Clinical Overview on Measurable Residual Disease (MRD) in Acute Myeloid Leukemia(AML)

Konstanze Döhner
Department of Internal Medicine III, University
Hospital of Ulm, Ulm Germany

DISCLOSURES OF COMMERCIAL SUPPORT

Konstanze Döhner

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

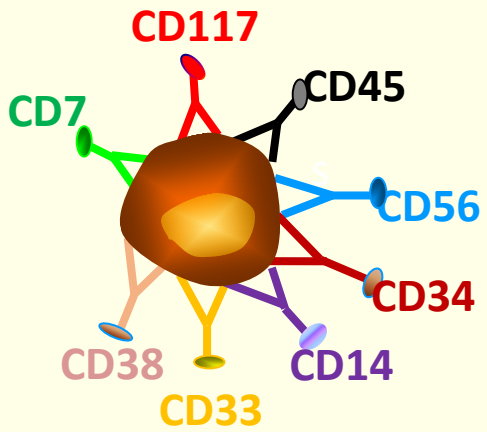
MRD monitoring: clinical implications

- Impact on prognosis
- 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)

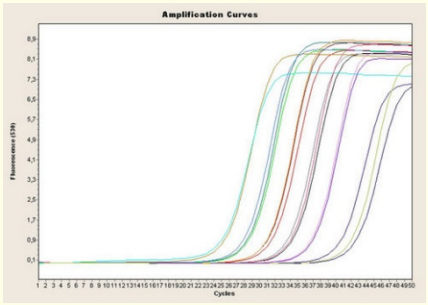
- Leukemia associated immunophenotype (LAIP)



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

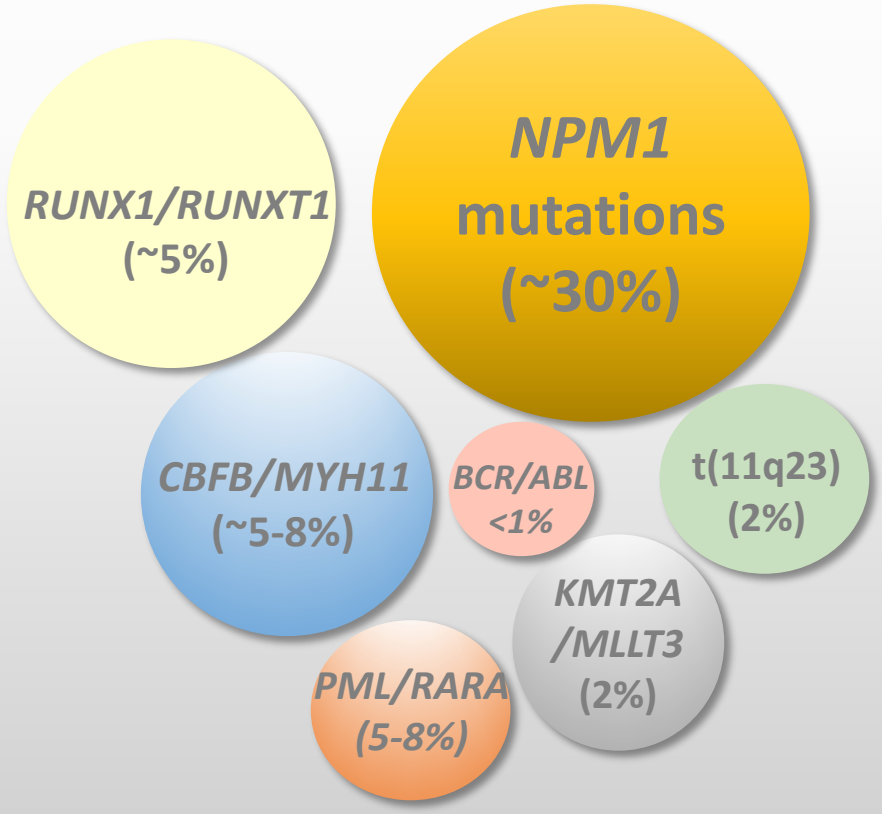
Sensitivity 10^{-4}

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



MRD monitoring in clinical AML trials

- 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
- However, 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 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)

Study design for MRD monitoring

- **Material:** paired BM and PB samples used for RQ-PCR analysis
- **Time points**: - after each treatment course
  - paired PB and BM samples obtained every 3 months until 36 months of post-consolidation
- **Quality control:** sensitivity of at least 1 in 10^4
- **Transport:** samples were sent by courier / overnight delivery
- **Report:** Clinicians were informed of PCR results

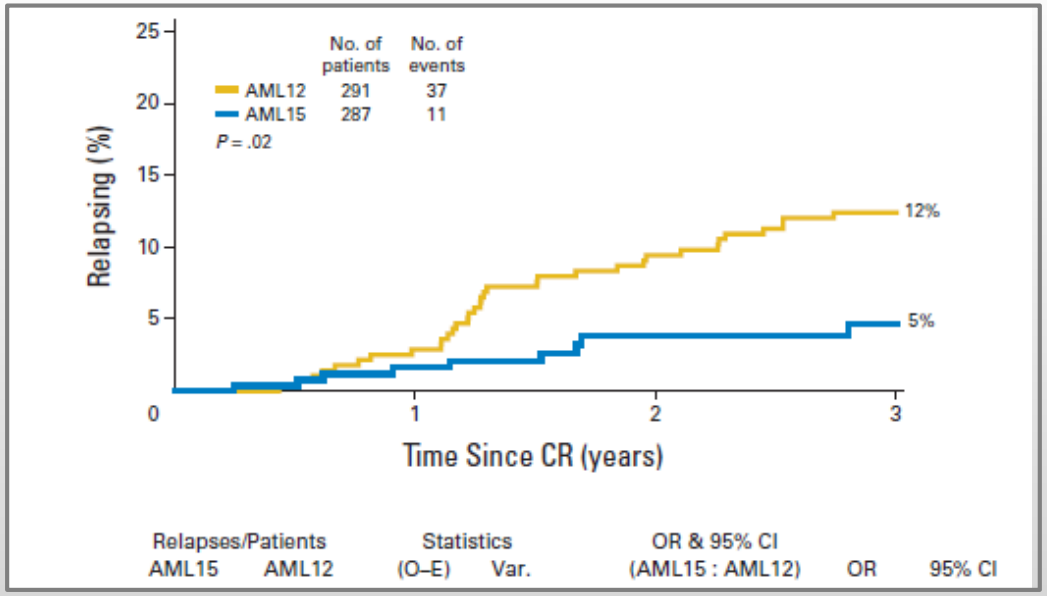
Prognostic impact of MRD in APL

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 in BM 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 *RUNX1/RUNX1T1* MRD in AML

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

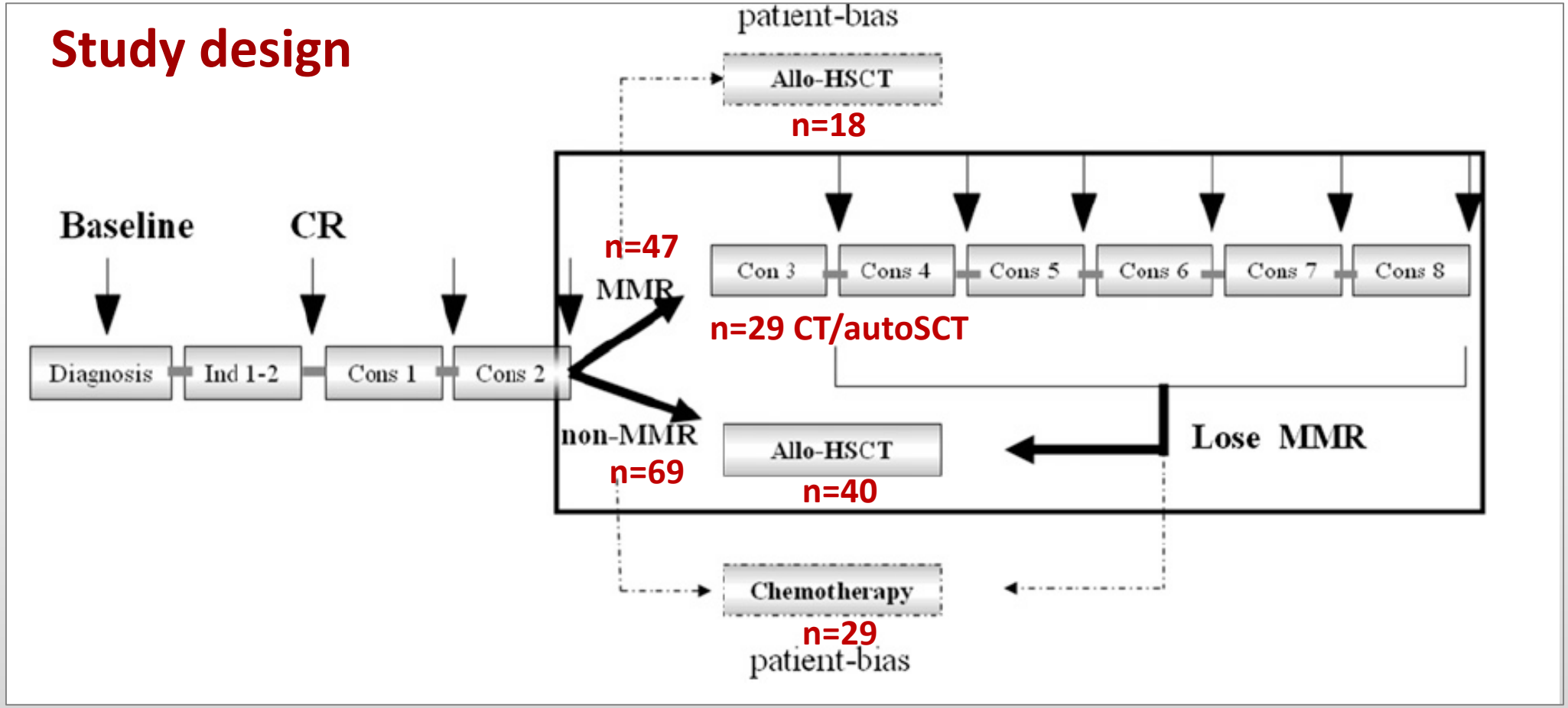
- **Prospective study** on 116 newly diagnosed pts with t(8;21)-positive AML achieving CR after 2 induction cyles
- MRD directed risk stratification treatment in pts in 1.CR

Study design for MRD monitoring

- **Material:** BM samples were used for RQ-PCR analysis
- **Time points**: - at diagnosis
  - after induction therapy
  - after each consolidation cycle
  - 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 *RUNX1/RUNX1T1* MRD in AML

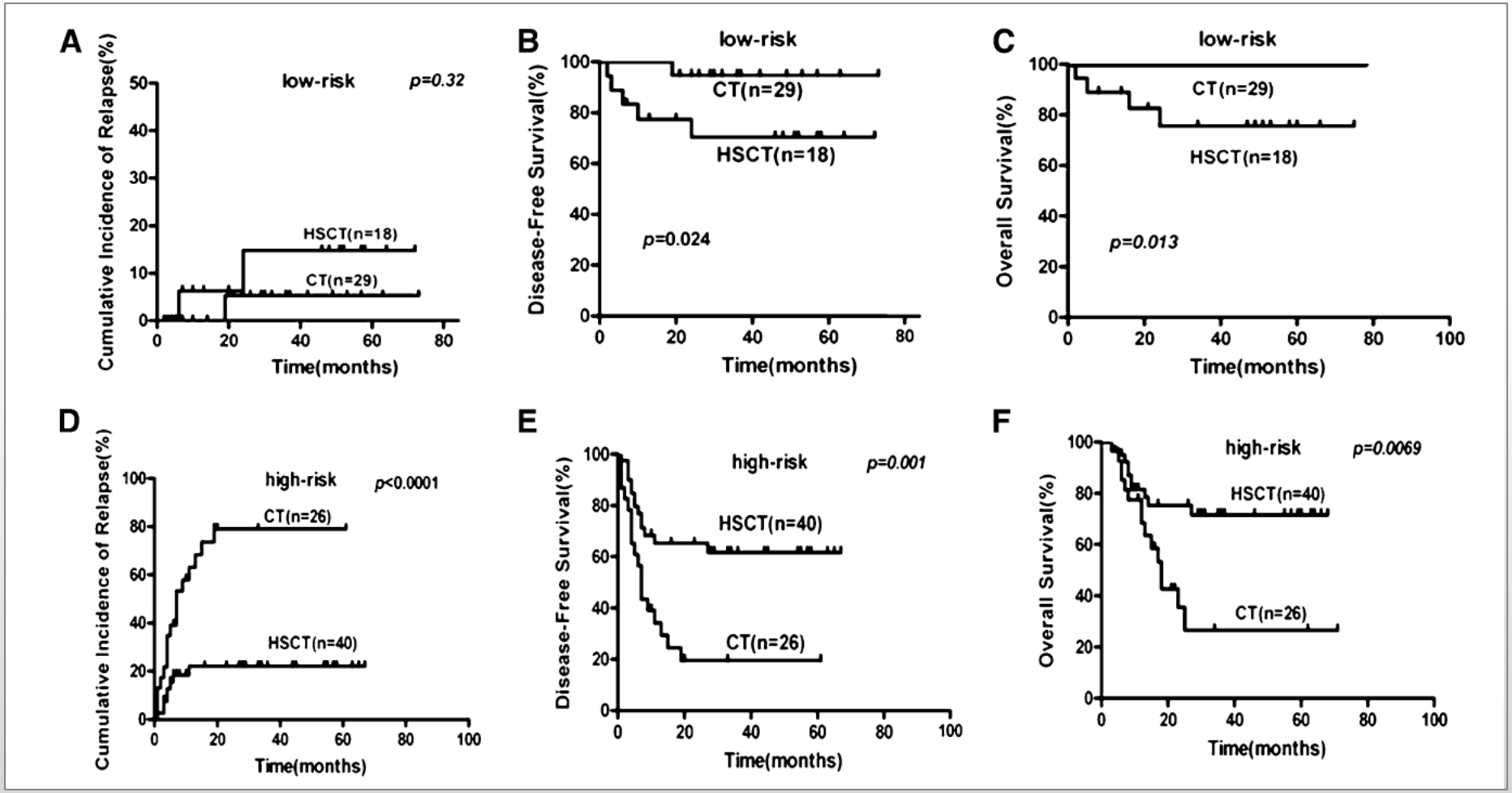
MRD directed risk stratification treatment in t(8;21)-positive AML in 1.CR:
The AML05 multicenter trial



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

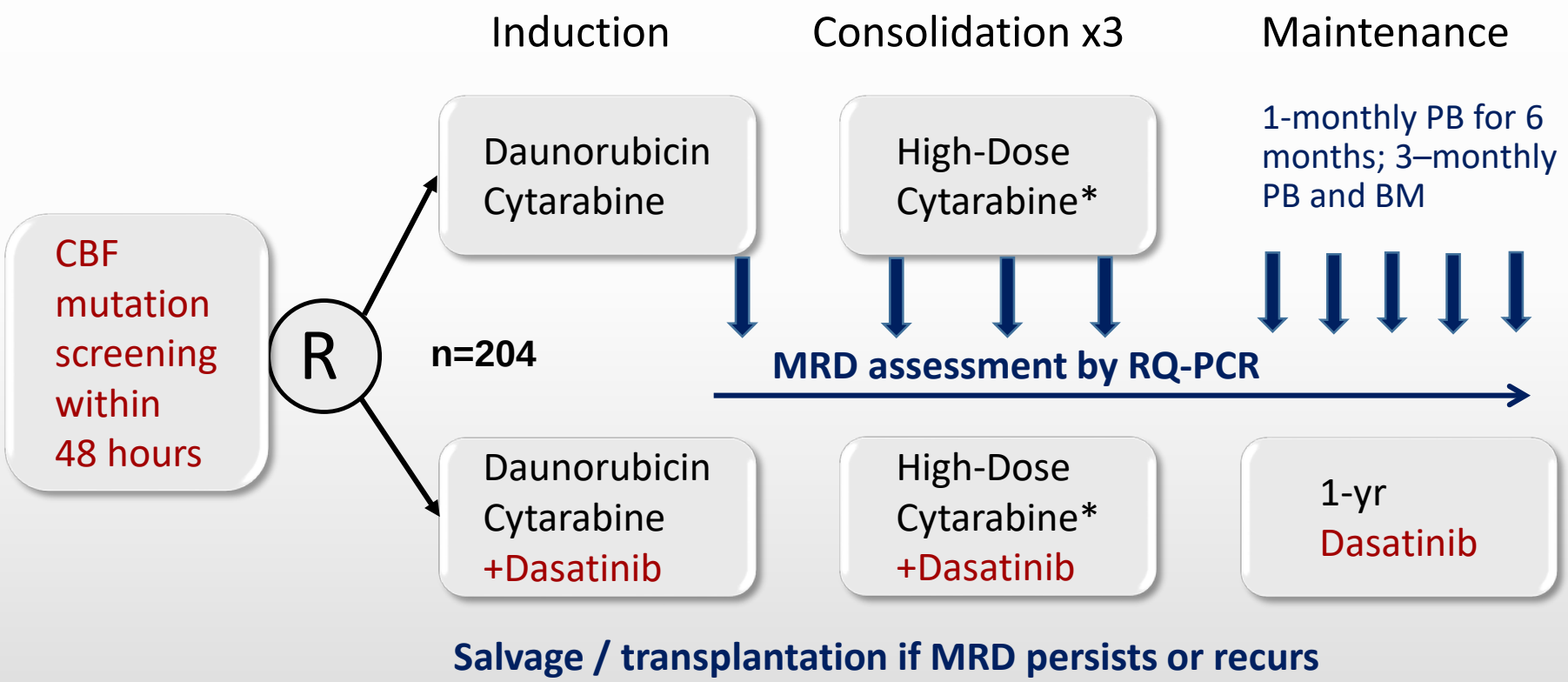
Prognostic impact of *RUNX1/RUNX1T1* MRD status in AML

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

Phase III study of chemotherapy with or without Dasatinib in CBF AML: AMLSG 21-13 Study

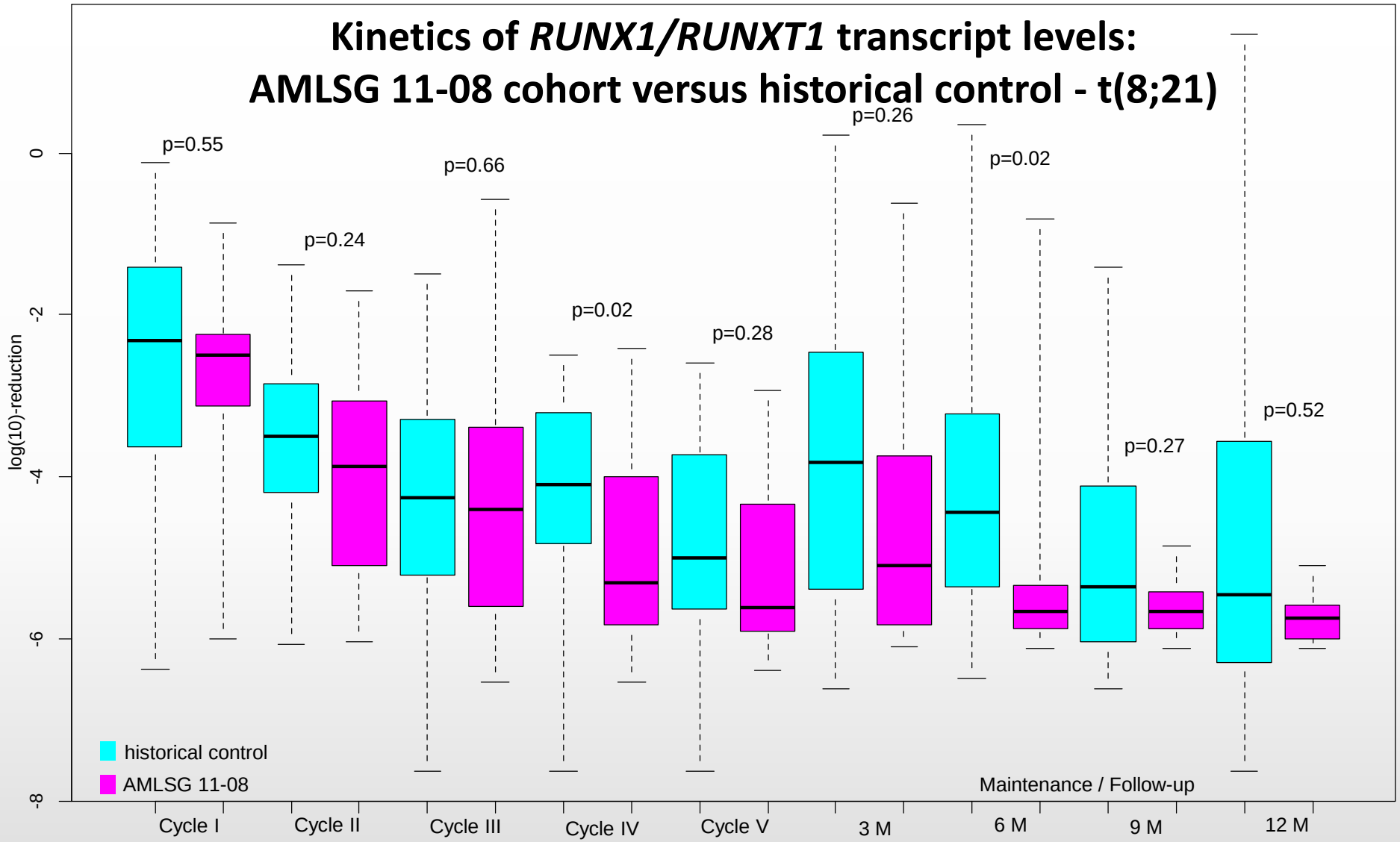


All adult patients eligible for intensive therapy, no upper age limit

* Cytarabine: 18-60yrs: 3g/m^2, q12hr, d1-3; >60yrs: 1g/m^2, q12hr, d1-3

ClinicalTrials.gov NCT02013648

Kinetics of *RUNX1/RUNXT1* transcript levels: AMLSG 11-08 cohort versus historical control - t(8;21)



N	34	28	34	25	35	24	33	20	26	17	27	15	23	11	16	11	16	10
Median	1861	680	62	26	28	12	32	0	8	0	21	0	17	0	0	0	0	0
Negative, n	1	1	3	7	9	9	5	12	12	11	9	8	11	9	9	11	10	10
Negative %	3.0	3.6	8.8	28.0	25.7	37.5	15.2	60.0	46.2	35.3	66.7	46.7	47.8	81.2	56.3	100	62.5	100

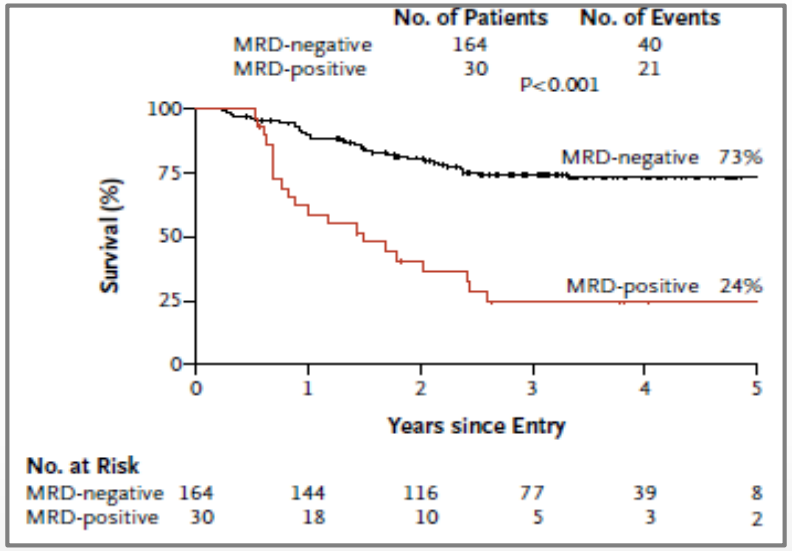
Paschka P et al., Leukemia 2018

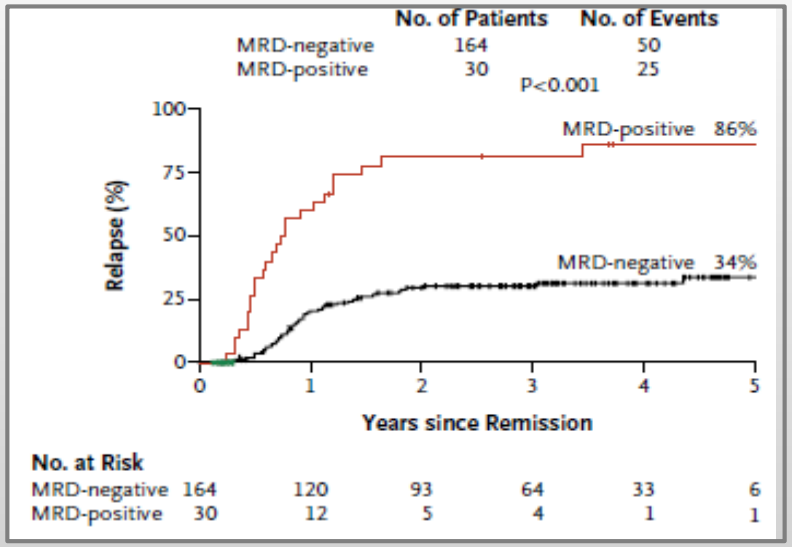
Prognostic impact of MRD in *NPM1* mutated AML

- *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 et al., N Engl J Med 2016; 374(5):422-33;* [n=437]

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)



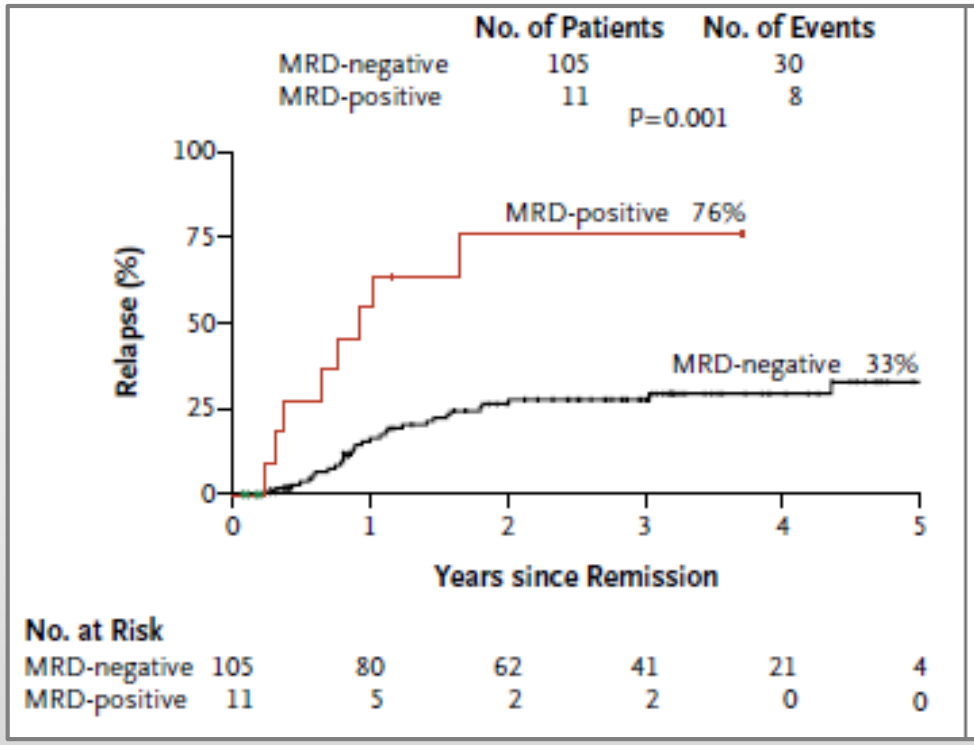


Ivey A et al. N Engl J Med 2016

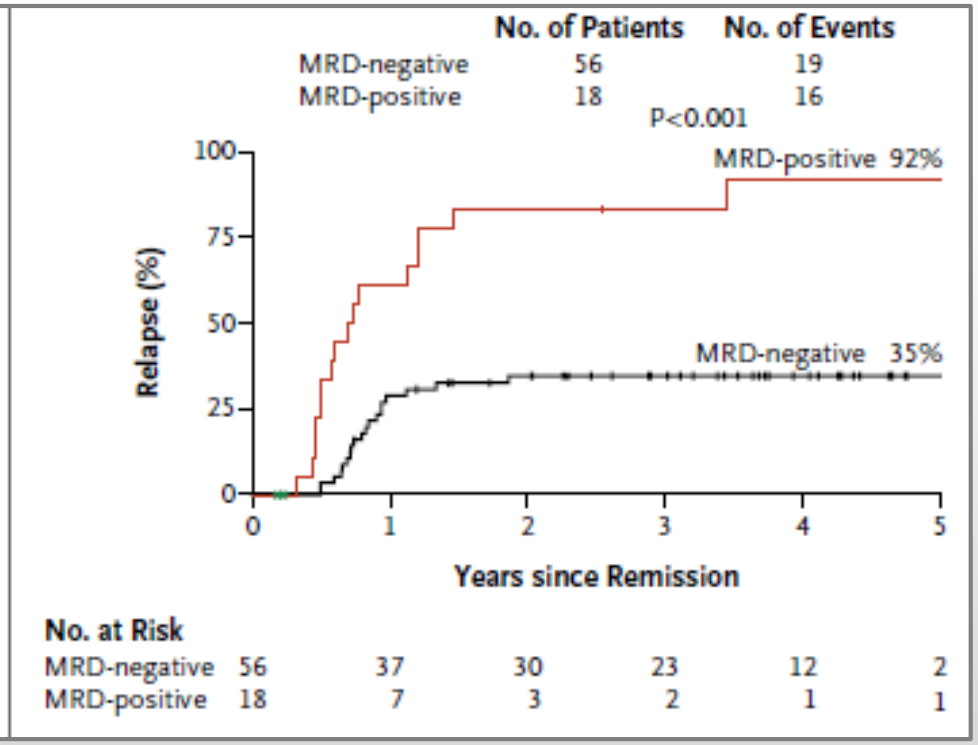
Prognostic impact of MRD in *NPM1* mutated AML

Impact of concurrent *FLT3*-ITD mutation

Relapse in pts without *FLT3*-ITD



Relapse in pts with *FLT3*-ITD

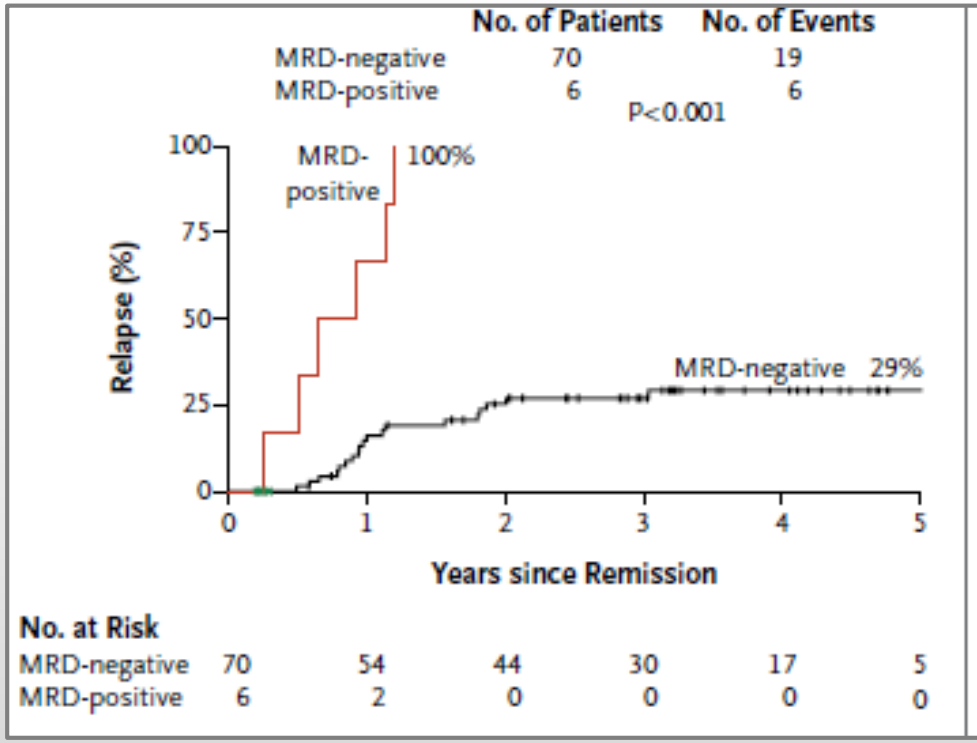


Ivey A et al. N Engl J Med 2016

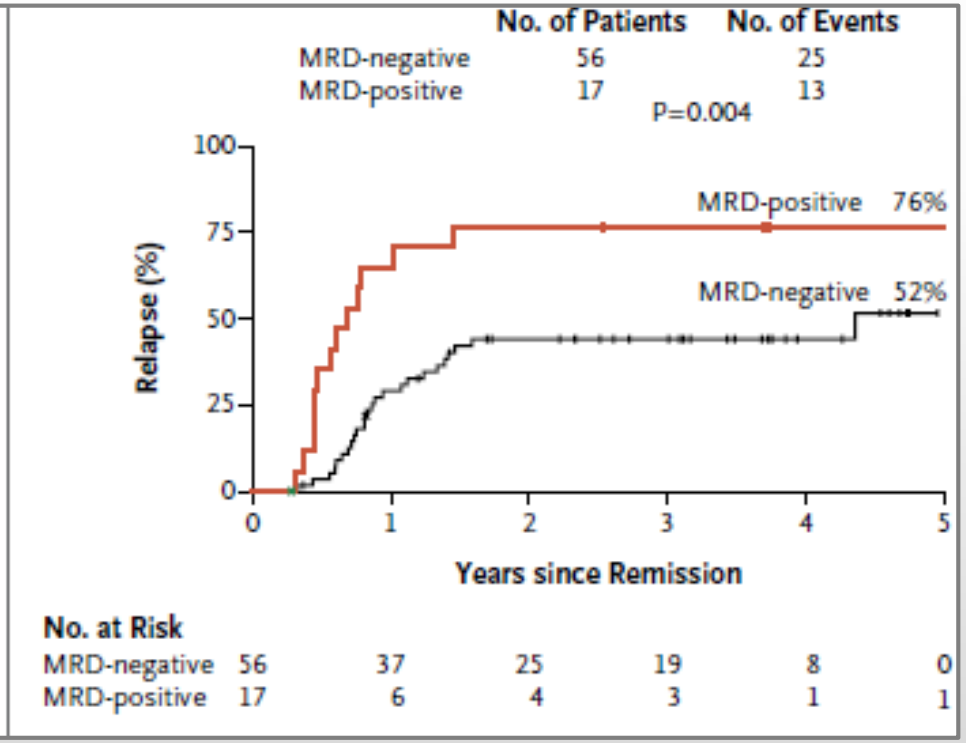
Prognostic impact of MRD in *NPM1* mutated AML

Impact of concurrent *DNMT3A*^{mut}

Relapse in pts without *DNMT3A*^{mut}



Relapse in pts with *DNMT3A*^{mut}

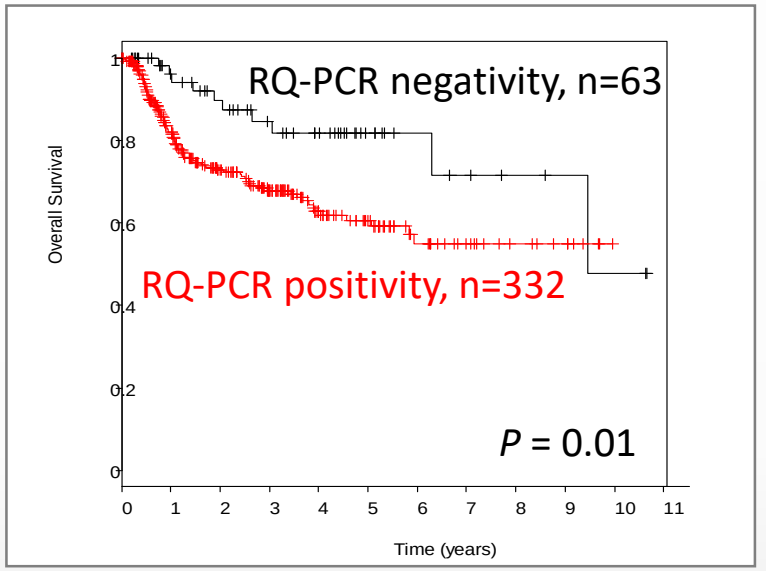


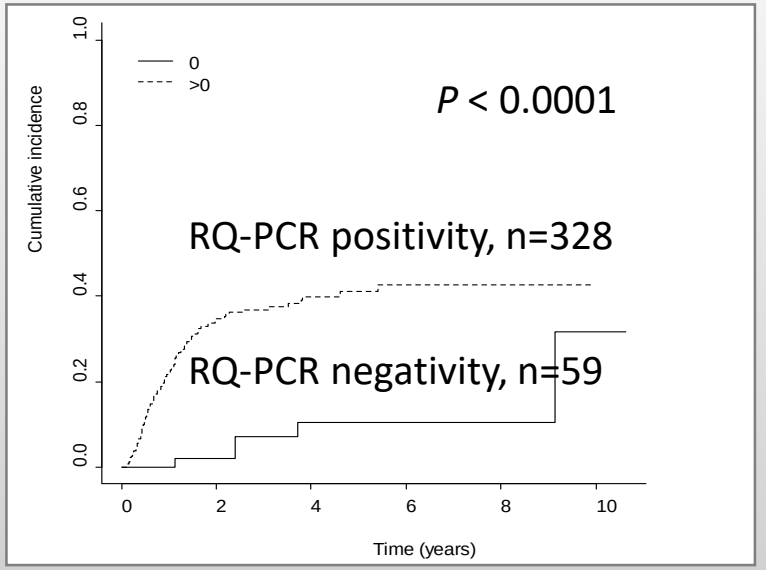
Ivey A et al. N Engl J Med 2016

Prognostic impact of MRD in *NPM1* mutated AML

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

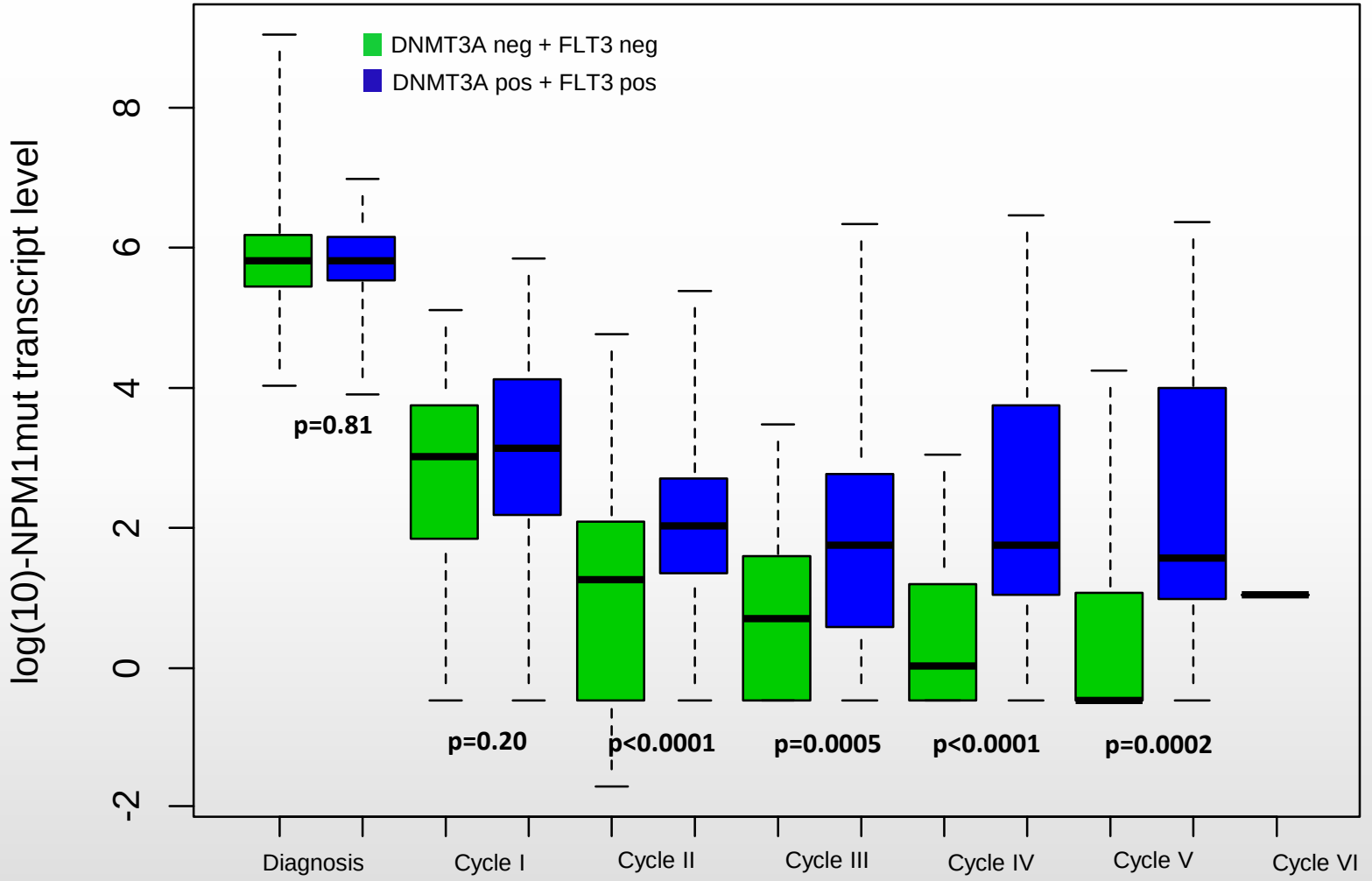
- Retrospective/since 2008 prospective study on 611 *NPM1*^{mut} adult AML pts enrolled in one of 4 AMLSG treatment trials; median follow-up for all patients/trials: 3.2 years
- 6339 BM/PB (3527/2812) samples were analyzed by RQ-PCR after each treatment cycle and during follow-up; sensitivity 10^{-5} 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 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	---

Kapp-Schwoerer S et al.,
ASH meeting 2017, #183

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

After 2 cycles of therapy

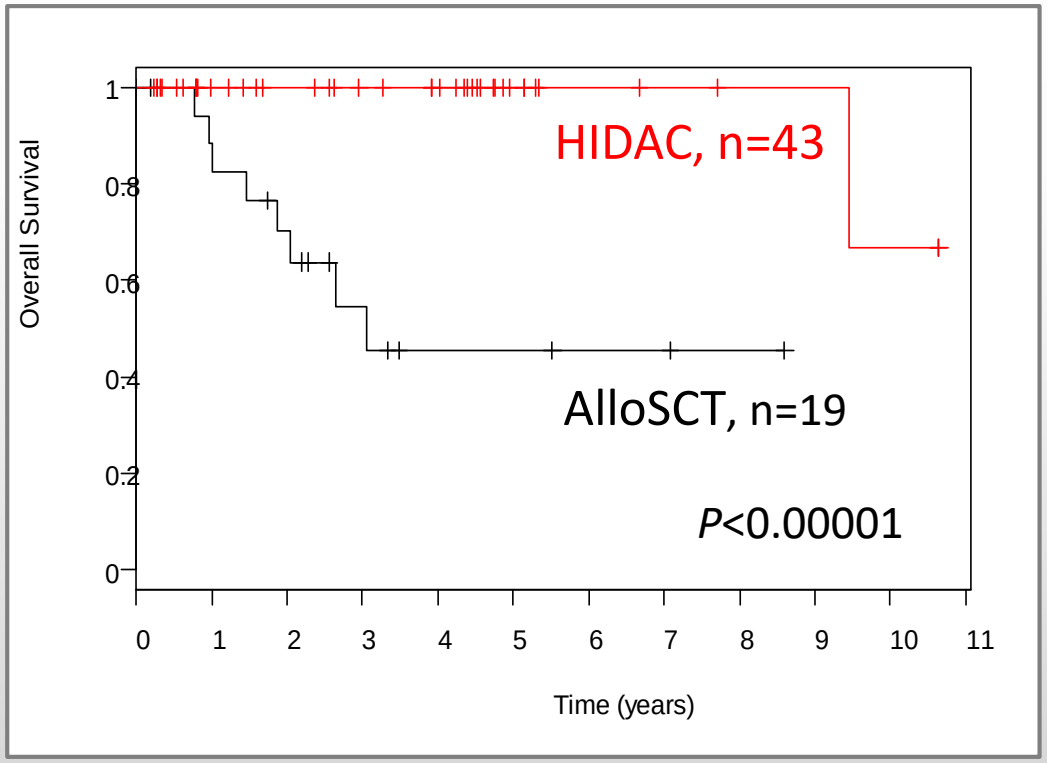
Genotype	*NPM1*^{mut} *FLT3*-ITD WT *DNMT3A* WT	*NPM1*^{mut} *FLT3*-ITD^{mut} *DNMT3A* WT	*NPM1*^{mut} *FLT3*-ITD WT *DNMT3A*^{mut}	*NPM1*^{mut} *FLT3*-ITD^{mut} *DNMT3*^{mut}
RQ-PCR negative (n)	30 (26%)	14 (25%)	10 (8%)	6 (8%)
RQ-PCR positive (n)	84 (74%)	41 (75%)	110 (92%)	65 (92%)
% negative	**26%**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

***P*=0.0002**

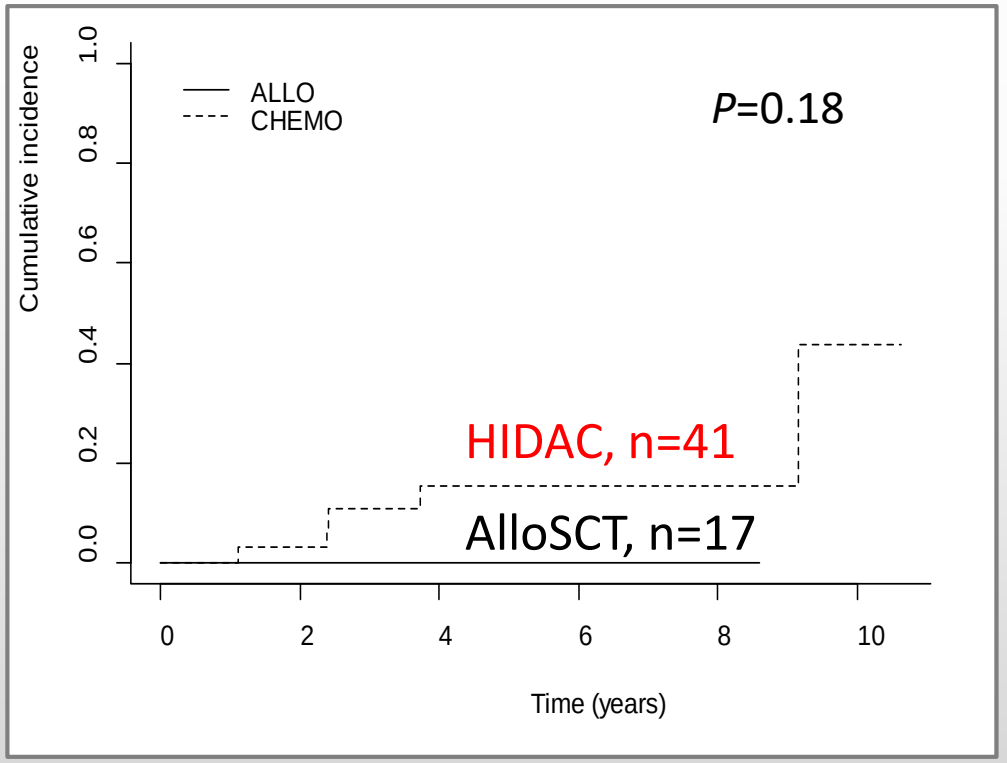
Kapp-Schwoerer S et al., unpublished data

Impact of MRD-negativity in BM after two cycles of intensive treatment on type of post-remission therapy

Overall survival



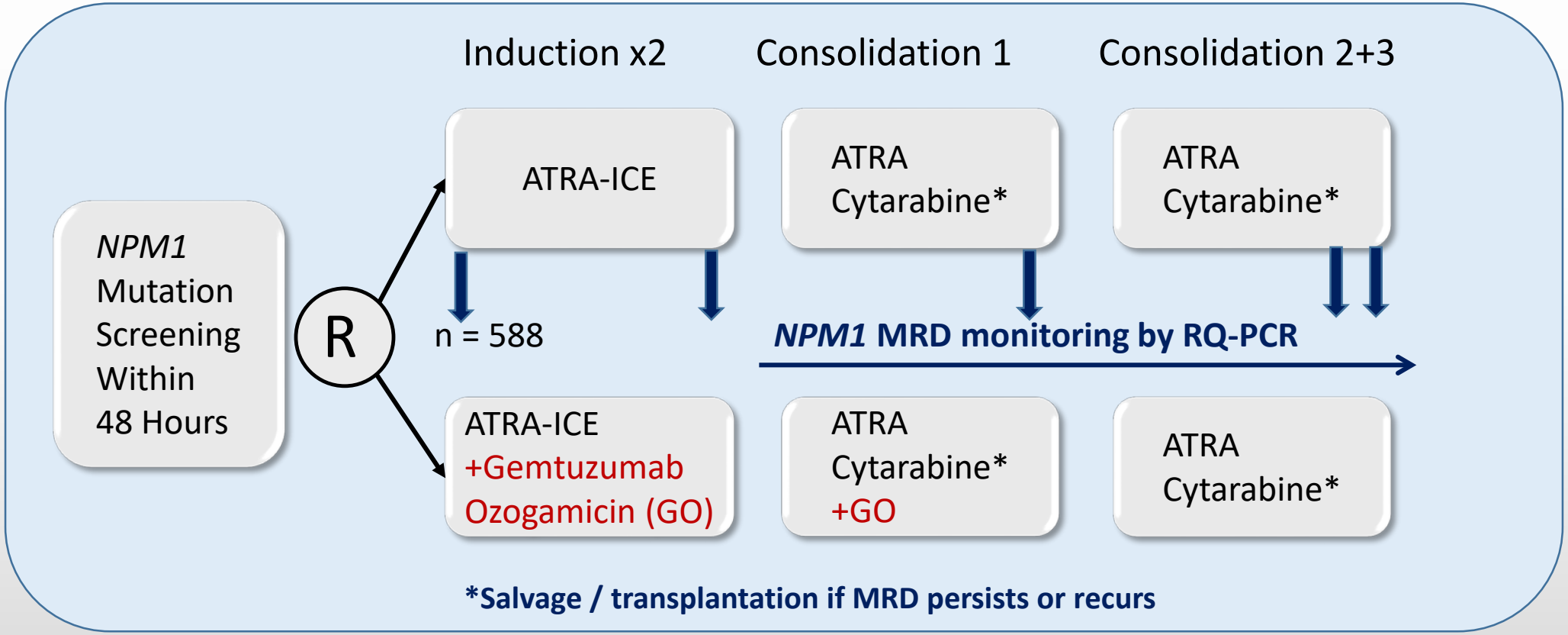
Cumulative incidence of relapse



Kapp-Schwoerer S et al., EHA 2018, PS973

Phase III Study of Chemotherapy in Combination with ATRA with or without Gemtuzumab Ozogamicin (GO) in *NPM1*^{mut} AML patients

[AMLSG 09-09 Trial]



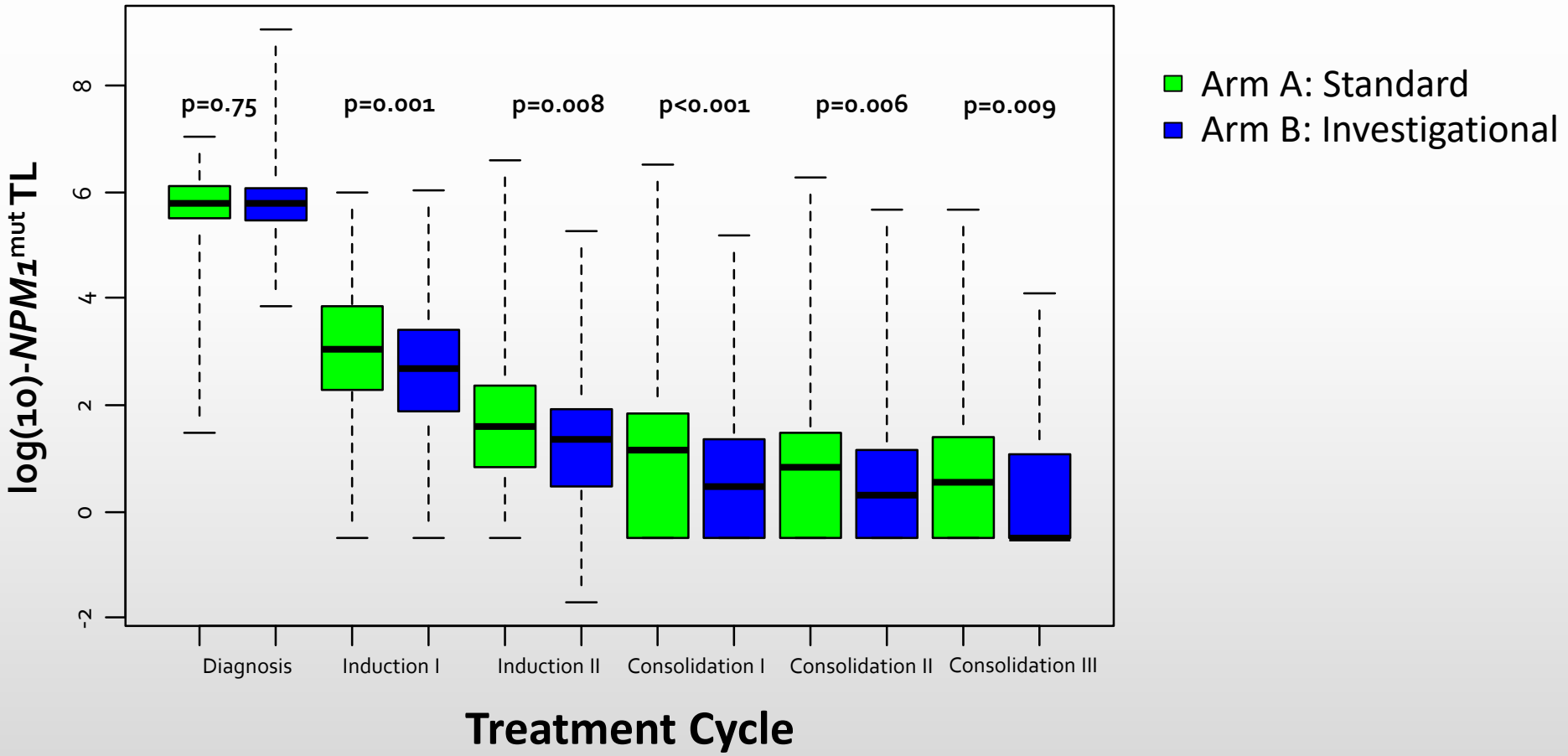
All adult patients eligible for intensive therapy, no upper age limit

* Cytarabine: 18-60yrs: 3g/m^2, q12hr, d1-3; >60yrs: 1g/m^2, q12hr, d1-3

PI: H. Döhner; supported by Else Kröner-Fresenius-Foundation & Pfizer

STUDY AML GROUP

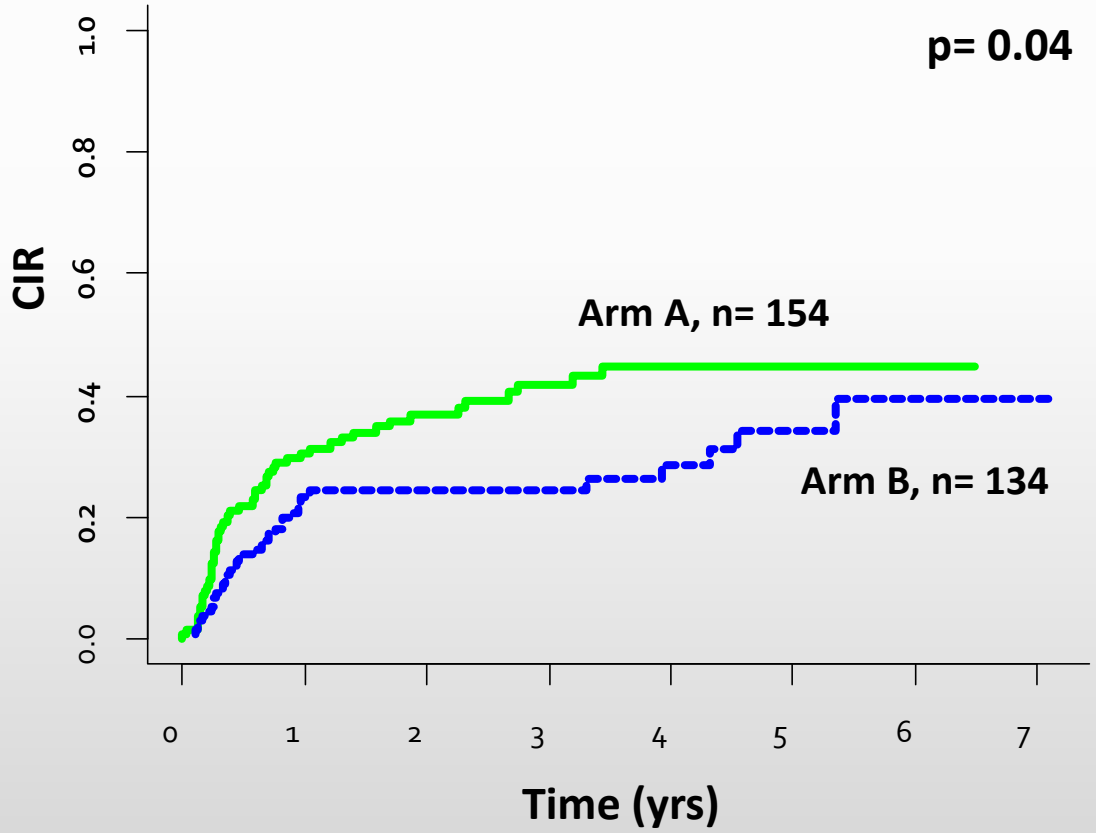
Impact of GO on kinetics of *NPM1*^{mut} transcript levels



Kapp-Schwoerer S et al., oral presentation ASH 2018, #991

Impact of GO on MRD at the end of treatment

BM samples (n=288); all pts in CR



Achievement of RQ-PCR negativity in BM

	Arm A	Arm B	p
RQ-PCR neg; n (%)	63/154 (41%)	74/134 (55%)	0.02

Kapp-Schwoerer S et al., oral presentation ASH 2018, #991

How to implement MRD detection in clinical AML trials

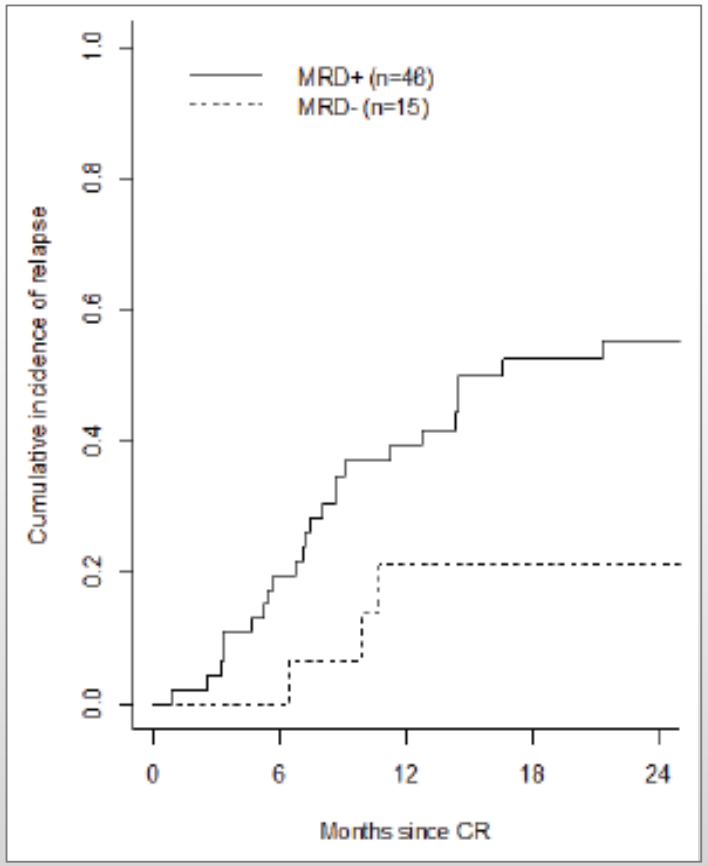
Lambert J et al. Oncotarget 2014; 5(15):6280-8

- Evaluation of the prognostic significance of MRD levels in adult AML pts treated in the randomized gemtuzumab ozogamicin (GO) ALFA-0701 trial
- 79 pts with monitorable *NPM1*^{mut} (GO-arm n=42, control arm n=37)
- BM/PB samples at diagnosis, after induction therapy and after each consolidation course
- 61 pts achieved CR and were subsequently tested for MRD (GO-arm n=33, control arm n=28)
- cDNA based RQ-PCR assay
- MRD positivity: > 0.1% in BM sample

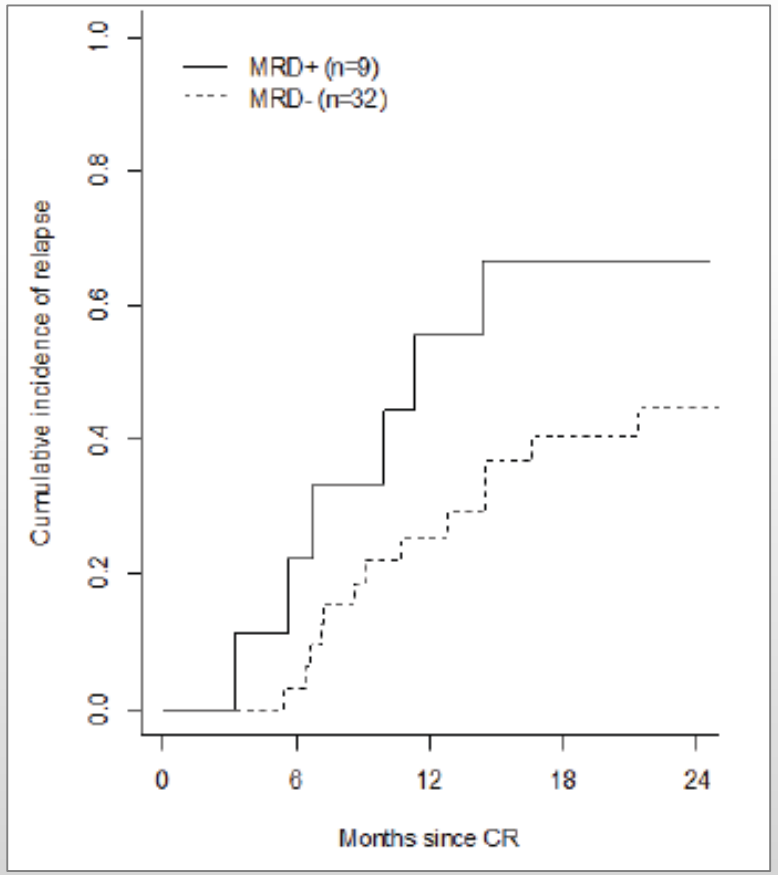
How to implement MRD detection in clinical AML trials

Lambert J et al. Oncotarget 2014; 5(15):6280-8

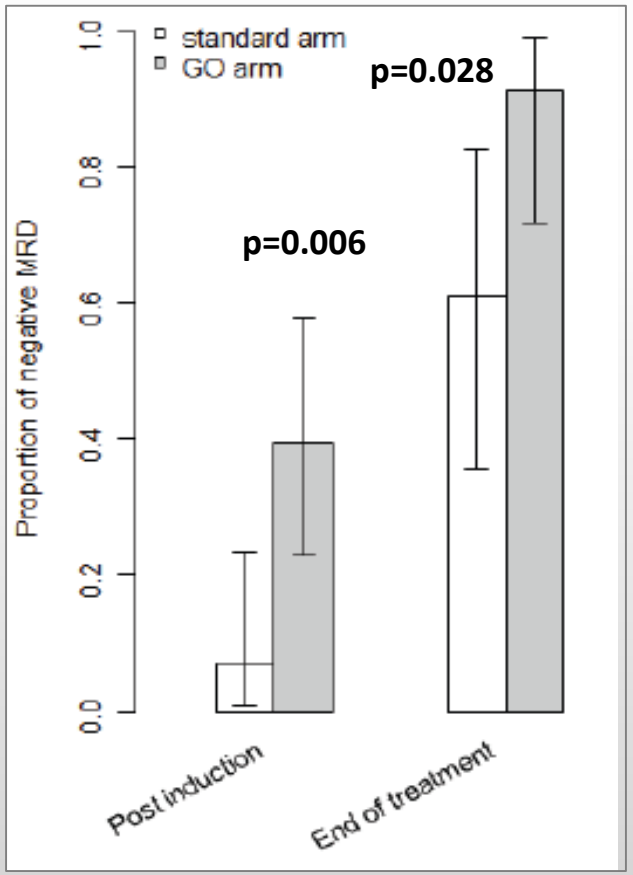
CIR after double induction



CIR at the end of treatment



Effect of GO on MRD



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

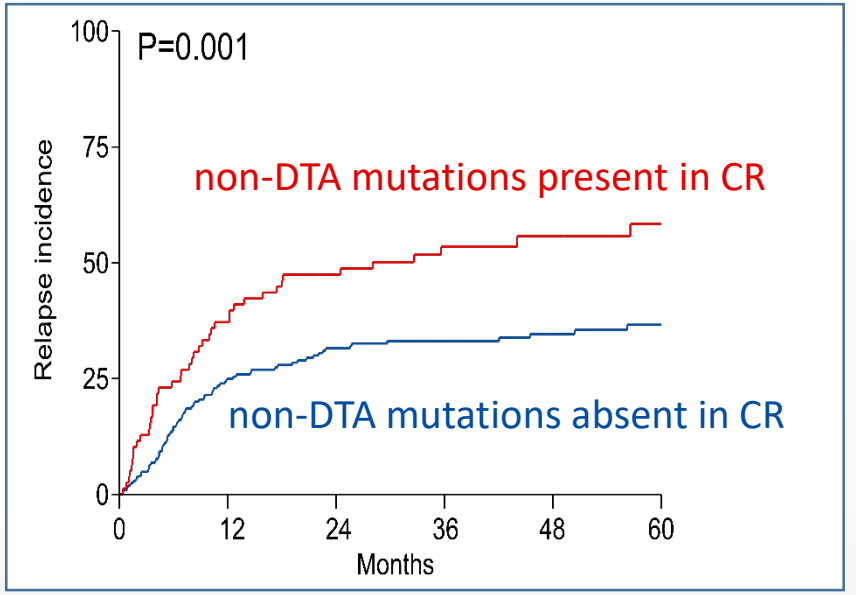
ABL1	DNMT3A	KDM6A	RAD21
ASXL1	ETV6/TEL	KIT	RUNX1
ATRX	EZH2	KRAS	SETBP1
BCOR	FBXWF	MLL	SF3B1
BCORL1	FLT3	MPL	SMC1A
BRAF	GATA1	MYD88	SMC3
CALR	GATA2	NOTCH1	SRSF2
CBL	GNAS	NPM1	STAG2
CBLB	IDH1	NRAS	TET2
CDKN2A	IDH2	PDGFRA	TP53
CEBPA	IKZF1	PHF6	U2AF1
CSF3R	JAK2	PTEN	WT1
CUX1	JAK3	PTPN11	ZRSR2

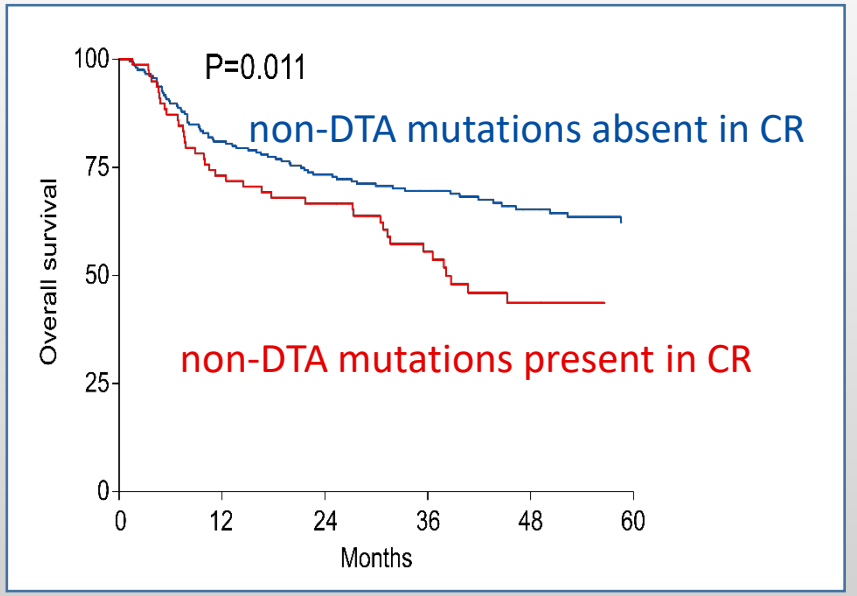
Illumina TruSight Myeloid Panel; mean coverage ~3500x

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

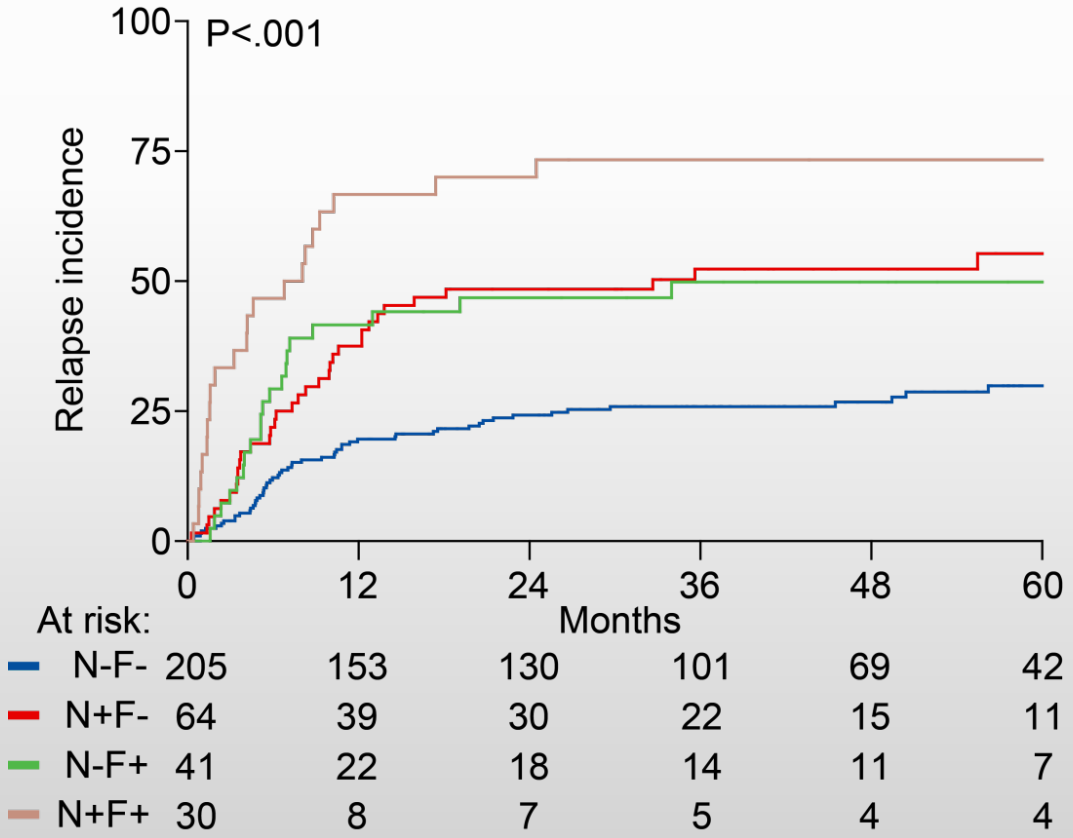
MRD monitoring by next generation sequencing (NGS)

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

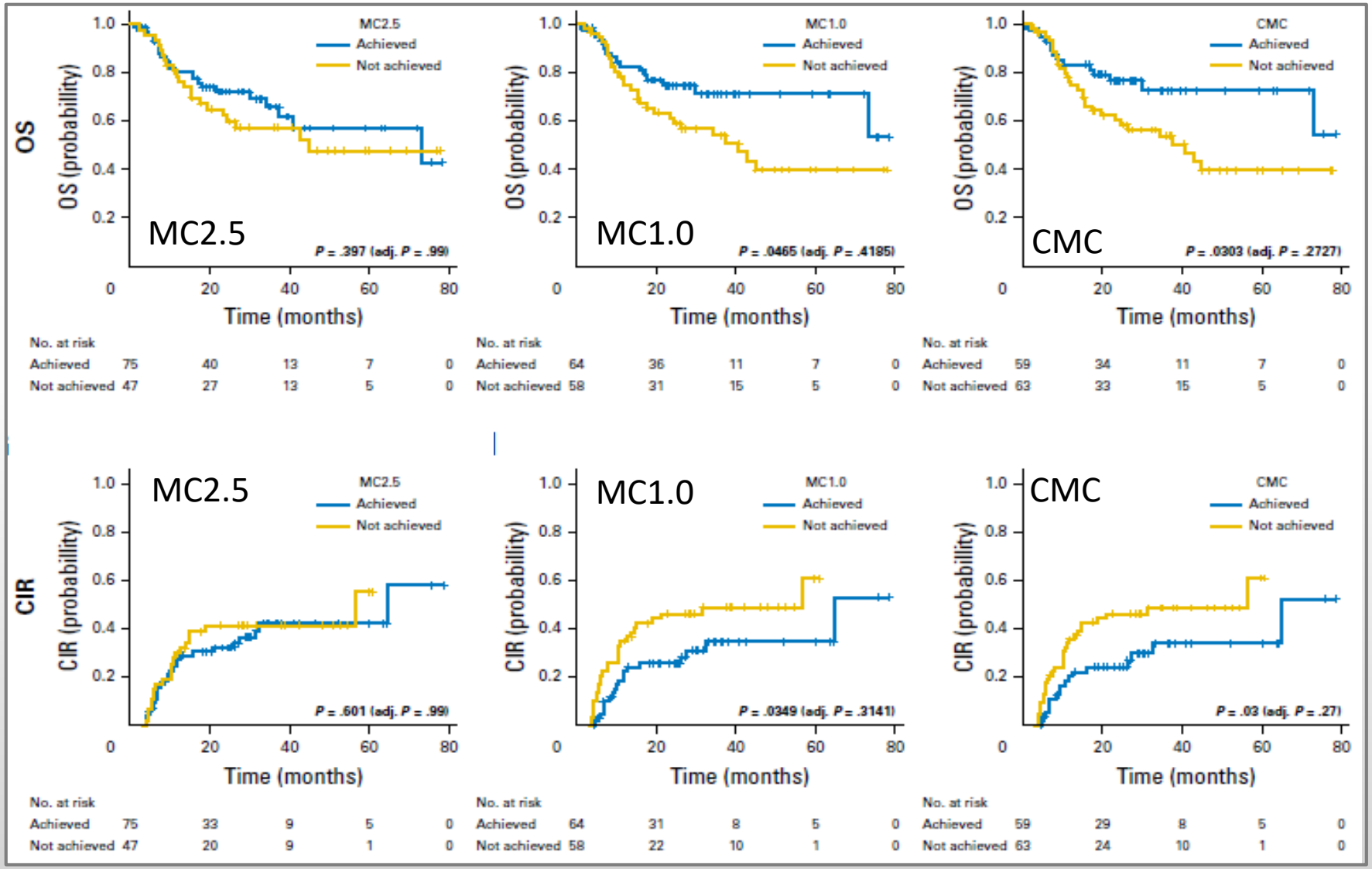


MRD monitoring by next generation sequencing (NGS)

- 131 AML pts (39 to 55 years) with intensive induction therapy and achievement of morphologic CR at day 30
- Detection of mutations in paired BM samples (Dx/CR) by targeted capture deep sequencing (coverage at DX 257 x/ in CR 575 x)
- Definition of three levels of mutation clearance (MC) of residual mutations in CR on the basis of VAF:
    - MC2.5: VAF < 2.5%
    - MC1.0: VAF < 1%
    - CMC: complete mutation clearance
- Correlation of mutation clearance with clinical endpoints (EFS, OS, CIR)

Morita K et al., J Clin Oncol; 2018

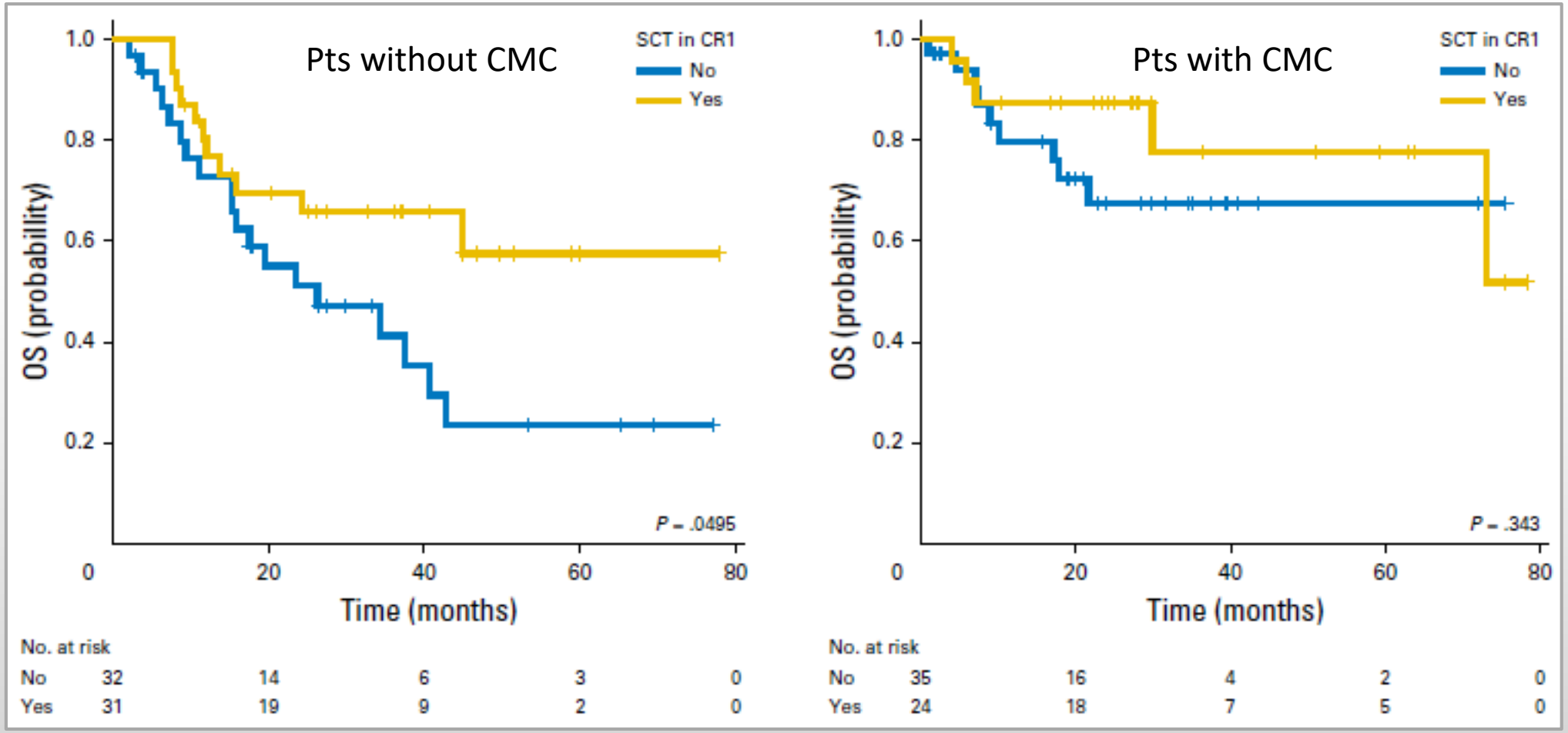
MRD monitoring by next generation sequencing (NGS)



Morita K et al., J Clin Oncol; 2018

MRD monitoring by next generation sequencing (NGS)

Prognostic impact of allogeneic stem cell transplantation in 1.CR



Morita K et al., J Clin Oncol; 2018

ELN recommendations for MRD assessment

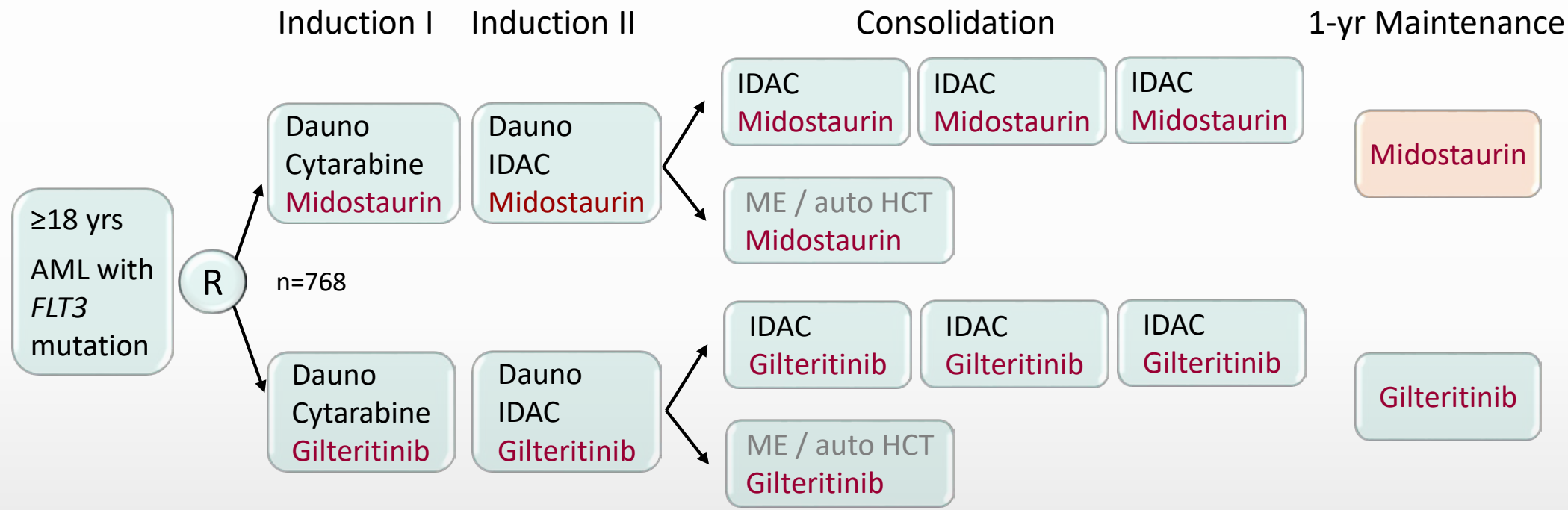
- Suitable markers for MRD: *NPM1, RUNX1-RUNXT1, CBFB-MYH11, PML-RARA; DNMT3A, ASXL1* or *TET2* are NOT usable
- Relevant MRD time points: after 2 treatment cycles, at the end of treatment (EOT); 3 monthly for 24 months after EOT
- Definitions for molecular remission, molecular progression and molecular relapse
- Molecular and/or MFC MRD should be integrated into all clinical trials at all times of evaluation of response, using the technical ELN guidelines

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”

Midostaurin vs Gilteritinib plus chemotherapy for AML with *FLT3* mutation – HOVON 156 / AMLSG 28-18



- Assessment of measurable residual disease (MRD) by RT-qPCR (*NPM1*, *CBF*), next-generation sequencing (NGS), and multiparameter flow cytometry (MFC) of leukemia-associated immunophenotypes (LAIP)
- Centralized diagnostic assessment (Ulm, Rotterdam, Amsterdam) – harmonized assays
- CR_{MRD-} as secondary endpoint

HCT, hematopoietic cell transplantation; IDAC, intermediate-dose cytarabine; ME, mitoxantrone, etoposide

Summary and Conclusions I

- Most of the studies were performed retrospectively >>> patient selection according 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/RQ-PCR-negativity was evaluated after 2 cycles of therapy or at the end of treatment in patients in 1.CR
- Here, achievement of MRD-negativity or significant reduction of transcript levels /mutations by RQ-PCR/NGS was associated with reduced relapse risk and improved survival
- However, a significant proportion of patients do not achieve MRD-negativity in BM at this early time point in particular when highly sensitive assays are used

Summary and Conclusions II

- MRD kinetics allow monitoring of treatment effects
- 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
- MRD monitoring (molecular / MCF) should be included in all clinical trials

S. Cocciardi
A. Corbacioglu
J. Herzig
S. Kapp-Schwoerer
J. Krönke
N. Jahn
E. Panina
L. Schmalbrock
D. Späth
F. Theis
F. Stegelmann
V. Teleanu

V. Gaidzik
P. Paschka
F. Rücker
H. Döhner
Ulm University

L. Bullinger
A. Dolnik
T. Blätte
Charité, Universitätsmedizin Berlin

M. Heuser
G. Göhring
F. Thol
B. Schlegelberger
A. Ganser
MHH, Hannover

DFG Deutsche Forschungsgemeinschaft

STUDY AML GROUP

SFB 1074

SFB 1074 Experimental Models and Clinical Translation in Leukemia

ulm university universität uulm