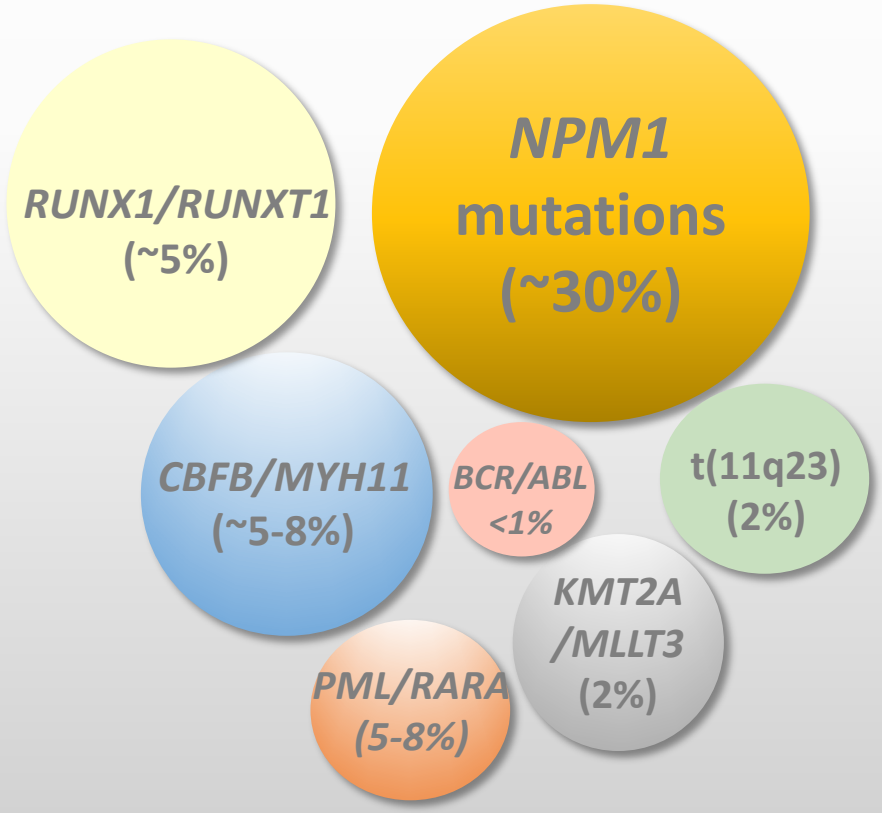
Sensitivity 10^{-4}

Molecular markers 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 or by hot spot mutations

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

~ 50% of all AML



Prognostic impact of MRD in APL

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)
- Paired BM and PB samples used for RQ-PCR analysis after each treatment course, every 3 months until 36 months of post-consolidation
- Sensitivity of at least 1 in 10^{-4}
- Samples were sent by courier / overnight delivery
- Clinicians were informed of PCR results

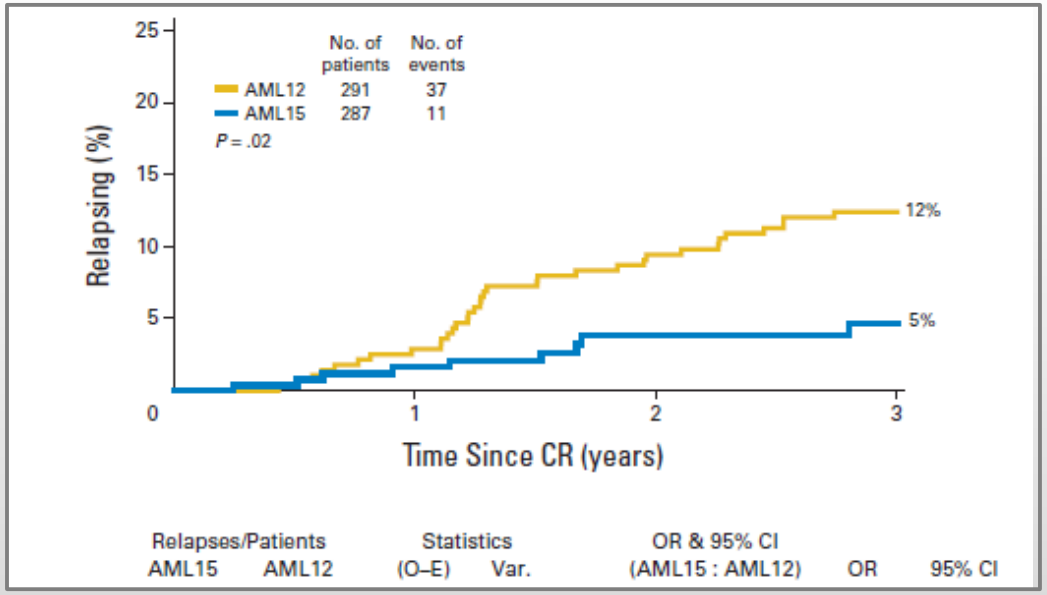
Prognostic impact of MRD in APL

Acute Promyelocytic Leukemia

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

- 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 in pts with persistent PCR positivity or molecular relapse prevented progression to overt relapse in the majority of the pts

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



Prognostic impact of MRD in Core-binding Factor (CBF) Leukemia

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

- MRD-negativity at the end of treatment in PB impacts clinical outcome – French Intergroup CBF-2006 trial. *Willekens et al., Haematologica 2016,* [t(8;21), n=94]
- MRD-negativity at end of treatment impacts clinical outcome – AML Study Group *Agrawal et al., ASH meeting 2016, abstract #1207* [t(8;21), n=120])
- 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]
- Minimal residual disease monitoring and mutational landscape in AML with RUNX1-RUNX1T1: a study on 134 patients. *Höllein et al., Leukemia 2018*

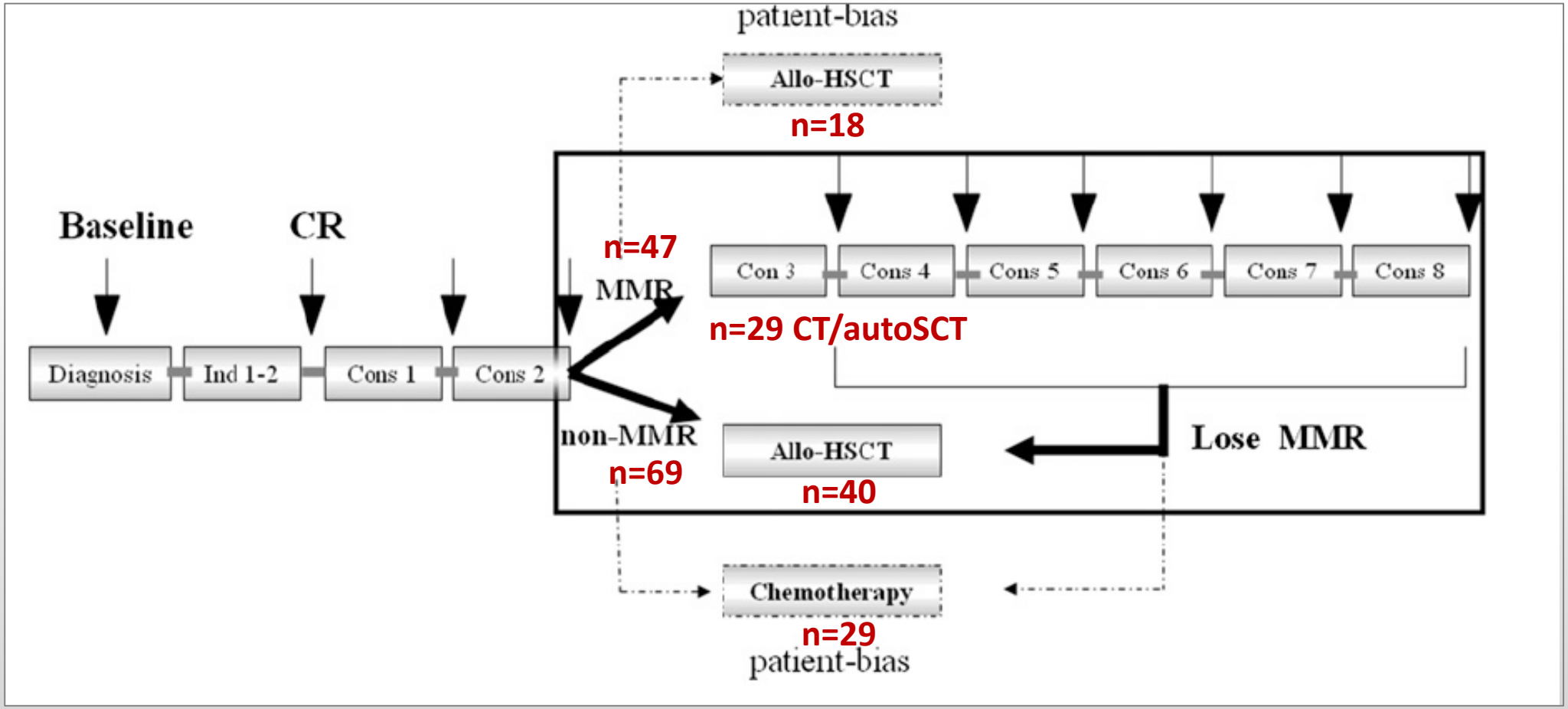
Prognostic impact of MRD in t(8;21)(q22;q22.1)-positive AML

Hong-Hu Zhu et al. Blood 2013; 121: 4056-4062

- **Prospective study** on 116 newly t(8;21)-positive AML pts achieving CR after 2 induction cyles
- MRD directed risk stratification treatment in pts in 1.CR
- BM samples were used for RQ-PCR analysis at diagnosis, after induction therapy, after each consolidation cycle, and 3-monthly for 1 year
- **Major molecular remission (MMR):** > 3 log reduction (<0.4%) in *RUNX1/RUNX1T1* transcripts compared to pretreatment sample
- **Loss of MMR :** *RUNX1/RUNX1T1* transcript levels > 0.4% in MMR pts

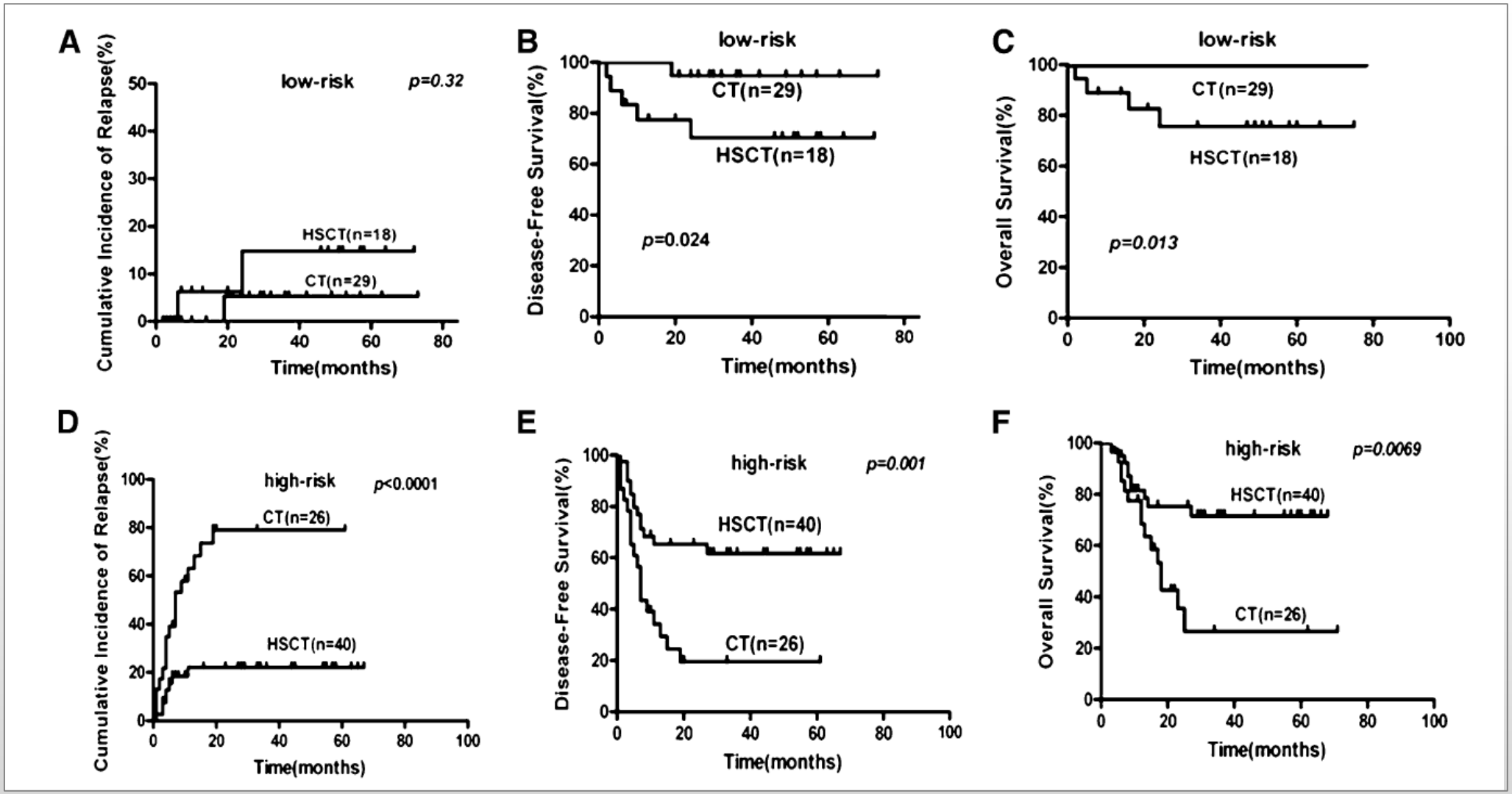
Prognostic impact of MRD in t(8;21)(q22;q22.1)-positive AML

MRD directed risk stratification treatment in t(8;21)-positive AML in 1.CR: The AML05 multicenter trial



Hong-Hu Zhu et al. Blood 2013; 121: 4056-4062

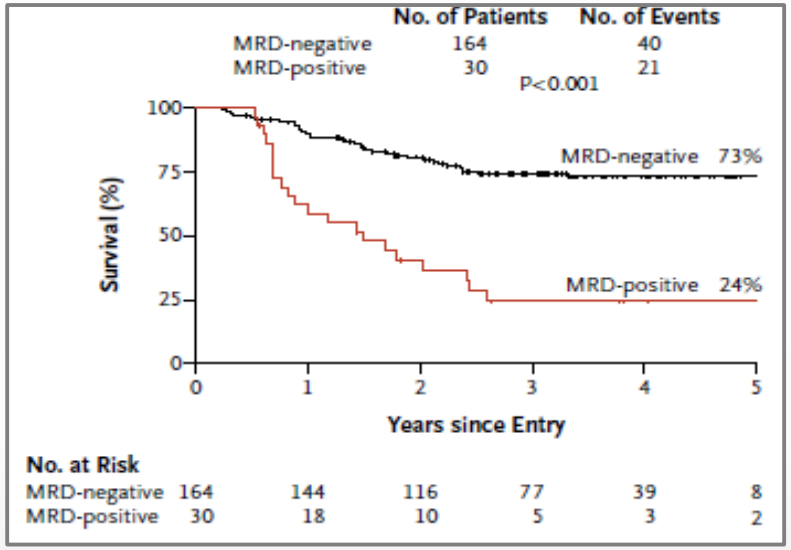
Prognostic impact of MRD in t(8;21)(q22;q22.1)-positive AML

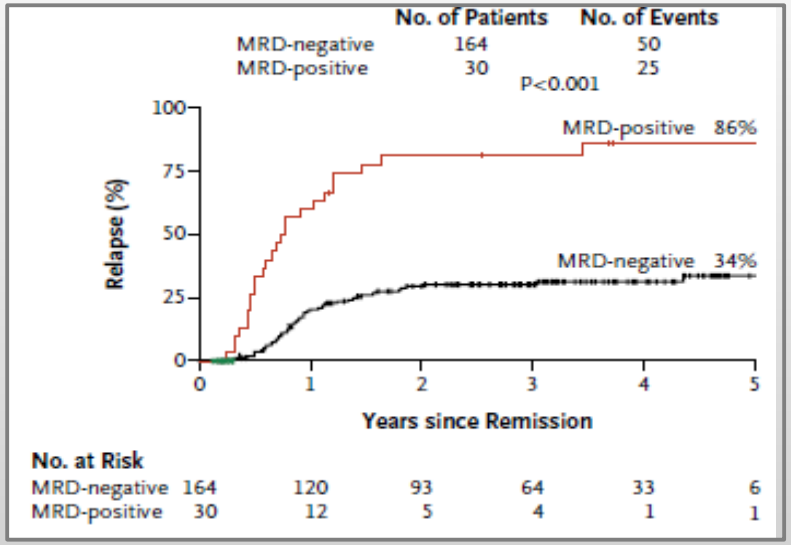


Hong-Hu Zhu et al. Blood 2013; 121: 4056-4062

Prognostic impact of MRD in *NPM1* mutated AML

- Retrospective study on 437 *NPM1* mutated 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)



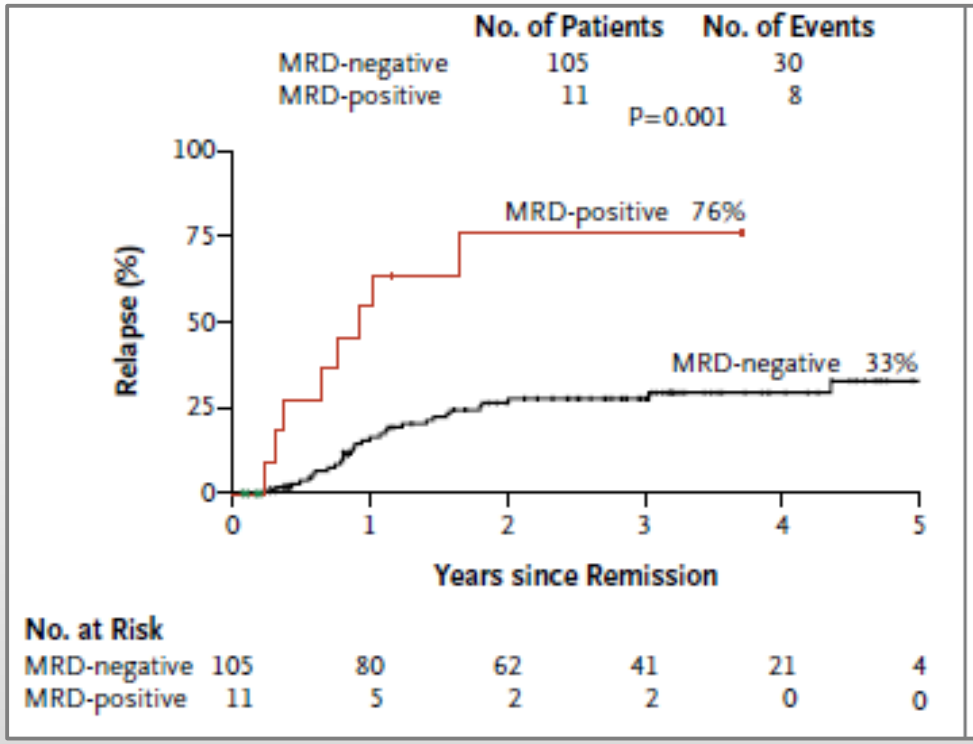


Ivey A et al. N Engl J Med 2016

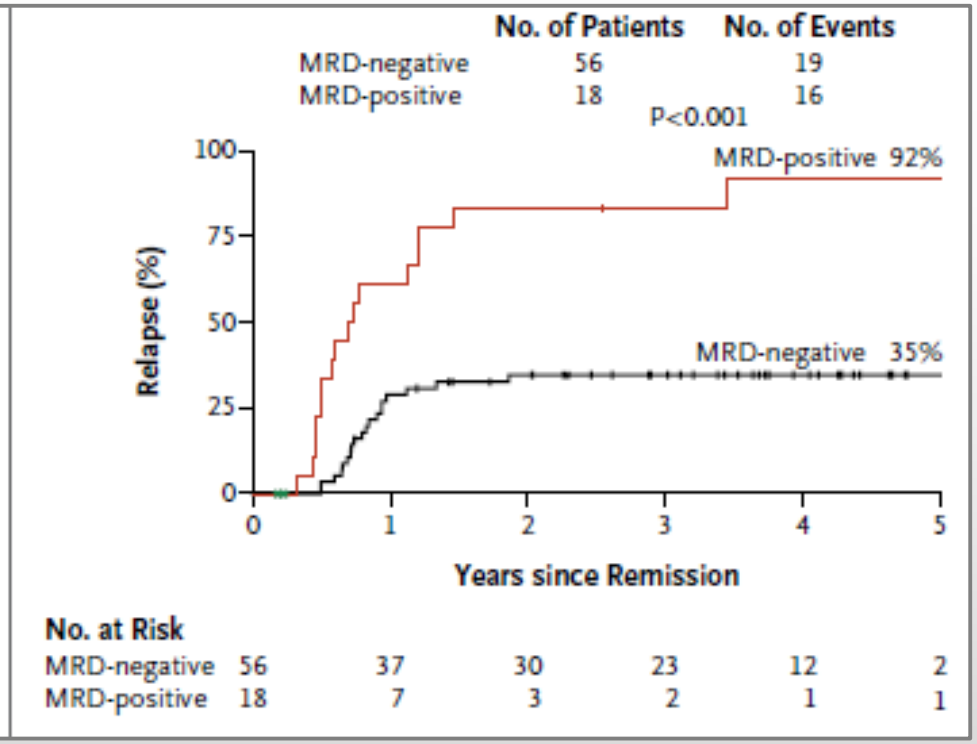
Prognostic impact of MRD in *NPM1* mutated AML

Impact of concurrent *FLT3*-ITD mutation

Relapse in pts without *FLT3*-ITD



Relapse in pts with *FLT3*-ITD

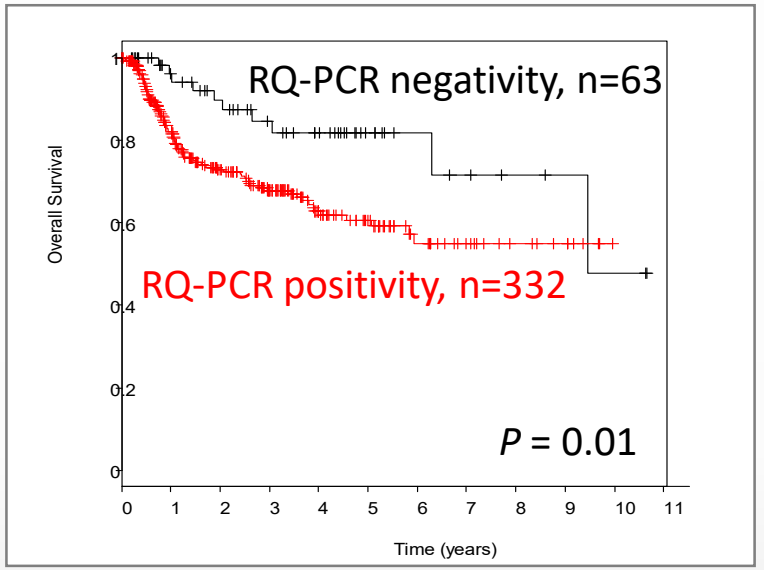


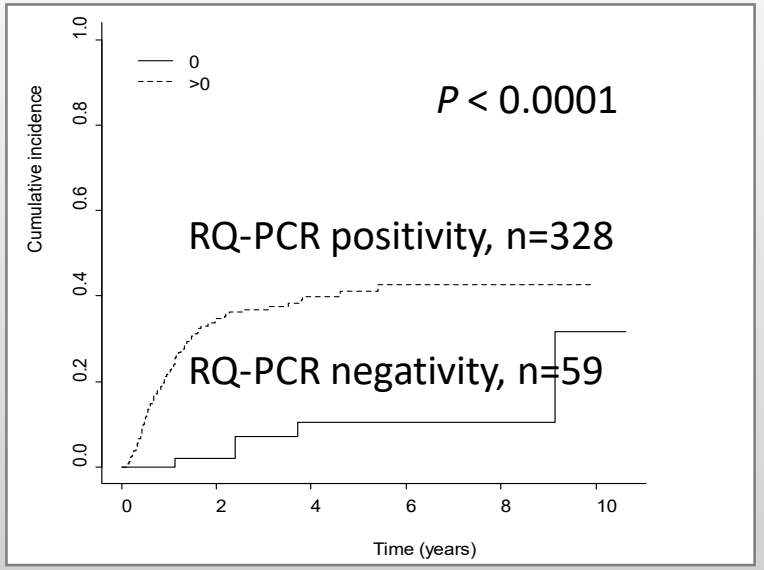
Ivey A et al. N Engl J Med 2016

Prognostic impact of MRD in *NPM1* mutated AML

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

- Retrospective/since 2008 prospective study on 611 *NPM1*^{mut} adult AML pts enrolled in 4 AMLSG treatment trials; median follow-up for all patients/trials: 3.2 years
- 6339 BM/PB (3527/2812) samples were analyzed by RQ-PCR after each treatment cycle and during follow-up; sensitivity 10^{-5} to 10^{-6}
- Achievement of RQ-PCR negativity in the BM after 2 treatment cycles was significantly associated with superior OS (at 4 yrs: 82% vs 63%) and lower CIR (at 4 yrs: 15% vs 40%) compared to RQ-PCR positive pts
- Multivariate analysis: *NPM1*^{mut} transcript levels (continuous variable) in BM were significantly associated with relapse (HR 1.87) and OS (HR 1.44)

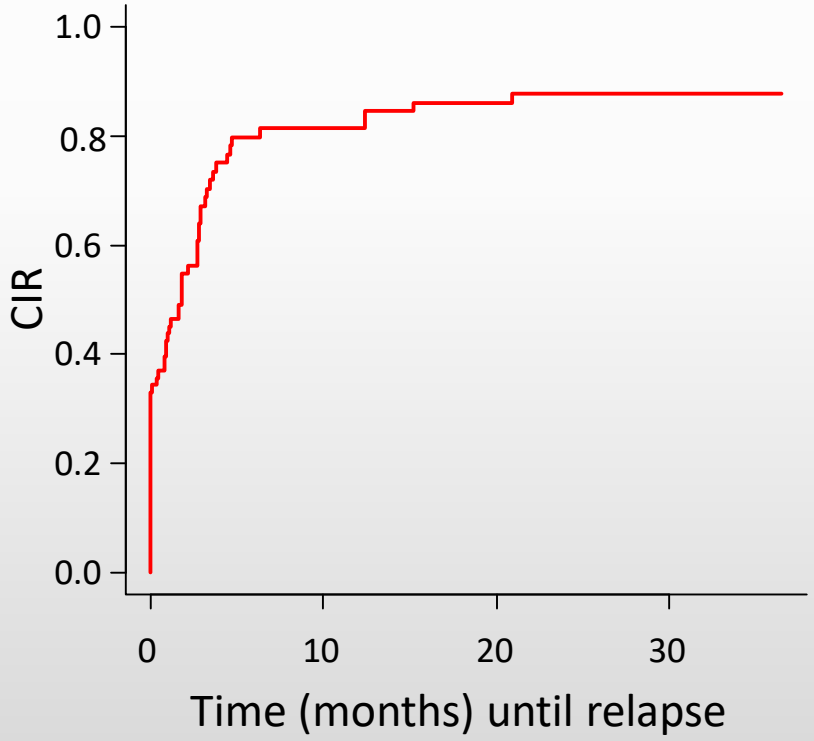




Krönke et al., JCO 2011;19:2709-2716; Kapp-Schwoerer S et al., ASH meeting 2017, #183

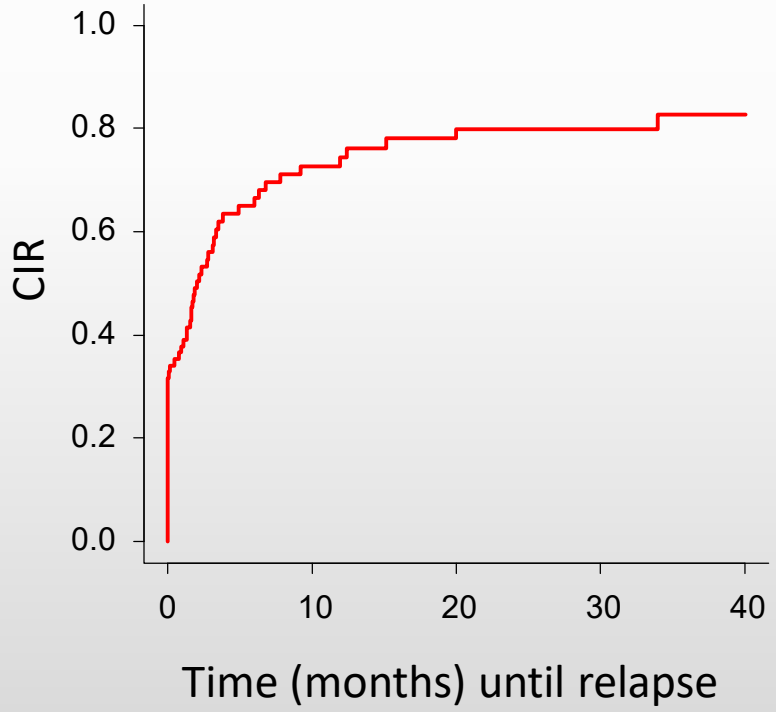
Impact of *NPM1*^{mut} transcript levels during follow-up

BM samples (n=82):
NPM1^{mut} TL > 200 (cut-off value)



Median time to relapse: 1.7 months

PB samples (n=82):
NPM1^{mut} TL > 50 (cut-off value)



Median time to relapse: 2.04 months

MRD monitoring by next generation sequencing (NGS)

- 482 AML pts (18 to 65 years) treated with 1 to 2 cycles of standard induction chemotherapy followed by consolidation in HOVON-SAKK clinical trials
- NGS panel of 54 genes (Illumina) at diagnosis and in BM in morphological CR after completion of induction therapy
- 430/482 (89.2%) pts had somatic driver mutations at diagnosis (2.9 mutations per case)
- 51.4% of pts had persisting mutations in BM in morphological CR at highly variable variant allele frequencies (VAF 0.02-47%), predominantly in *DNMT3A* (78.7%), *TET2* (54.2%) and *ASXL1* (51.6%) >> DTA mutations

Mutation detection at diagnosis and in CR

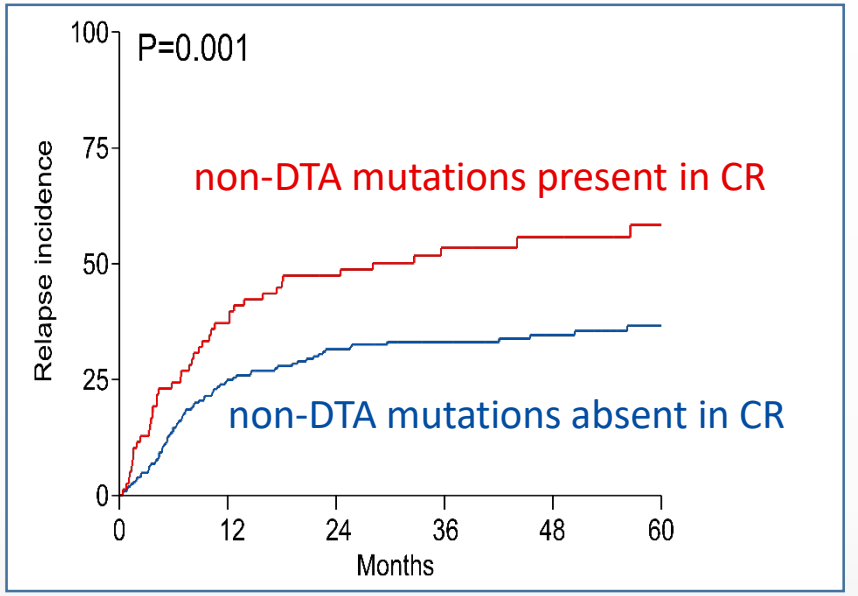
ABL1	DNMT3A	KDM6A	RAD21
ASXL1	ETV6/TEL	KIT	RUNX1
ATRX	EZH2	KRAS	SETBP1
BCOR	FBXWF	MLL	SF3B1
BCORL1	FLT3	MPL	SMC1A
BRAF	GATA1	MYD88	SMC3
CALR	GATA2	NOTCH1	SRSF2
CBL	GNAS	NPM1	STAG2
CBLB	IDH1	NRAS	TET2
CDKN2A	IDH2	PDGFRA	TP53
CEBPA	IKZF1	PHF6	U2AF1
CSF3R	JAK2	PTEN	WT1
CUX1	JAK3	PTPN11	ZRSR2

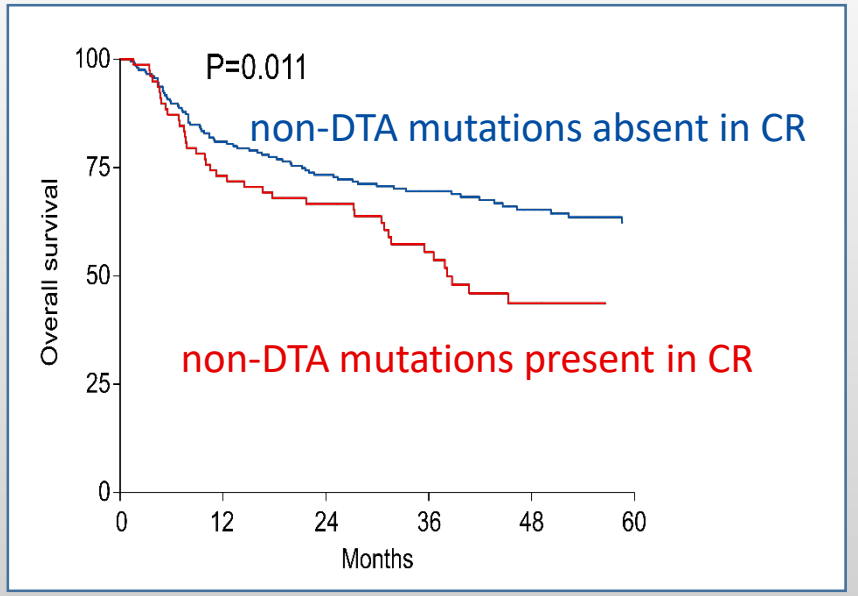
Illumina TruSight Myeloid Panel; mean coverage ~3500x

Mojca Jongen-Lavrencic et al., N Engl J Med; 2018

MRD monitoring by next generation sequencing (NGS)

- *DTA* mutations were not associated with the incidence of relapse at any VAF cut-off >> stage of clonal hematopoiesis rather than impending relapse
- After exclusion of persistent DTA mutations, NGS MRD was significantly associated with higher relapse rate (55.4% vs 31.9%), lower RFS (36,6% vs 58.1%) and inferior OS (41.9% vs 66.1%) than no detection
- Persistence on non-DTA mutations revealed as an independent prognostic variable in multivariate analysis for relapse (HR 1.89), RFS (HR 1.64) and OS (HR 1.64)





Mojca Jongen-Lavrencic et al., N Engl J Med; 2018

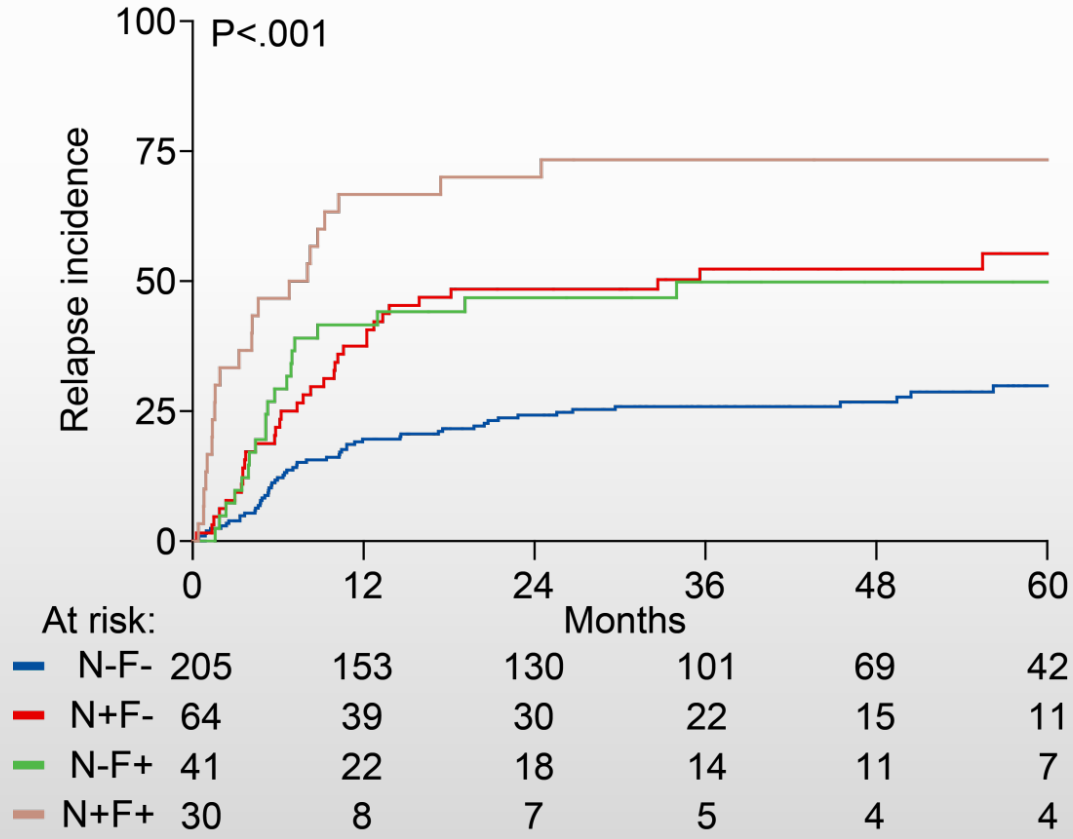
MRD monitoring by next generation sequencing (NGS)

Relapse incidence of residual leukemia:

NGS MRD and multiparameter flow MRD both were significantly associated with relapse in AML

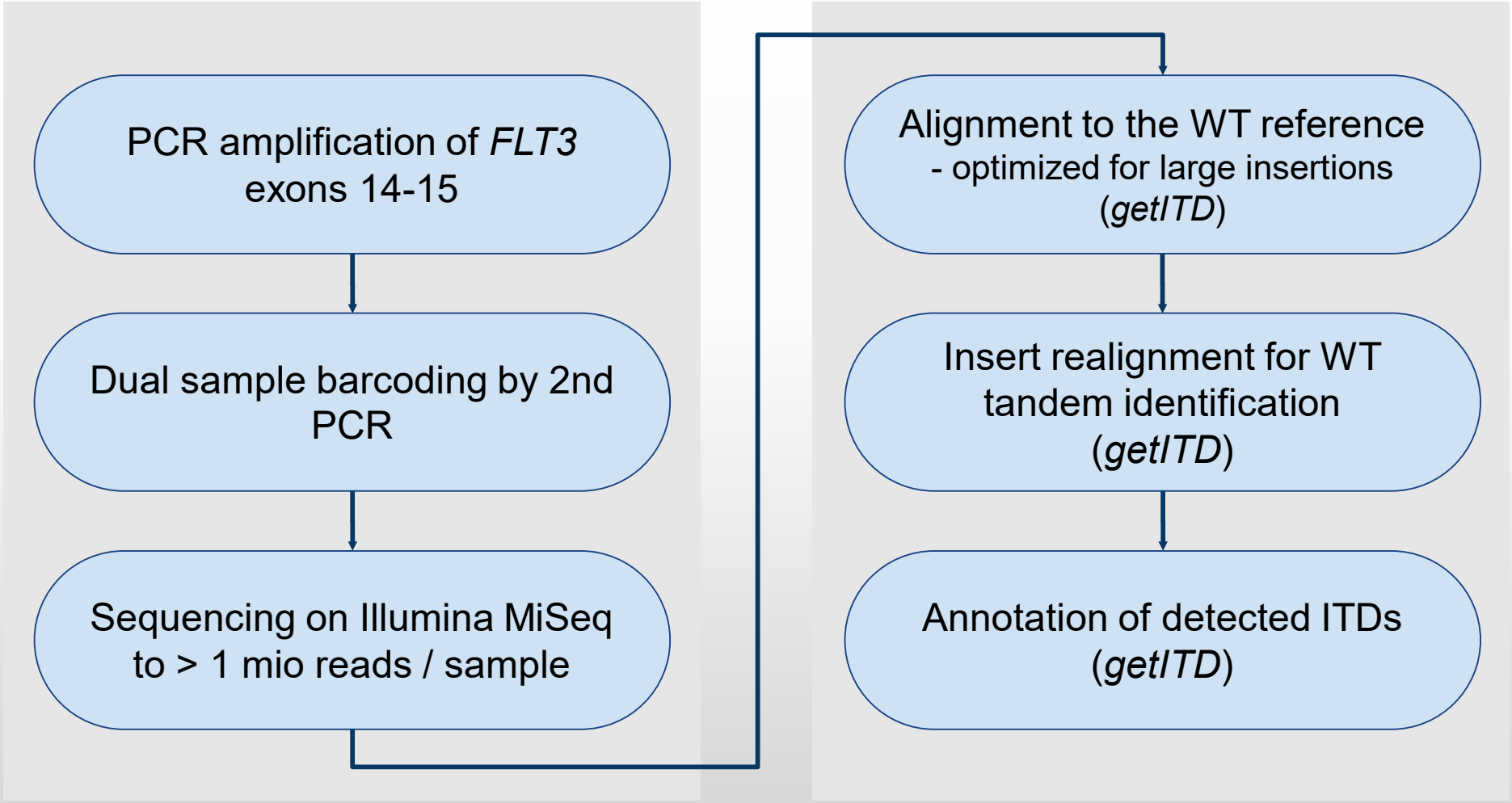
		NGS MRD -	NGS MRD +
flow MRD	-	205	64
	+	41	30

Multivariate analysis: the combined use of the two assays conferred independent prognostic value with respect to RFS and OS



Mojca Jongen-Lavrencic et al., N Engl J Med; 2018

NGS based MRD monitoring of *FLT3*-ITD mutated AML
- assay & analysis workflow -

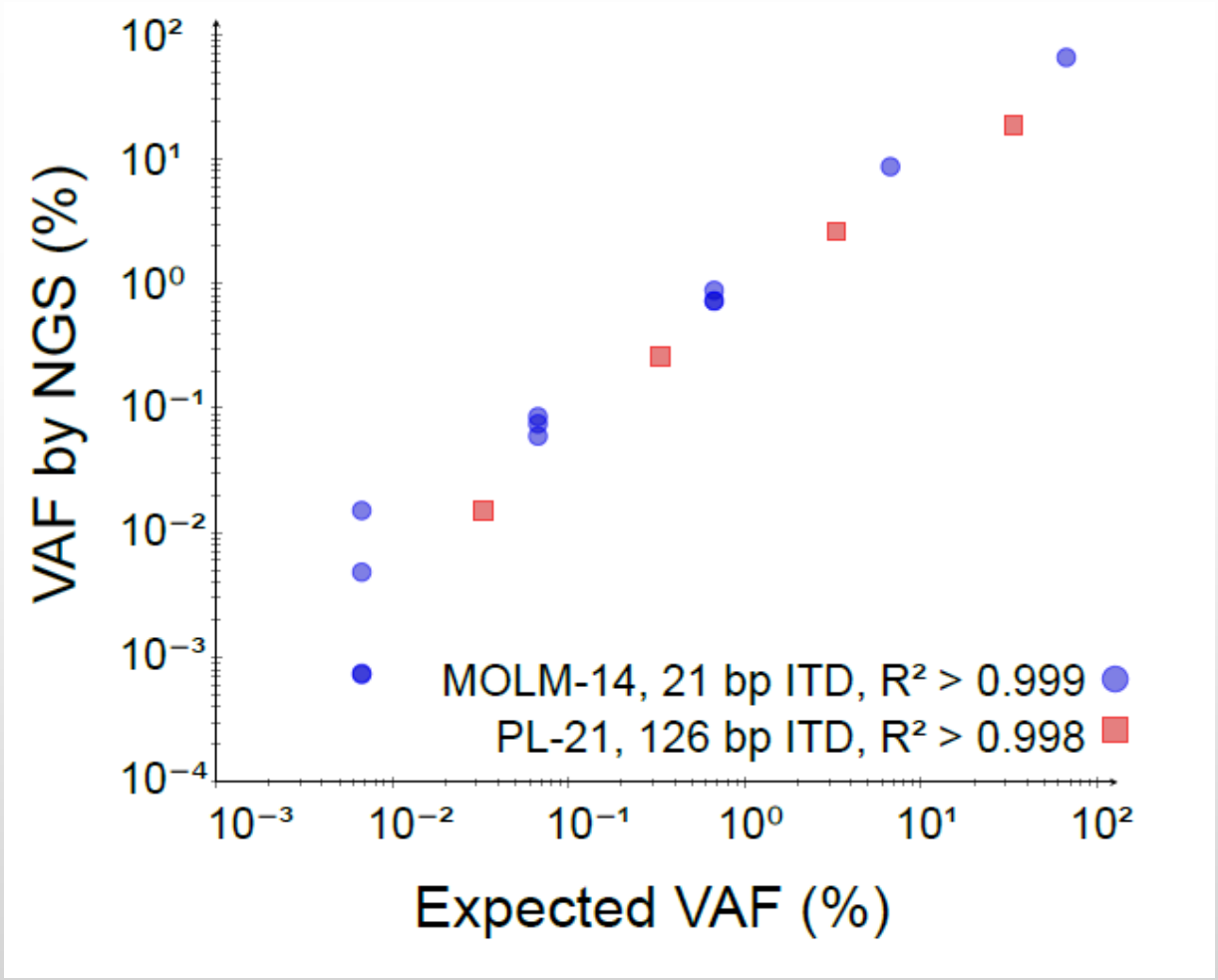


Blätte T et al., Leukemia; accepted for publication

NGS based MRD monitoring of *FLT3*-ITD mutated AML - assay sensitivity-

Serial dilution of ITD$^+$ (MOLM 14; 21bp and PL-21 126bp) in HL-60 DNA

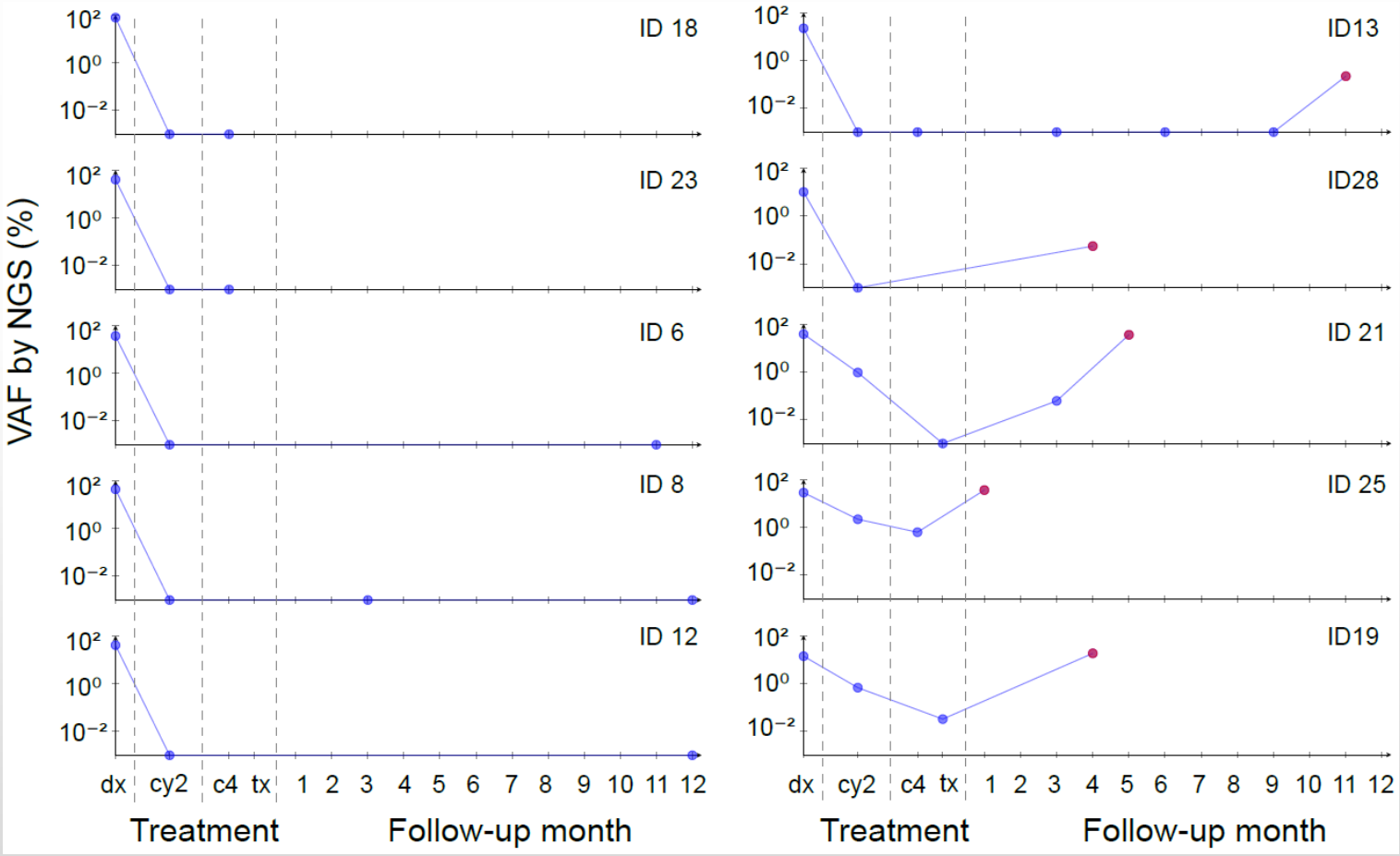
- Expected ITDs were detected in all samples
- VAF estimates were accurate and decreased linearly as expected
- The most diluted sample was MOLM-14:HL-60 1:10000
- detection of ITD at 0.0067% VAF $(6.7x10)^{-5}$ > limit of detection



Blätte T et al., Leukemia; accepted for publication

Serial NGS based MRD monitoring of *FLT3*-ITD mutated AML patients

FLT3-ITD mutated AML pts in continuous CR; n=5



FLT3-ITD mutated AML pts with relapse; n=5

Blätte T et al., Leukemia; accepted for publication

blood®

Special Report

Minimal/measurable residual disease in AML: a consensus document from the European LeukemiaNet MRD Working Party

Gerrit J. Schuurhuis,[1] Michael Heuser,[2,*] Sylvie Freeman,[3,*] Marie-Christine Béné,[4] Francesco Buccisano,[5] Jacqueline Cloos,[1,6] David Grimwade,[7] Torsten Haferlach,[8] Robert K. Hills,[9] Christopher S. Hourigan,[10] Jeffrey L. Jorgensen,[11] Wolfgang Kern,[8] Francis Lacombe,[12] Luca Maurillo,[5] Claude Preudhomme,[13] Bert A. van der Reijden,[14] Christian Thiede,[15] Adriano Venditti,[5] Paresh Vyas,[16] Brent L. Wood,[17,18] Roland B. Walter,[17,19] Konstanze Döhner,[20,†] Gail J. Roboz,[21,†] and Gert J. Ossenkoppele[1]

Schuurhuis GJ et al., Blood 2018;131:1275-1291

The U.S. Food and Drug Administration (FDA) recently issued a draft guidance titled

”Hematologic Malignancies: Regulatory Considerations for Use of Minimal Residual Disease (MRD) in Development of Drug an Biologic Products for Treatment”

Summary and Conclusions

- Most of the studies were performed retrospectively >>> patient selection based to the presence of a molecular marker, the availability of a BM/PB sample at defined time points, and the CR status (1. CR)
- Achievement of MRD-negativity or significant reduction of transcript levels/mutations by RQ-PCR/NGS after 2 cycles of therapy or at EOT was associated with reduced relapse risk and improved survival
- NGS-based MRD monitoring has been shown to be useful in ~ 90% of AML patients; further development of the techniques is ongoing; sensitivities are still low and data analysis is challenging
- Standardization/harmonization guidelines for MRD monitoring are needed
- MRD monitoring (molecular/MCF) should be included in all clinical trials

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STUDY AML GROUP

SFB 1074

SFB 1074 Experimental Models and Clinical Translation in Leukemia

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