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Is it time for MRD-based management of AML?

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MEMORIAL BAHÇELİEVLER HASTANESİ-HEMATOLOJİ KLİNİĞİ

21 September 2018 – Turkish Republic of Northern Cyprus

Relapse is the most important problem in adult AML

- More than %50 of patients with AML will relapse after achieving CR
- Whereas risk assessment has evolved to adopt cytogenetic and molecular profiling, response criteria are still largely determined by bone marrow morphologic assessment and peripheral cell count recovery (assessment of CR is traditionally defined as <5% blasts in the BM)
- Pretreatment risk factors (age, cytogenetic and certain mutations) are used for prediction of relapse.

Current genetic risk classification per ELN-2017 and NCCN-2017 guidelines

Risk category*	ELN criteria[10]	NCCN criteria[6]
Favorable	t(8;21)(q22;q22.1); *RUNX1-RUNX1T1*	Core binding factor: inv(16)†,‡ or t(16;16)†,‡ or t(8;21)†,‡ or t(15;17)‡
	inv(16)(p13.1;q22) or t(16;16)(p13.1;q22); *CBFB-MYH11*	Normal cytogenetics: *NPM1* mutation in absence of *FLT3*-ITD or isolated biallelic (double) *CEBPA* mutation
	Mutated *NPM1* without *FLT3*-ITD or with *FLT3*-ITD^{low}§	
	Biallelic mutated *CEBPA*	
Intermediate	Mutated *NPM1* and *FLT3*-ITD^{high}§	Normal cytogenetics
	Wild-type *NPM1* without *FLT3*-ITD or with *FLT3*-ITD^{low}§ (without adverse-risk genetic lesions)	+8 alone
	t(9;11)(p21.3;q23.3); *MLLT3-KMT2A*‖	t(9;11)
	Cytogenetic abnormalities not classified as favorable or adverse	Other nondefined
		Core binding factor with *KIT* mutation
Poor/adverse	t(6;9)(p23;q34.1); *DEK-NUP214*	Complex (≥3 clonal chromosomal abnormalities)
	t(v;11q23.3); *KMT2A* rearranged	Monosomal karyotype
	t(9;22)(q34.1;q11.2); *BCR-ABL1*	−5, 5q−, −7, 7q−
	inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2); *GATA2,MECOM(EVI1)*	11q23 – non t(9;11)
	−5 or del(5q); −7; −17/abn(17p)	inv(3), t(3;3)
	Complex karyotype,¶ monosomal karyotype#	t(6;9)
	Wild-type *NPM1* and *FLT3*-ITDhigh	t(9;22)
	Mutated *RUNX1***	Normal cytogenetics: with *FLT3*-ITD mutation††
	Mutated *ASXL1***	*TP53* mutation
	Mutated *TP53*‡‡	

Döhner H et al. Blood 2017; 129(4): 424-47
NCCN Guidelines: acute myeloid leukemia, version 1.2018

Integrated risk profiles-based ELN recommendations for allo-HCT in AML CR1

AML risk group‡	AML risk assessment criteria at diagnosis	MRD after cycle 2	Risk of relapse following consolidation approach		Prognostic scores for NRM that indicate alloHSCT as preferred consolidation		
			Chemotherapy or autoHSCT (%)	AlloHSCT (%)	EBMT score[52]	HCT-CI score[53]	NRM risk (%)
Good	–t(8;21) or *AML1-ETO*, WBC <20 –inv16/t(16;16) or *CBFB-MYH11* –CEBPA-biallelic mutant-positive –FLT3-ITD-negative/NMP1-positive	Positive or negative	35-40	15-20	NA (≤1)	NA (<1)	10-15
Intermediate	–CN –X –Y, WBC <100, CRe –t(8;21) or *AML1-ETO* plus WBC >20 or mutant KIT	Negative	50-55	20-25	≤2	≤2	<20-25
Poor	–CN –X –Y, WBC <100, CRe –t(8;21) or *AML1-ETO*, WBC >20 and/or mutant KIT –CN –X –Y, WBC <100, no CRe –CN –X –Y, WBC >100 –CA, but non-CBF, MK-negative, no abn3q26	Positive Positive Negative Negative	70-80	30-40	≤3-4	≤3-4	<30
Very poor	–CN –X –Y, WBC >100 –CA, but non-CBF, MK-negative, no abn3q26, EVI1-negative –MK-positive –abn3q26 –Non-CBF, EVI1-positive –Non-CBF with mutant p53, or –mutant RUNX1, or mutant ASXL1 –or biallelic FLT3-ITD with –FLT3-ITD:FLT3 WT ratio of >0.6	Positive Positive Positive or negative	>90	40-50	≤5	≤5	<40

Cornelissen JJ et al. Blood 2016; 127(1): 62-70

Definition of MRD

Risk of relapse is associated with post-CT persistence of minimal residual disease

MRD is defined as existence of leukemic cells below morphologic detection limits

Detection of any disease with sensitive technics is in fact associated with poor prognosis

Persistence of leukemia even in morphologic CR should not be regarded as minimal

MRD better defined as measurable residual disease

MRD in routine clinical practice

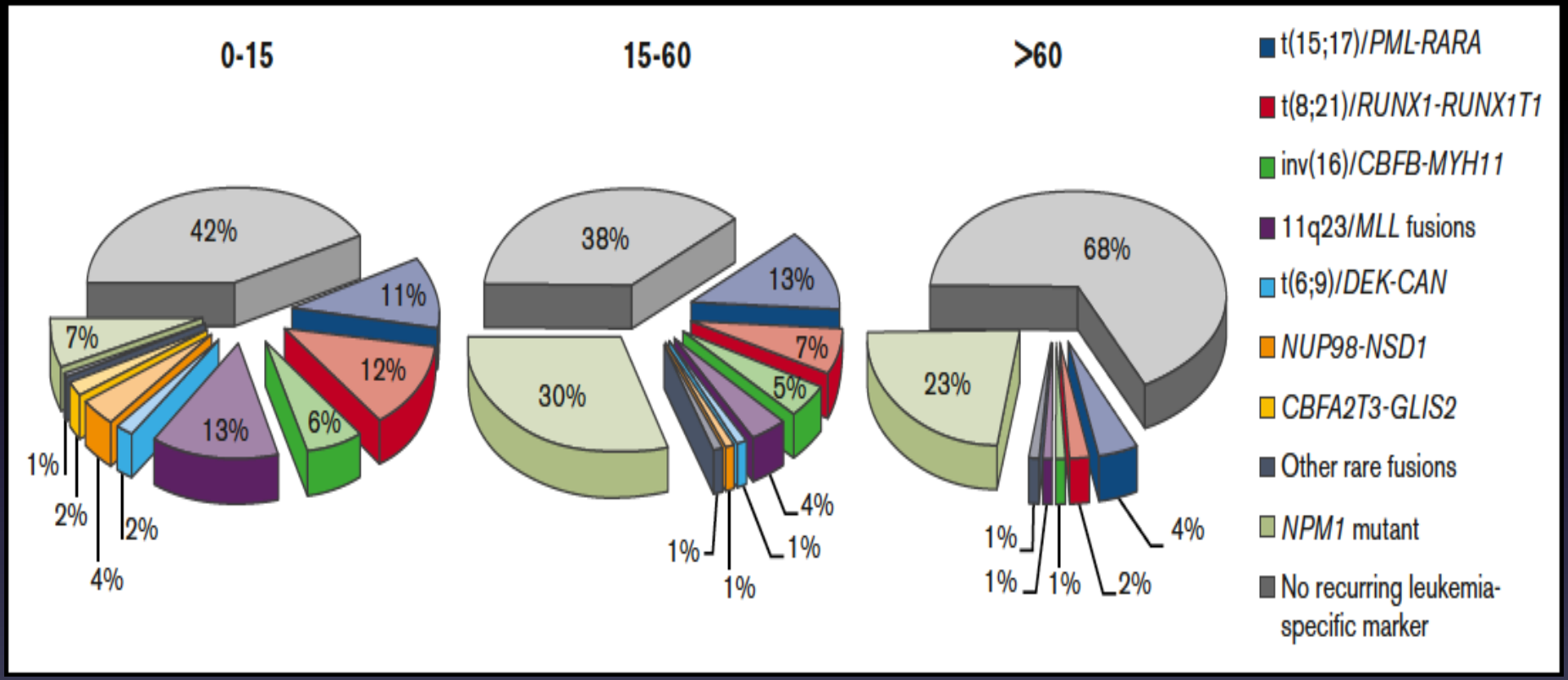
- ALL
- APL
- CML

Comparison of major MRD evaluation methods

Modality	Sensitivity	Pros	Cons
Real-time quantitative PCR (RQ-PCR)	10^{-4} to 10^{-5}	Generally affords a higher sensitivity compared to flow cytometry A sizeable percentage of patients with AML have a mutated gene (*NPM1*, *RUNX1*, *MYH11*, etc.) that is stable and suitable for tracking Can be run in most laboratories RQ-PCR capacity	Marked heterogeneity of mutations necessitates an array of standardized assays No single target sequence (i.e., *PML-RARA* in M3 AML) Relative quantification approach (needs reference standard curve)
Multiparameter flow cytometry (MFC)	10^{-3} to 10^{-4}	Near universal applicability in most cases with suitable LAIP Allows detection of cells with leukemia stem-cell phenotype Can often be run in a single day	Antigenic shifts and emergence of initially minor subpopulations excluded by the initial assay may hinder follow-up sensitivity Lack of standardization between laboratories Requires highly trained pathologists
Next-generation sequencing (NGS)	10^{-3} to 10^{-5}	Can be used in subsets of AML that are not amenable to an established RQ-PCR assay Particularly useful for targets such as FLT3-ITD, which can have mutational shifts between diagnosis and relapse	Substantial intrinsic complexity and major costs Broadly applicable NGS-based protocols are still not available in most laboratories
Digital droplet PCR (ddPCR)	10^{-4} to 10^{-5}	No need for a standard curve Easier data interpretation Less susceptible to potential PCR performance inhibitors	Has not been adequately studied prospectively Higher costs

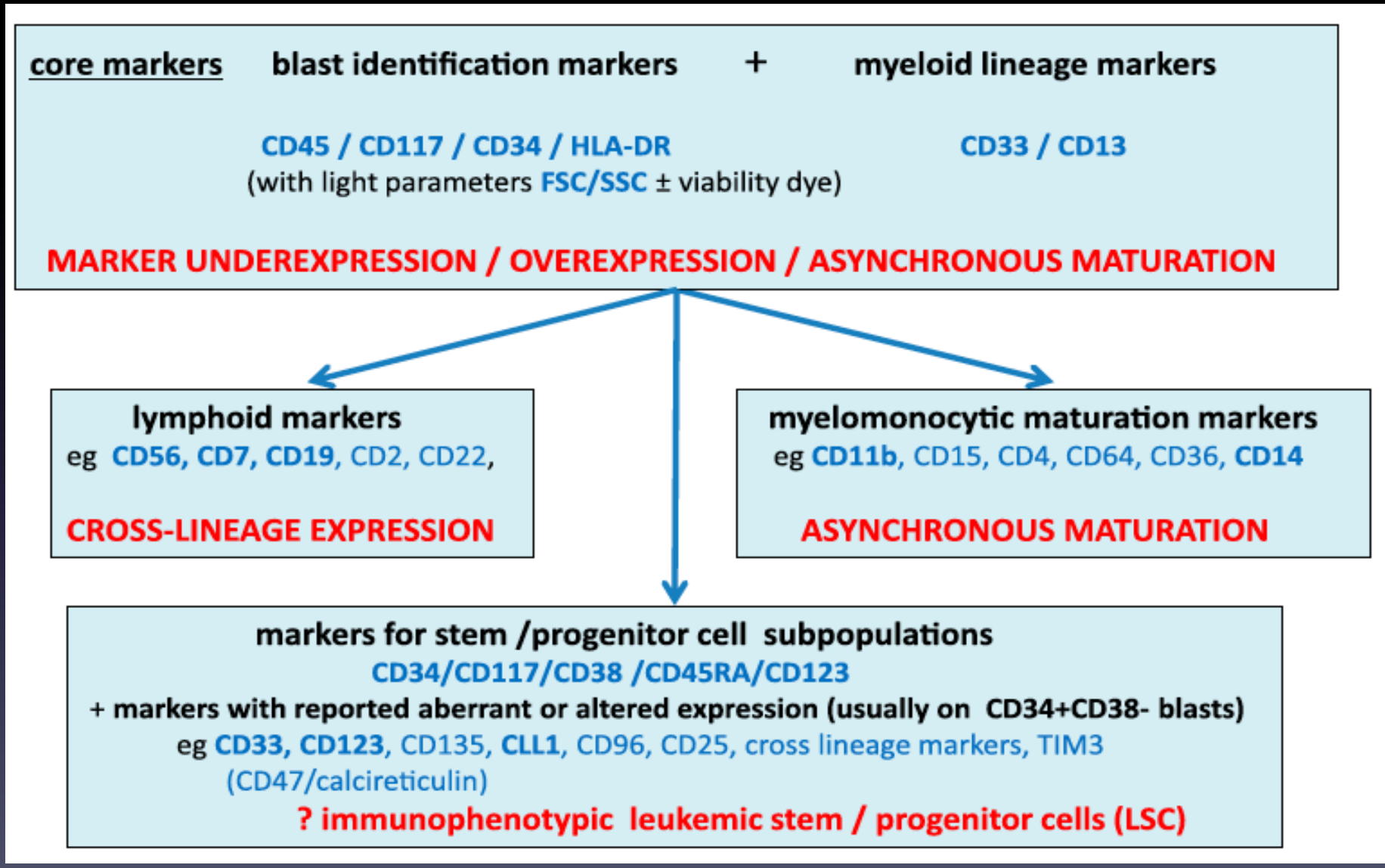
Coltoff A et al. Ann Hematol 2018; 97(7): 1155-67

AML specific targets detected by qRT-PCR



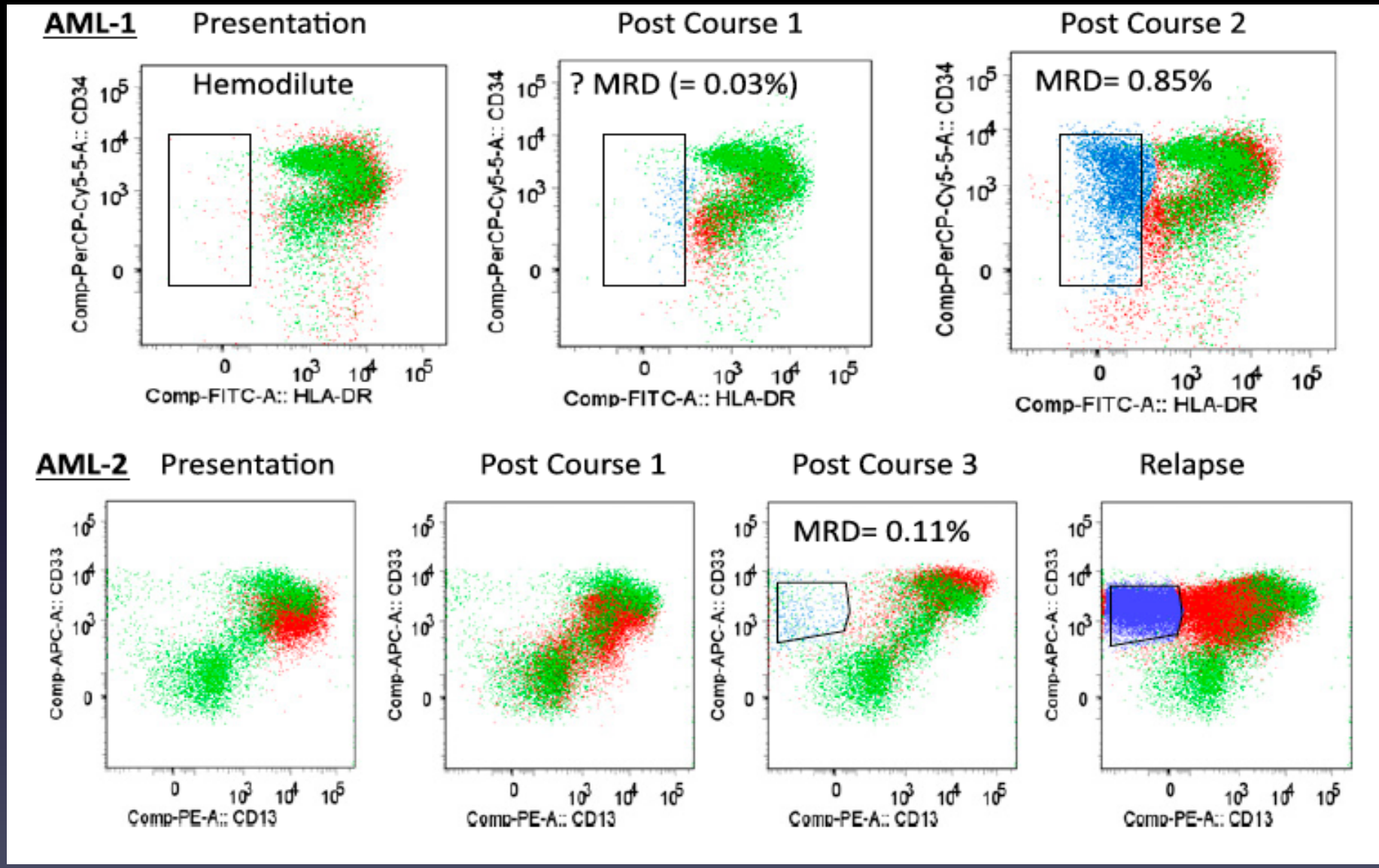
Grimwade D et al. Blood 2014; 124(23): 3345-55

Screening antibody panel and selecting Ag combinations at diagnosis (leukemia-associated immunophenotype-LAIP)



Grimwade D et al. Blood 2014; 124(23): 3345-55

Screening for established immunophenotypic profiles to distinguish abnormal leukemic cells from normal cells (different from normal-DfN)



Grimwade D et al. Blood 2014; 124(23): 3345-55

MFC-based MRD Studies in AML-1

Reference	Multicenter, yes/no	Study population, adult/children	% LAIP	Number of patients	MRD measurement following	Cutoff MRD level			Univariate analysis significant for	Multivariate analysis significant for	Study details
						I	C	Post-Tx			
81	N	A	46	53	I, C	<0.05%	0.2%		RFS, OS	RFS	
82	Y	A	70	56	I, C	0.045%	0.035%		I;-C: RFS, OS	I:-C: RFS, OS	
83		A	75	126	I	<0.01%, 0.01%-0.1% 0.1%-1% >1%	- - - -		RFS, OS	RFS	MRD >1%: 3 y RR: 85% MRD 0.1%-1.0%: 3 y RR: 45% MRD 0.01%-0.1%: 3 y RR: 14% MRD < 0.01%: 3 y RR: 0%
84	Y	Ch	?	252	I_1	0.5%			RFS, OS	RFS, OS	3 y OS 69% (MRD neg) vs 41% (MRD pos)
85	Y	A	100	106	Day 16	Log difference 2.11			CR, EFS, RFS, OS	EFS, RFS	
86	N	A	100	62	I, C	Log difference 2.11	Log difference 2.53		I: RFS C: RFS, OS	I: RFS C: RFS	
34	N	A	100	72	I_1, I_2, C, PBSCT	I_1: 1% I_2: 0.14%	0.11%	0.13%	I_1, I_2, C, PBSCT; RFS, OS	I_1, I_2, C, PBSCT; RFS, OS	
87	Y	A	89	100	I, C	0.035%	0.035%		I and C: RR, RFS, OS	I:-C: RR, RFS, OS	5 y RFS 72% (MRD neg) vs 11% (MRD pos)
88	Y	A, Ch	?	150	Day 15, I, I_2, C	0.1%-2%	0.1%-1.3%		Day 15, I; RFS	—	MRD similar EFS as traditional risk factors
89	Y	A	?	142	I, C	0.035%	0.035%		I and C: RFS, OS	I and C: RFS, OS	5 y RR 60% (MRD pos) vs 16% (MRD neg)
90	N	A	94	54	I, C	0.15%	0.15%		I: RFS, OS C: RFS,OS	I: RFS, OS C:-	

Schuurhuis GJ et al. Blood 2018; 131(12): 1275-91

MFC-based MRD Studies in AML-2

Reference	Multicenter, yes/no	Study population, adult/children	% LAIP	Number of patients	MRD measurement following	Cutoff MRD level			Univariate analysis significant for	Multivariate analysis significant for	Study details
						I	C	Post-Tx			
91	Y	Ch	?	94	I_1, I_2, C, end of Tx	<0.1% 0.1%-0.5% >0.5%			I_1: RFS, OS	I_1: RFS, OS	3 y RFS 64% (MRD pos) vs 14% (MRD neg)
61	Y	Ch	100	188	I_1, I_2, end of Tx	>0%, 0-1%			I_1: OS, RFS I_2: RFS, OS	I_1: OS, RFS I_2: RFS, RR	RR at 3 y 60% vs 29%
62	Y	Ch	?	203	I_1, I_2, end of Tx		<0.1% 0.1%-1% >1%		I_1: EFS, RFS I_2: EFS, RFS	I_1: EFS, RFS I_2: EFS, RFS	Morphological assessment has limited value in comparison with flow cytometry.
28	Y	A	89	517	I_1, I_2,	<0.1%	<0.1%		I_1: RFS, OS I_2: RFS, OS	I_1: RFS, OS I_2: RFS, OS	Cutoff points between 0.05 and 0.8 are all significant.
30	Y	A	93	427	I_1, I_2,	<0.1%	<0.1%			I_1: RFS, OS I_2: RFS, OS	3 y OS 38% (MRD pos) vs 18% (MRD neg) after cycle 2
32	N	A, Ch	100	253	Pre-Tx	<0.1%			DFS OS		MRD predictive in CR1 and CR2
92	Y	A	?	210	I, C	0.035%	0.035%		I, C: DFS, OS	I, C: DFS, OS	MRD negativity gives 5 y DFS: 57 vs 13% in elderly AML
76	N	A	100	359	Pre-Tx	0.1%				OS, PFS, RFS	3 y RR 67% (MRD pos) vs 22% (MRD neg)
93	Y	A	100	306	At the time of morphological CR	<0.01% 0.01%-0.1% >0.1%			RFS	RFS	Multivariate analysis revealed MRD, age. and cytogenetics as independent variables. Cytogenetics and MRD are complementary in a scoring system.
94	Y	Ch	78	101	Day 15, pre-C	0.1%	0.1%		Day 15: EFS, OS Pre-C: EFS, OS	Day 15: EFS, OS Pre-C: EFS, OS	EFS at 5 y 65% (MRD neg) vs 22% (MRD pos)
95	Y	Ch (1-21 y)	?	216	I_1, I_2	<0.1%, 0.1%-1% >1%	- -		EFS OS	I_1, I_2: EFS I_1, I_2: OS	I_1: CIR at 3 y 38.6% for MRD pos and 16.9% for MTD neg I_2: 56.3% vs 16.7%
96	N	A	100	241	Pre-Tx	0.1%			DFS, OS, relapse	DFS, OS, relapse	Negative impact of MRD on posttransplant MRD is similar after NMA and MA conditioning.

Schuurhuis GJ et al. Blood 2018; 131(12): 1275-91

MFC-based MRD Studies in AML: Summary

- It is now well established that MRD detected by MFC is an independent prognostic factor for relapse, RFS and OS.
- In younger patients < 65 years of age with AML who received ARA-C plus antracyline based induction and consolidation therapy, MFC-based MRD negative status was identified as the most important independent predictor of RFS and OS.
- Data in older patients with AML have also demonstrated the prognostic impact of MRD monitoring by MFC in patients receiving intensive induction chemotherapy.
- Detection of MFC-based MRD before conditioning was also associated with substantially higher likelihood of relapse and worse OS in allo-HCT setting.

Molecular MRD markers in AML: NPM1

Gene	Number of patients	Time point	PB vs BM	cDNA vs DNA	Favorable prognostic cutoff (proportion of patients)	Associated risk	Sensitivity of the assay
NPM1	194	After 2 cycles of chemotherapy	PB	cDNA	Negative (84.5%)	3-y CIR 30% (vs 82% if positive), 3-y OS 75% (vs 24% if positive)	10^{-5} (range, $10^{-3.7}$ to $10^{-7.1}$)
NPM1	137	After 2 cycles of chemotherapy	BM	cDNA	Negative (19%)	4-y CIR 6.4% (vs 53% if positive), 4-y OS 90% (vs 56% if positive)	10^{-5} to 10^{-6}
NPM1	82	After 2 cycles of chemotherapy	BM	cDNA	Negative (26%)	3-y OS 84% (vs 76% if NPM1/ABL ≤1% vs 47% if NPM1/ABL >1%)	
NPM1	194	At end of treatment	PB	cDNA	Negative (92%)	3-y OS 80% (vs not estimable if positive)	10^{-5} (range, $10^{-3.7}$ to $10^{-7.1}$)
NPM1	131 (for PB)	After 1 or 2 induction cycles	PB	cDNA	≥4log10 reduction (55%)	3-y CIR 20.5% (vs 65.8% if <4log10 reduction); 3-y OS 91%-93% (vs 40.8% if <4log10 reduction)	0.01%
NPM1	129	At end of treatment	BM	cDNA	Negative (48%)	4-y CIR 15.7% (vs 66.5% if positive), 4-y OS 80% (vs 44% if positive)	10^{-5} to 10^{-6}
NPM1	80	At end of treatment	BM	cDNA	Negative (49%)	1-y CIR 37% (vs 63% if NPM1/ABL ≤1% vs 85% if NPM1/ABL >1%); 2-y OS 82% (vs 61% if NPM1/ABL ≤1% vs 45% if NPM1/ABL >1%)	10^{-5}
NPM1	136	In follow-up	BM	cDNA	<200 copies (68% of patients completing chemotherapy)	No relapses occurred.	10^{-5} to 10^{-6}

Schuurhuis GJ et al. Blood 2018; 131(12): 1275-91

Molecular MRD markers in AML: RUNX1-RUNX1T1

Gene	Number of patients	Time point	PB vs BM	cDNA vs DNA	Favorable prognostic cutoff (proportion of patients)	Associated risk	Sensitivity of the assay
RUNX1-RUNX1T1	94	At end of treatment	PB	cDNA	Negative (70%)	4-y CIR 23.6% (vs 50.9% if positive), 4-y OS 96% (vs 63.6% if positive)	10^{-5}
RUNX1-RUNX1T1	120	At end of treatment	BM	cDNA	Negative (49%)	4-y EFS 81% (vs 61% if positive), 4-y OS 93% (vs 67% if positive)	10^{-6}
	94	At end of treatment	BM	cDNA	Negative (30%)	4-y CIR 28.2% (vs 33.8% if positive), 4-y OS 86.4% (vs 87.7% if positive, n.s.)	10^{-5}
RUNX1-RUNX1T1	163	In follow-up	PB	cDNA	<100 copies/10^5 ABL copies (85%)	5-y CIR 7% (vs 100% if ≥100), 5-y OS 95% (vs 59% if ≥100)	10^{-5}
RUNX1-RUNX1T1	163	In follow-up	BM	cDNA	<500 copies/10^5 ABL copies (83.5%)	5-y CIR 7% (vs 100% if ≥500), 5-y OS 94% (vs 57% if ≥500)	10^{-5}

Schuurhuis GJ et al. Blood 2018; 131(12): 1275-91

Molecular MRD markers in AML: CBF-MYH11

Gene	Number of patients	Time point	PB vs BM	cDNA vs DNA	Favorable prognostic cutoff (proportion of patients)	Associated risk	Sensitivity of the assay
CBFB-MYH11	115	At end of treatment	PB	cDNA	<10 copies/10^5 ABL copies (80%)	5-y CIR 36% (vs 78% if ≥10)	10^{-5}
CBFB-MYH11	115	In follow-up	PB	cDNA	<10 copies/10^5 ABL copies (69%)	5-y CIR 7% (vs 97% if ≥10), 5-y OS 91% (vs 57% if ≥10)	10^{-5}
CBFB-MYH11	115	In follow-up	BM	cDNA	<50 copies/10^5 ABL copies (73%)	5-y CIR 10% (vs 100% if ≥50), 5-y OS 100% (vs 25% if ≥50)	10^{-5}

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Molecular MRD markers in AML: PML-RARA

Gene	Number of patients	Time point	PB vs BM	cDNA vs DNA	Favorable prognostic cutoff (proportion of patients)	Associated risk	Sensitivity of the assay
PML-RARA	301	At end of treatment (ATRA + anthracycline based)	BM	cDNA	Negative (95%)	3-y CIR 11% (vs 34% if positive)	At least 10^{-3}
PML-RARA	115	At end of treatment (ATO + ATRA, low and intermediate risk APL)	BM	cDNA	Negative (100%)	4.2-y CIR 1.9%	n.d.

Schuurhuis GJ et al. Blood 2018; 131(12): 1275-91

Molecular MRD markers in AML: WT1

Gene	Number of patients	Time point	PB vs BM	cDNA vs DNA	Favorable prognostic cutoff (proportion of patients)	Associated risk	Sensitivity of the assay
WT1	129	After induction	PB or BM	cDNA	≥2 log reduction in the same tissue (PB or BM) (62%)	5-y CIR 40% (vs 75% if <2 log)	10^{-4}
WT1	584	At end of treatment	BM	cDNA	<10 copies (32%)	3-y CIR 25% (vs 45% if 10-100 copies vs 72 of >100 copies), 3-y OS 72% (vs 59% if 10-100 copies vs 30% if >100 copies)	10^{-4}

Schuurhuis GJ et al. Blood 2018; 131(12): 1275-91

Molecular MRD Studies in AML: Summary

- Molecular MRD detected by RT-PCR is an independent prognostic factor for relapse, RFS and OS.
- Surveillance of MRD by molecular methods in suitable patients (NPM1, RUNX1-RUNX1T1, CBF-MYH11, PML-RARA) is an important tool for predicting relapse

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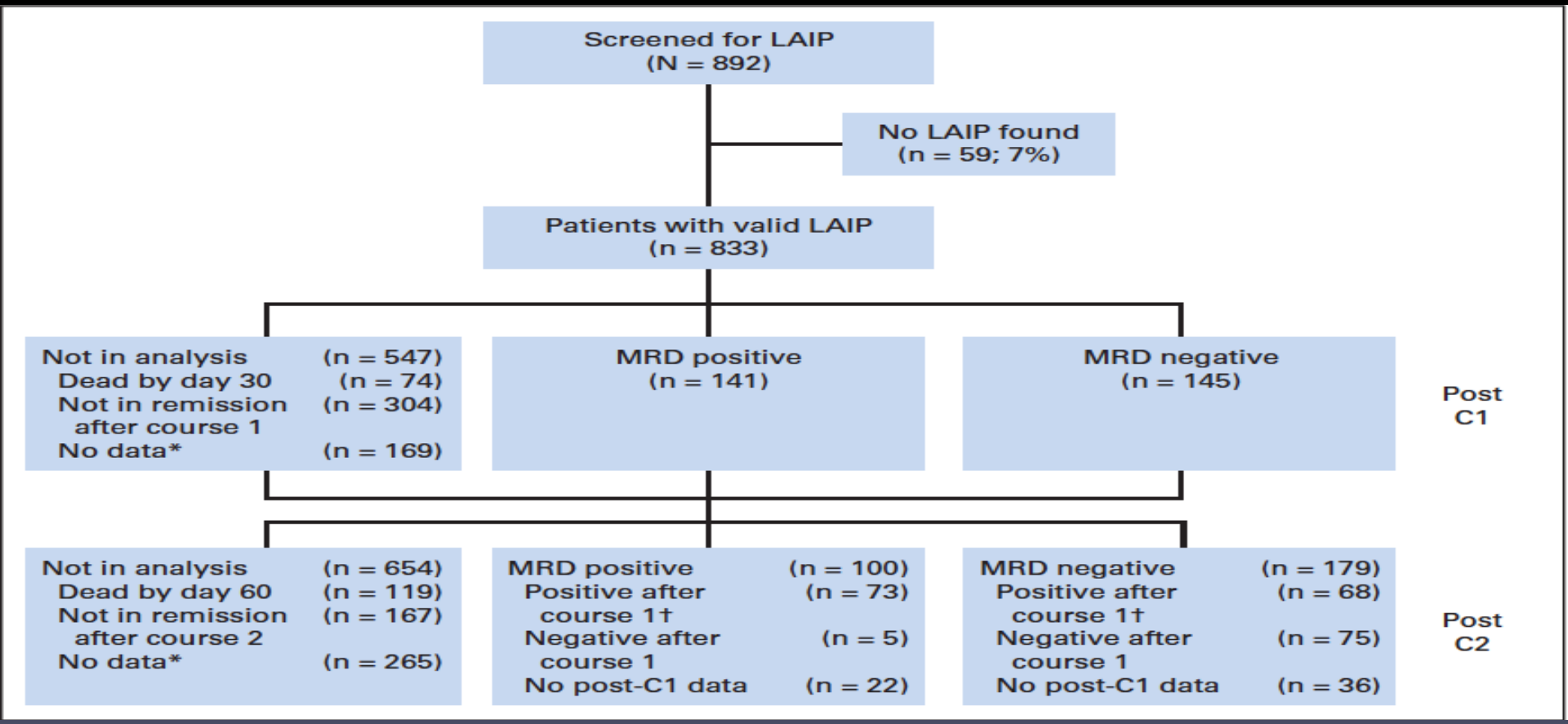
ORIGINAL REPORT

Prognostic Relevance of Treatment Response Measured by Flow Cytometric Residual Disease Detection in Older Patients With Acute Myeloid Leukemia

Sylvie D. Freeman, Paul Virgo, Steve Couzens, David Grimwade, Nigel Russell, Robert K. Hills, and Alan K. Burnett



Freeman SD et al. J Clin Oncol 2013; 31: 4123-31

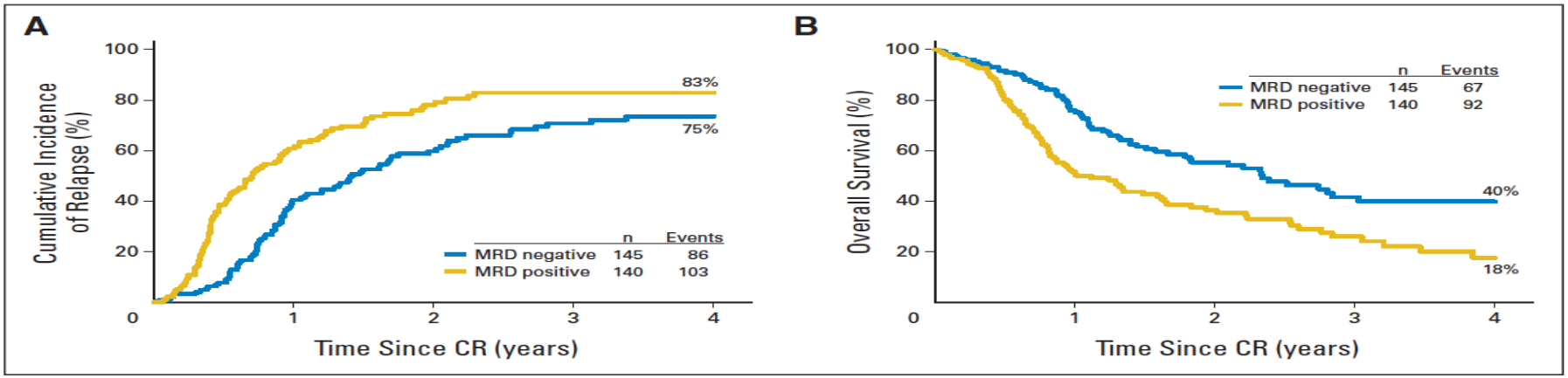


Fig 2. Effect of minimal residual disease (MRD) positivity on patients in complete remission (CR) after course 1. (A) Cumulative incidence of relapse. (B) Survival from CR.

Table 2. Outcomes

Outcome	MRD Positive (%)	MRD Negative (%)	Unadjusted HR	Unadjusted 95% CI	Unadjusted *P*	Adjusted HR	Adjusted 95% CI	Adjusted *P*
After course 1								
CIR at 3 years	83	71	2.05	1.52 to 2.75	< .001	1.95	1.34 to 2.84	< .001
Survival from CR at 3 years	26	42	1.85	1.35 to 2.53	< .001	1.96	1.39 to 2.76	< .001
After course 2								
CIR at 3 years	91	79	1.89	1.38 to 2.59	< .001	1.56	1.10 to 2.21	.01
Survival from CR at 3 years	18	38	1.90	1.35 to 2.68	< .001	1.48	1.01 to 2.15	.04

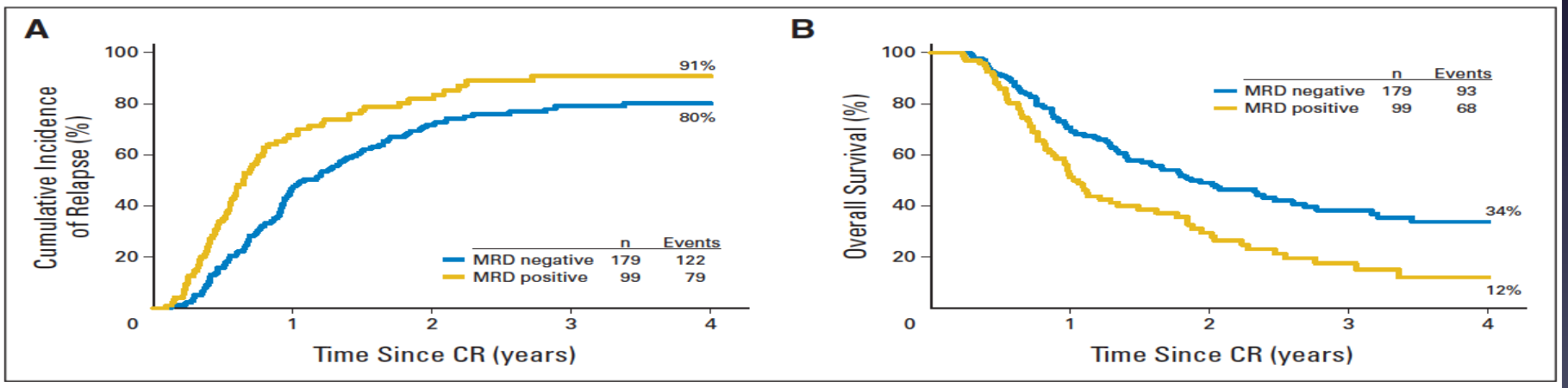
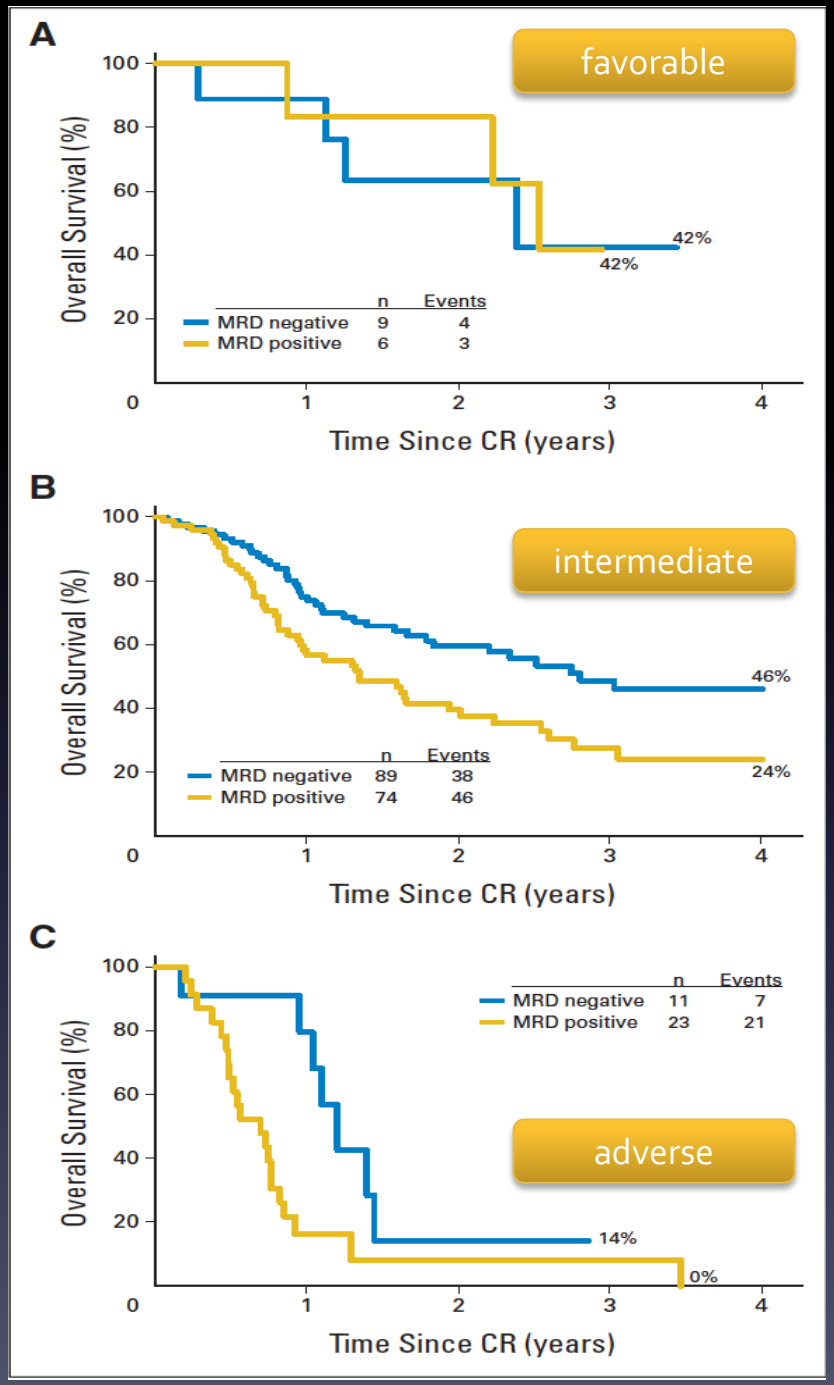
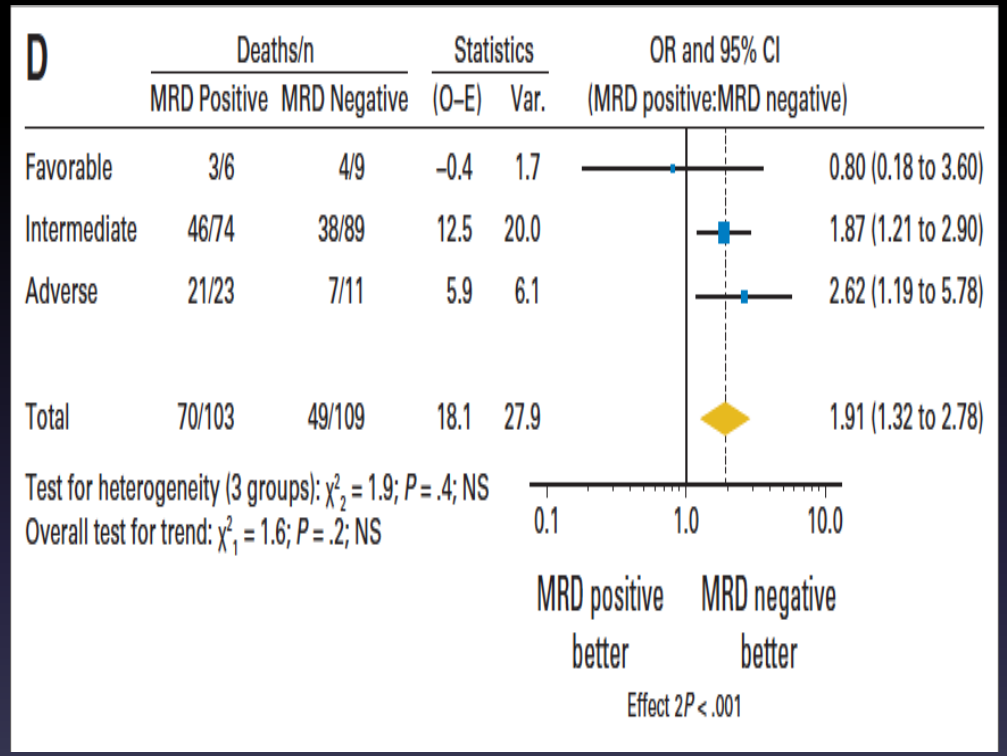


Fig 3. Effect of minimal residual disease (MRD) positivity on patients in complete remission (CR) after course 2. (A) Cumulative incidence of relapse. (B) Survival from CR.

Freeman SD et al. J Clin Oncol 2013; 31: 4123-31





Freeman SD et al. J Clin Oncol 2013; 31: 4123-31

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JOURNAL OF CLINICAL ONCOLOGY

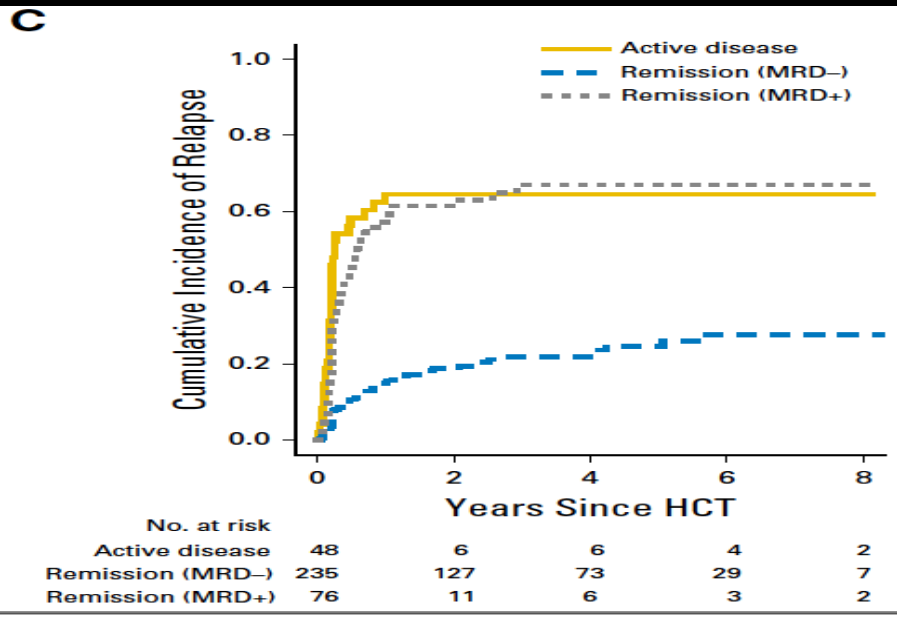
ORIGINAL REPORT

Allogeneic Hematopoietic Cell Transplantation for Acute Myeloid Leukemia: Time to Move Toward a Minimal Residual Disease–Based Definition of Complete Remission?

Daisuke Araki, Brent L. Wood, Megan Othus, Jerald P. Radich, Anna B. Halpern, Yi Zhou, Marco Mielcarek, Elihu H. Estey, Frederick R. Appelbaum, and Roland B. Walter

2006-2014 (n: 356)
AML: MA-alloHCT

Araki D et al. J Clin oncol 2016; 34: 329-36



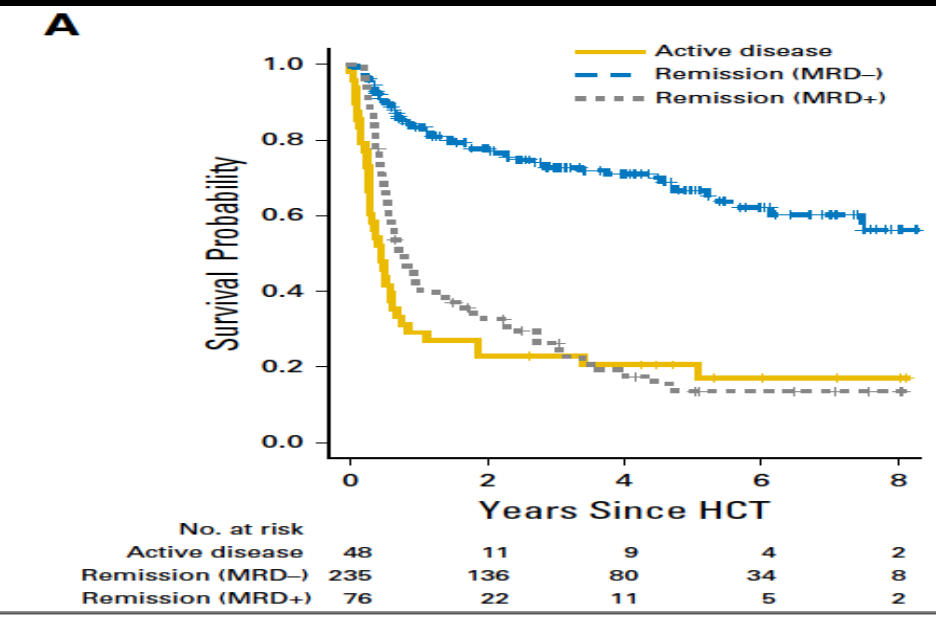


Table 4. Multivariable Regression Models for Disease Status

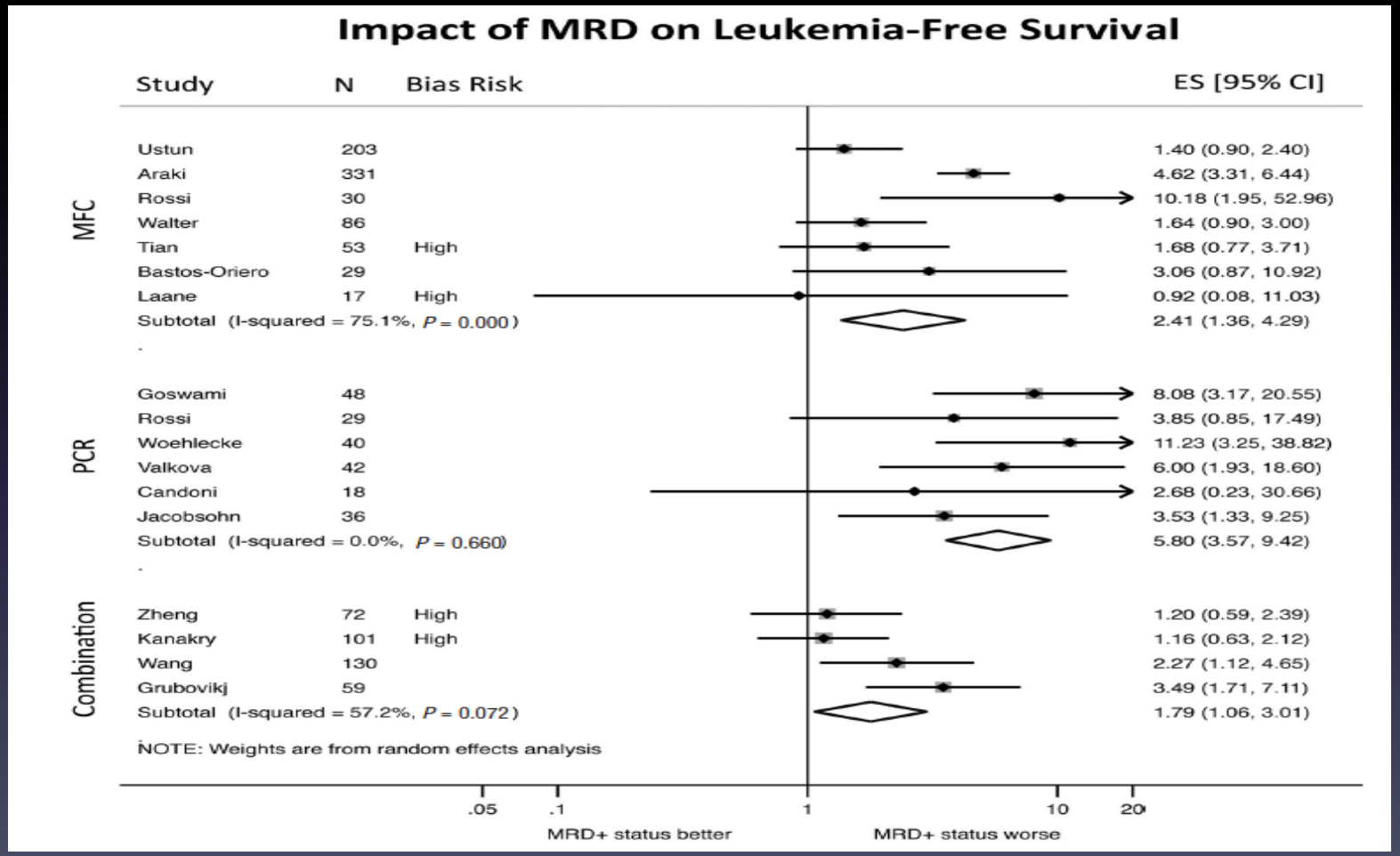
Regression Model	No. of Patients	Overall Mortality		Failure for PFS		Relapse		NRM	
		HR (95% CI)	*P*	HR (95% CI)	*P*	HR (95% CI)	*P*	HR (95% CI)	*P*
Disease status									
MRD-negative remission	235	1 (reference)		1 (reference)		1 (reference)		1 (reference)	
MRD-positive remission	76	3.69 (2.51 to 5.42)	< .001	4.38 (3.04 to 6.33)	< .001	4.17 (2.69 to 6.47)	< .001	1.72 (0.93 to 3.18)	.083
Active disease	48	4.40 (2.56 to 7.55)	< .001	5.30 (3.18 to 8.81)	< .001	4.87 (2.50 to 9.72)	< .001	1.37 (0.52 to 3.65)	.530
Age (per 10 years)		1.12 (0.98 to 1.29)	.089	0.98 (0.87 to 1.10)	.704	0.87 (0.75 to 1.00)	.050	1.27 (0.99 to 1.65)	.065
Cytogenetic risk group									
Intermediate/favorable	264	1 (reference)		1 (reference)		1 (reference)		1 (reference)	
Adverse	89	0.90 (0.61 to 1.32)	.583	1.00 (0.70 to 1.45)	.979	1.19 (0.77 to 1.86)	.430	0.66 (0.34 to 1.32)	.240
Type of AML									
De novo	144	1 (reference)		1 (reference)		1 (reference)		1 (reference)	
Secondary	97	1.01 (0.73 to 1.41)	.953	0.99 (0.72 to 1.35)	.945	0.91 (0.62 to 1.35)	.640	1.03 (0.55 to 1.93)	.880
Pre-HCT karyotype									
Normalized	132	1 (reference)		1 (reference)		1 (reference)		1 (reference)	
Not normalized	85	1.48 (0.94 to 2.33)	.088	1.58 (1.02 to 2.43)	.039	1.26 (0.75 to 2.10)	.380	1.43 (0.70 to 3.12)	.360
Pre-HCT blood counts*									
Recovered	258	1 (reference)		1 (reference)		1 (reference)		1 (reference)	
Not recovered	101	0.87 (0.59 to 1.29)	.490	0.86 (0.59 to 1.24)	.418	0.97 (0.61 to 1.57)	.910	1.08 (0.58 to 2.03)	.800

Araki D et al. J Clin oncol 2016; 34: 329-36

EUROPEAN HEMATOLOGY ASSOCIATION

Ferrata Storti Foundation

Minimal residual disease prior to allogeneic hematopoietic cell transplantation in acute myeloid leukemia: a meta-analysis



Buckley SA et al. Haematologica 2017; 102: 865-73

Challenges to clinical application of MRD testing-1

- The genetic heterogeneity of AML and lack of universal antigenic surface markers of leukemic stem cells make it difficult to standardize MRD evaluation protocols.
- There is no consensus regarding optimal key parameters like type of specimen (BM vs PB), MRD target, timing of MRD assessment, technology (MFC vs RT-PCR), testing protocols and cutoff values defining positivity. Several of these issues has been addressed in current ELN guideline.
- Antigenic (immunophenotypic) shift of blast cells and emerging subclones

Challenges to clinical application of MRD testing-2

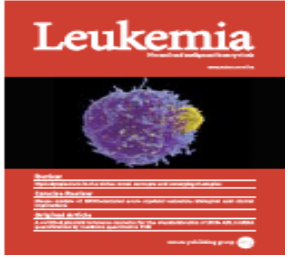
- Commonly mutated genes in AML, have been observed especially in older people without any hematologic abnormalities (age-related clonal hematopoesis with indeterminate potential, CHIP 10% in >65 subjects)
- Detection of the so-called DTA (DNMT3A, TET2, ASXL1) does not always indicate pending relapse unless these were not associated with other mutations.
- The kinetics of molecular relapse is associated with the type of AML in terms of mutations. (CBF-MYH11 AML clone grows slowly compared to PML-RARA. The recommendation of MRD testing for CBF-MYH11 AML is every 6 months which is 2 months for APL)

Jongen-Lavrencic M et al. N Engl J Med 2018; 378 (13): 1189

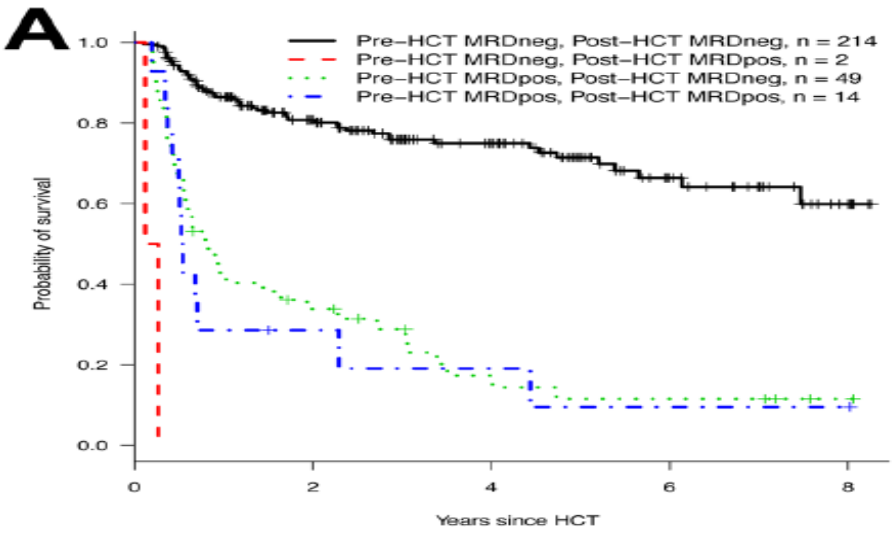
Challenges to clinical application of MRD testing-3

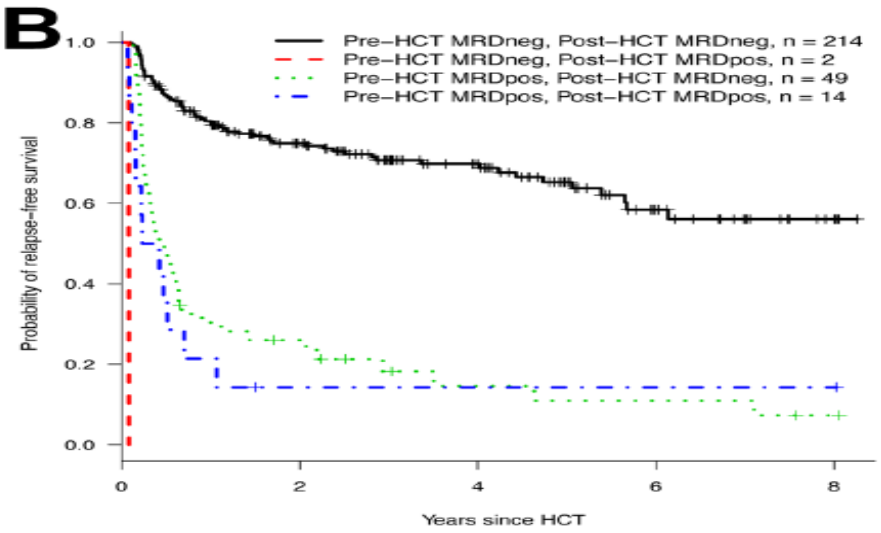
- Using a specified cutoff for MRD detection and analyzing patients in 2 groups (MRD^+ and MRD^-) is an oversimplification. Long-term OS can be achieved in MRD^+ patients, while a minority of MRD^- patients will still relapse as well.
- Data in patients receiving chemotherapy indicate that clinical impact of MRD positivity depends on certain parameters like cut-off levels defining MRD, risk groups of patients and time of evaluation
- The prognostic impact of MRD status in patients on non-intensive therapies like hypomethylating agents is currently unknown.

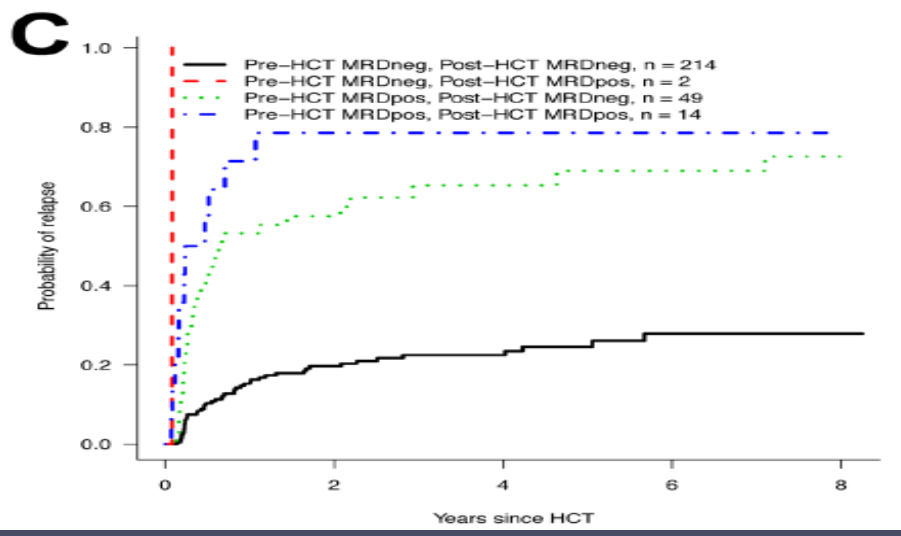
PREDICTIVE OR JUST A PROGNOSTIC MARKER?

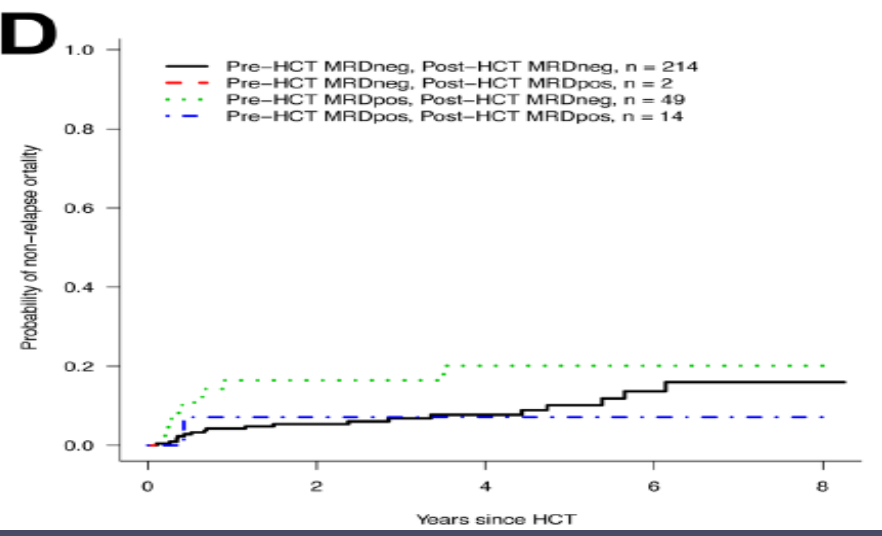


Pre- and post-transplant quantification of measurable ('Minimal') residual disease via multiparameter flow cytometry in adult acute myeloid leukemia







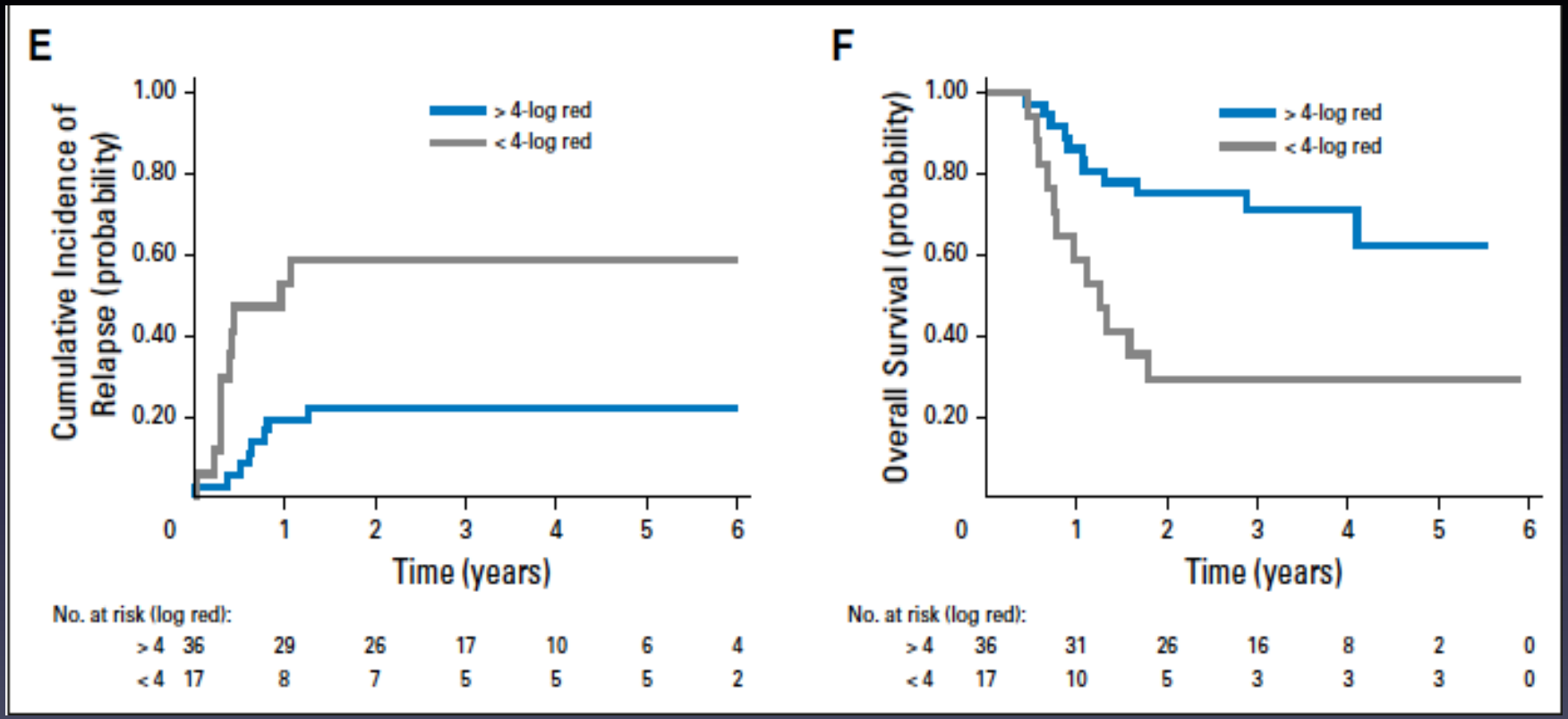


Zhou Y et al. Leukemia 2016; 30(7): 1456-64

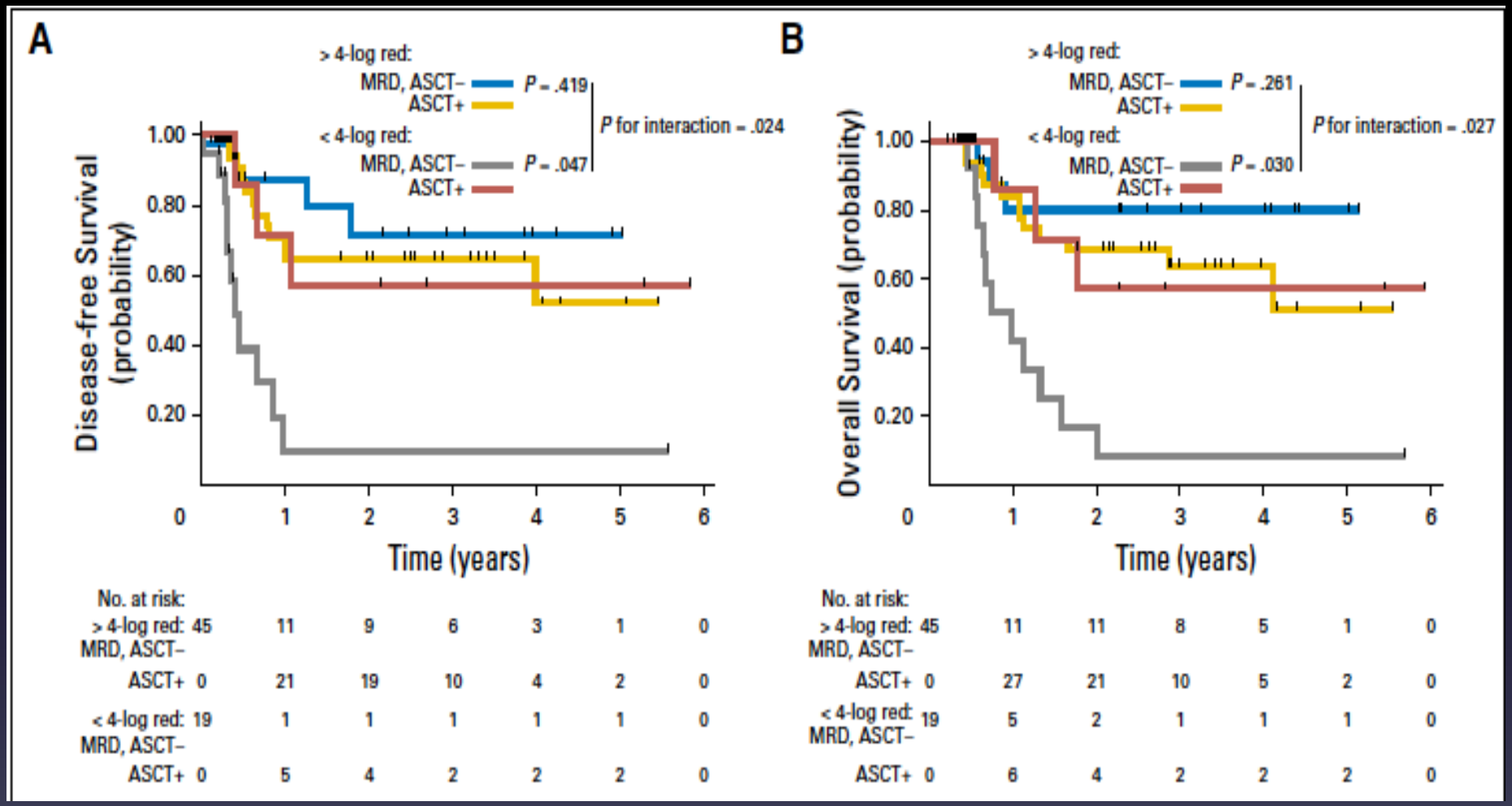
JOURNAL OF CLINICAL ONCOLOGY

ORIGINAL REPORT

Postinduction Minimal Residual Disease Predicts Outcome and Benefit From Allogeneic Stem Cell Transplantation in Acute Myeloid Leukemia With *NPM1* Mutation: A Study by the Acute Leukemia French Association Group



Balsat M et al. J Clin Oncol 2017; 35: 185-93



Balsat M et al. J Clin Oncol 2017; 35: 185-93

Preemptive AZA in NPM1^{+} AML

- n: 10
- NMP1^{+} AML CR1/CR2 with molecular relapse
- RT-PCR (NMP1/ABL > 1% after last therapy in BM)
- Preemptive AZA at molecular relapse
- Median follow-up: 10 months
- 3 (30%) patients had clinical relapse

Sockel K et al. Haematologica 2011; 96(10): 1568-70

LEADING ARTICLE

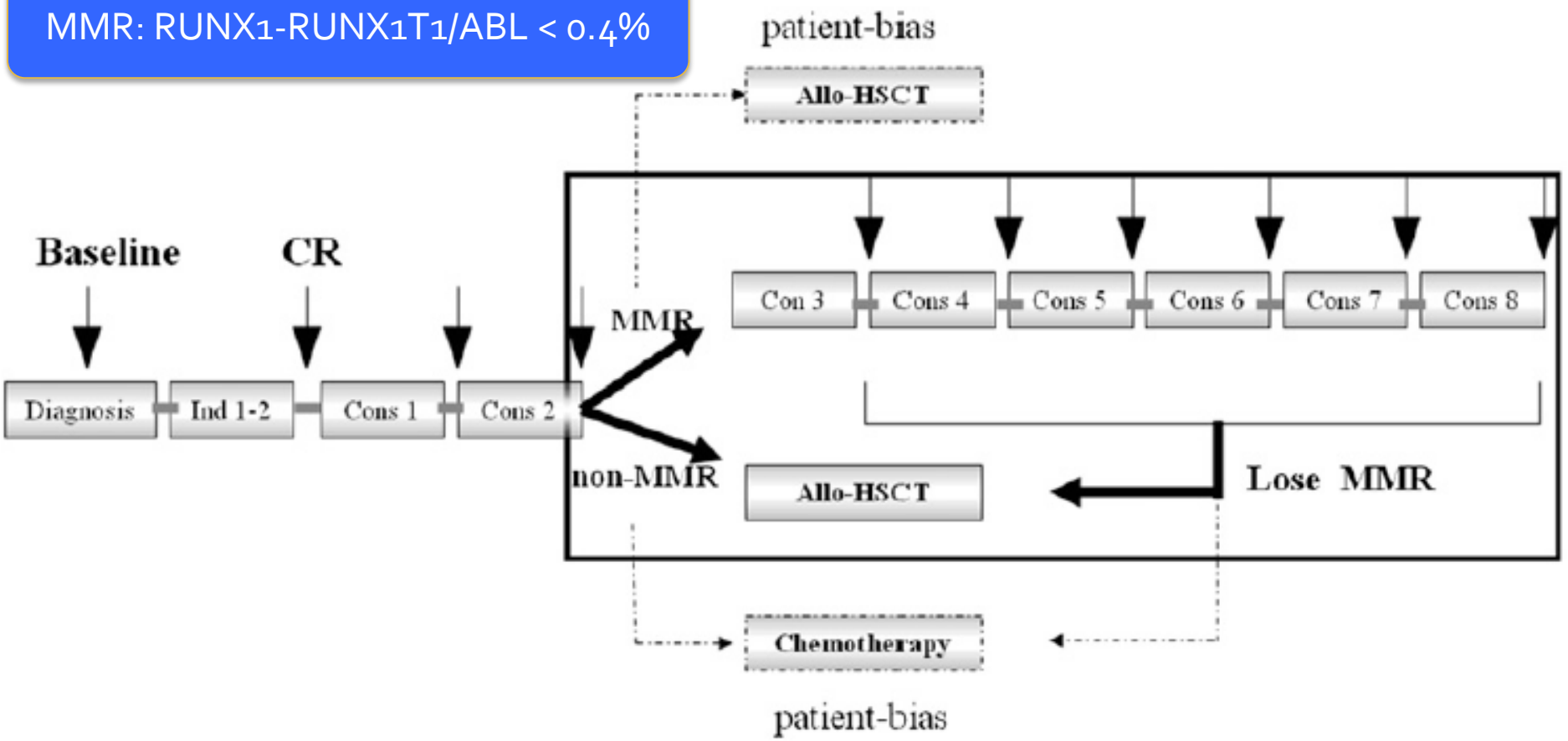
Azacitidine for treatment of imminent relapse in MDS or AML patients after allogeneic HSCT: results of the RELAZA trial

- Treatment of MRD with preemptive AZA in MDS/AML patients after allo-HCT
- MRD by a sensitive donor chimerism analysis of $CD34^+$ blood cells
- 20/59 patients experienced a decrease of donor chimerism < 80% while in CR
- 4 cycles of AZA (11 patients received additional 4 cycles)
- 16 (80%) responded (increasing donor chimerism > 80%: n:10; 50%) (stabilization in the absence of relapse: n:6; 30%)
- Hematologic relapse (n:13; 65%) after a median of 231 days after loss of donor chimerism

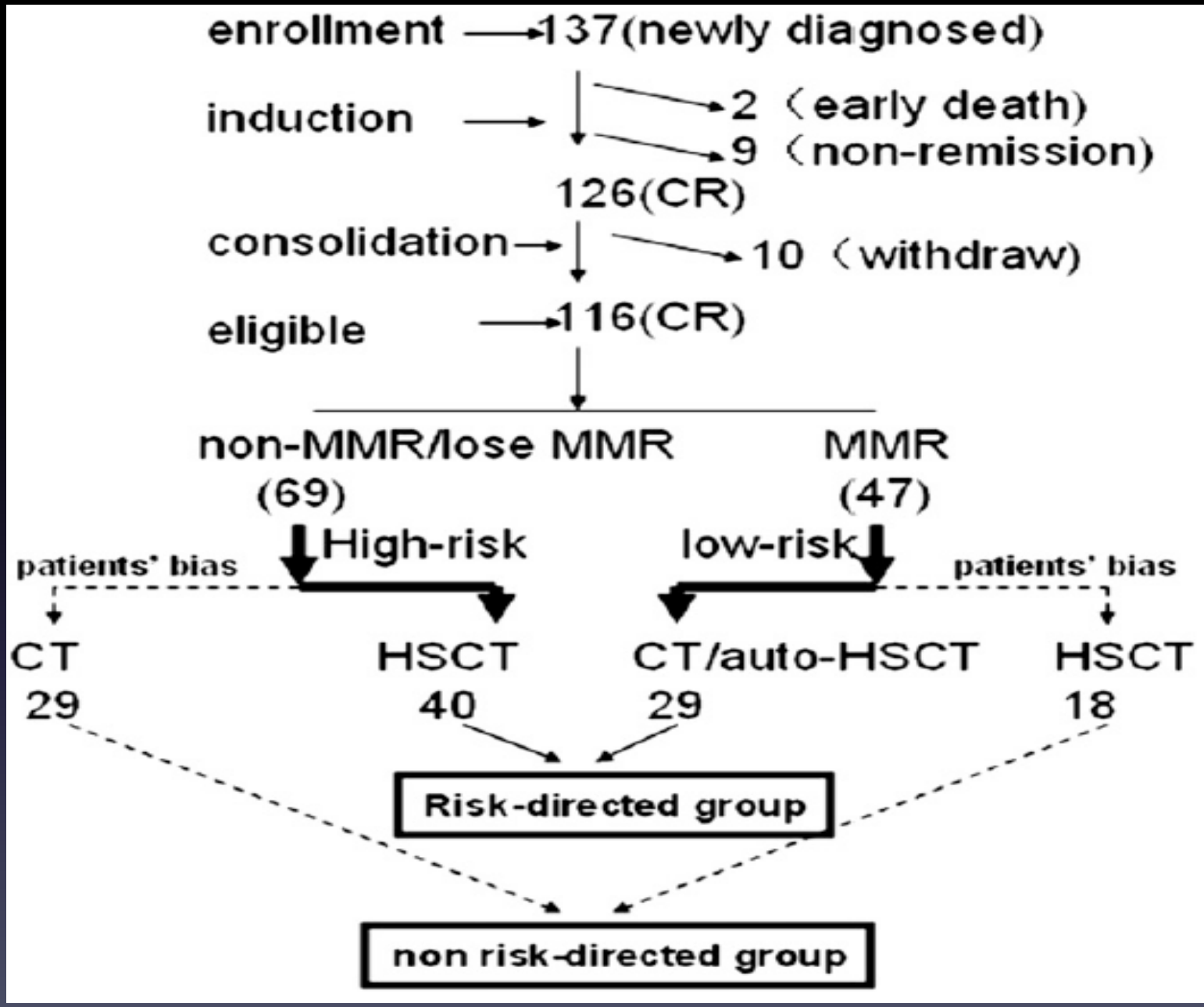
Platzbecker U et al. Leukemia 2012;26 (3): 381-89

MRD-directed risk stratification treatment may improve outcomes of t(8;21) AML in the first complete remission: results from the AML05 multicenter trial

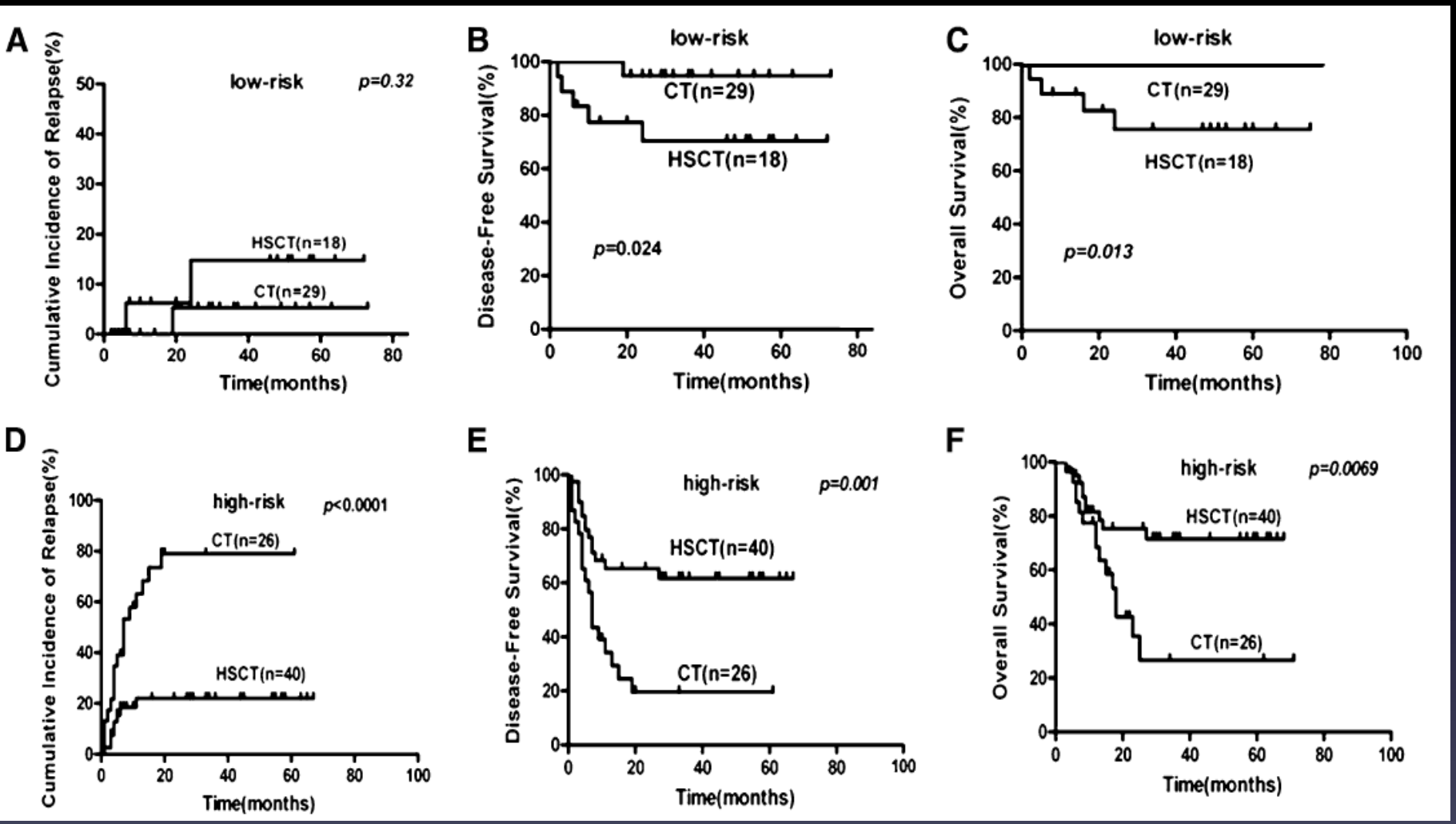
MMR: RUNX1-RUNX1T1/ABL < 0.4%



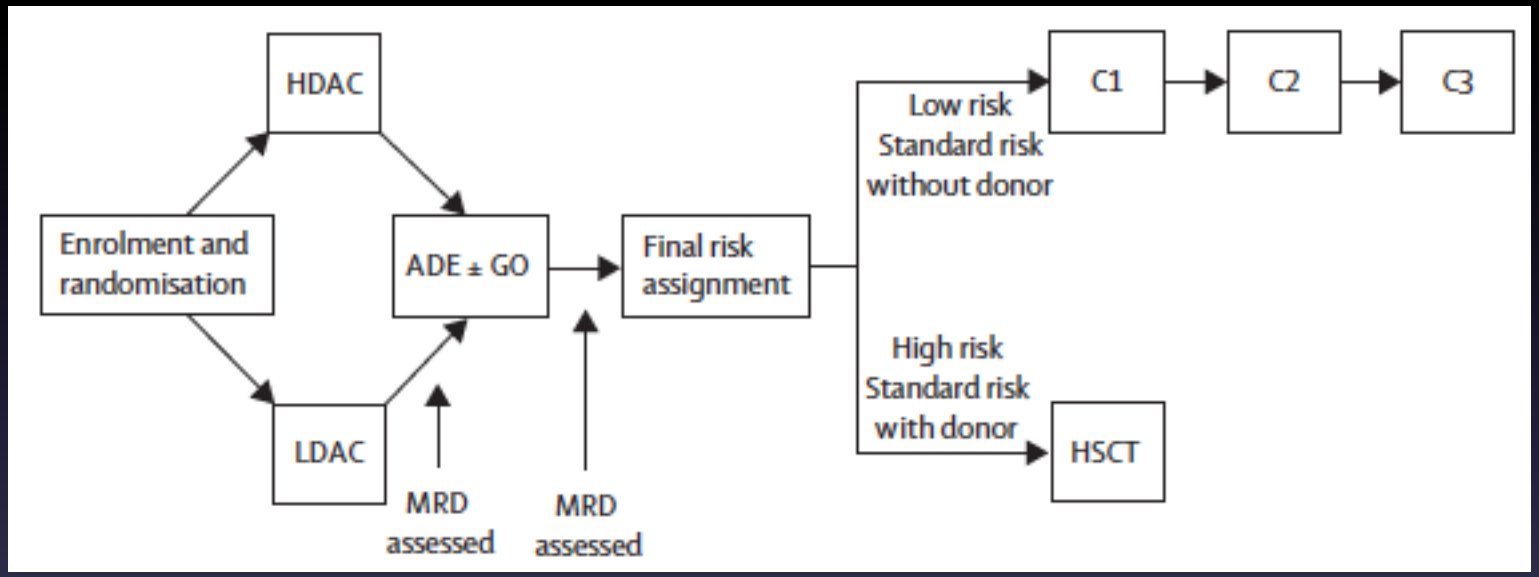
Zhu HH et al. Blood 2013; 121(20): 4056-62



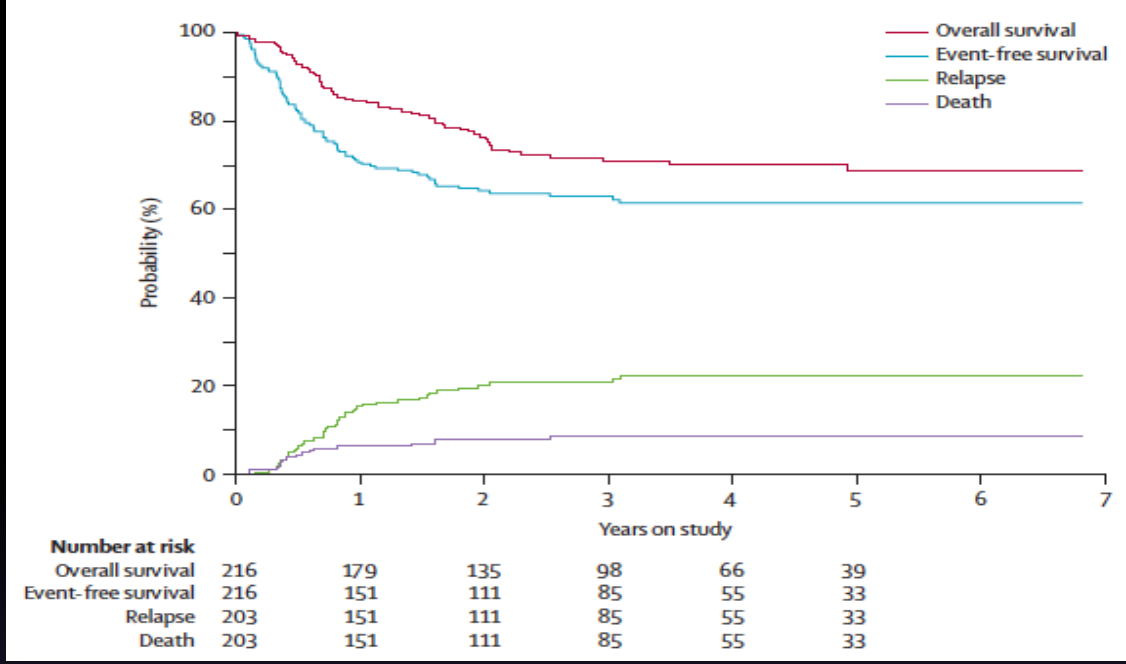
Zhu HH et al. Blood 2013; 121(20): 4056-62

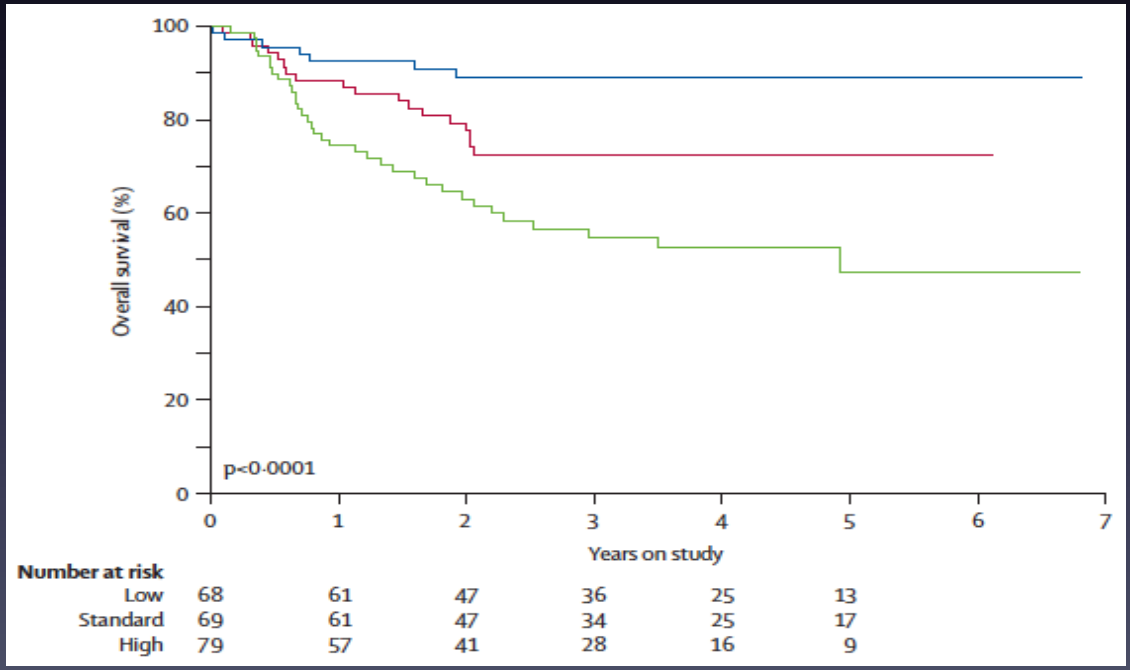

A
low-risk
p=0.32
Cumulative Incidence of Relapse(%)
HSCT(n=18)
CT(n=29)
Time(months)
B
low-risk
Disease-Free Survival(%)
CT(n=29)
HSCT(n=18)
p=0.024
Time(months)
C
low-risk
Overall Survival(%)
CT(n=29)
HSCT(n=18)
p=0.013
Time(months)
D
high-risk
p<0.0001
Cumulative Incidence of Relapse(%)
CT(n=26)
HSCT(n=40)
Time(months)
E
high-risk
p=0.001
Disease-Free Survival(%)
HSCT(n=40)
CT(n=26)
Time(months)
F
high-risk
p=0.0069
Overall Survival(%)
HSCT(n=40)
CT(n=26)
Time(months)

Minimal residual disease-directed therapy for childhood acute myeloid leukaemia: results of the AML02 multicentre trial



Rubnitz JE et al. Lancet Oncol 2010; 11(6): 543-52





Rubnitz JE et al. Lancet Oncol 2010; 11(6): 543-52

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

Schuurhuis GJ et al. Blood 2018; 131(12): 1275-91

	Recommendations
Flow cytometry	
1	Use the following markers in an MRD panel: CD7, CD11b, CD13, CD15, CD19, CD33, CD34, CD45, CD56, CD117, HLA-DR (backbone: CD45, CD34, CD117, CD13, CD33, forward scatter/sideward scatter) If necessary, add a "monocytic tube" containing: CD64/CD11b/CD14/CD4/CD34/HLA-DR/CD33/CD45.
2	Integrate the classic LAIP approach with the DfN approach. To trace all aberrancies (at and beyond diagnosis, including newly formed postdiagnosis aberrancies) apply a full panel both at diagnosis and at follow-up.
3	Aspirate 5-10 mL of BM and use the first pull for MRD assessment. At present, PB, with its lower MRD content, should not be used for MRD assessment. Pull as low as desirable BM volume because contamination with PB increases with BM volume
4	Estimate the contamination with PB, especially when a first pool of BM was impossible.
5	Use 500 000 to 1 million WBCs; use the best aberrancy available and relate it to $CD45^+$ WBCs.
6	To define "MRD-negative" and "MRD-positive" patient group, a cutoff of 0.1% is recommended.
7	If true MRD <0.1% is found, report this as "MRD-positive <0.1%, may be consistent with residual leukemia." If applicable, the comment "this level has not been clinically validated" should be added.
8	In a multicenter setting, transport and storage of full BM at room temperature for a period of 3 d are acceptable.
9	Single center studies with no extensive experience on MFC MRD are strongly discouraged.

Schuurhuis GJ et al. Blood 2018; 131(12): 1275-91

	Recommendations
Molecular biology	
1	Molecular MRD analysis is indifferent to the anticoagulant used during cell sampling, and thus both heparin and EDTA can be used as anticoagulant.
2	Aspirate 5-10 mL of BM, and use the first pull for molecular MRD assessment.
3	WT1 expression should not be used as MRD marker, unless no other MRD marker is available in the patient.
4	Do not use mutations in FLT3-ITD, FLT3-TKD, NRAS, KRAS, DNMT3A, ASXL1, IDH1, IDH2, MLL-PTD and expression levels of EVI1 as single MRD markers. However, these markers may be useful when used in combination with a second MRD marker.
5	We define molecular progression in patients with molecular persistence as an increase of MRD copy numbers ≥1 log10 between any 2 positive samples. Absolute copy numbers should be reported in addition to the fold increase to enable the clinician to make his/her own judgments.
6	We define molecular relapse as an increase of the MRD level of ≥1 log10 between 2 positive samples in a patient who was previously tested negative.
	The conversion of negative to positive MRD in PB or BM should be confirmed 4 wk after the initial sample collection in a second sample from both BM and PB. If MRD increases in the follow-up samples ≥1 log10, molecular relapse should be diagnosed.

Schuurhuis GJ et al. Blood 2018; 131(12): 1275-91

	Recommendations
Clinical	
1	Refine morphology-based CR by assessment of MRD, because CR_{MRD}^- is a new response criterion according to the AML ELN recommendation 2017.
	Use MRD to refine risk assessment prior to consolidation treatment, the postinduction time point closest to consolidation treatment is recommended.
2	MRD monitoring should be considered part of the standard of care for AML patients.
	Monitoring beyond 2 y of follow-up should be based on the relapse risk of the patient and decided individually.
	Patients with mutant NPM1, RUNX1-RUNX1T1, CBFB-MYH11, or PML-RARA should have molecular assessment of residual disease at informative clinical time points.
3	Not to assess molecular MRD in subtypes other than APL, CBF AML, and NPM1-mutated AML.
4	For AML patients not included in the molecularly defined subgroups above, MRD should be assessed using MFC.
	During the treatment phase, we recommend molecular MRD assessment at minimum at diagnosis, after 2 cycles of standard induction/consolidation chemotherapy and after the end of treatment in PB and BM.
	During follow-up of patients with PML-RARA, RUNX1-RUNX1T1, CBFB-MYH11, mutated NPM1, and other molecular markers, we recommend molecular MRD assessment every 3 mo for 24 mo after the end of treatment in BM and in PB. Alternatively, PB may be assessed every 4-6 wk.
5	Failure to achieve an MRD-negative CR, or rising MRD levels during or after therapy are associated with disease relapse and inferior outcomes and should prompt consideration of changes in therapy.
6	In APL, the most important MRD end point is achievement of PCR negativity for PML-RARA at the end of consolidation treatment.
	For patients with PML-RARA fusion and low/intermediate-risk Sanz score who are treated with ATO and ATRA, MRD analysis should be continued until the patient is in CR_{MRD}^- in BM and then should be terminated.
7	Detectable levels of PML-RARA by PCR during active treatment of APL should not change the treatment plan for an individual patient.
8	A change in status of PML-RARa by PCR from undetectable to detectable, and confirmed by a repeat sample, should be regarded as an imminent disease relapse in APL.
9	Patients with CBF AML should have an initial assessment of MRD after 2 cycles of chemotherapy, followed by serial measurements every 3 mo for at least the first 2 y after the end of treatment.
10	MRD should be assessed pretransplant.
11	MRD should be performed posttransplant.
12	All clinical trials should require molecular and/or MFC assessment of MRD at all times of evaluation of response.

Schuurhuis GJ et al. Blood 2018; 131(12): 1275-91

Take home messages from ELN

- MRD monitoring should be part of standard care of AML patients, because MRD is a new response criterion (CR with/without MRD)
- MRD status has no effect on OS in multivariate analysis of patients with CBF-MYH11 AML and therefore no recommendation is made for a change in therapy (high response rate to salvage)
- There is no time point or MRD threshold during active treatment phase of patients with RUNX1-RUNX1T1 AML that should lead to change therapy.
- If an upward trend of MRD , as defined by a log increase in either BM or PB, is detected in patients with $NMP1^{+}$ AML, consideration should be given to salvage therapy.
- No randomized, controlled trials of MRD-directed therapy has been conducted.

Schuurhuis GJ et al. Blood 2018; 131(12): 1275-91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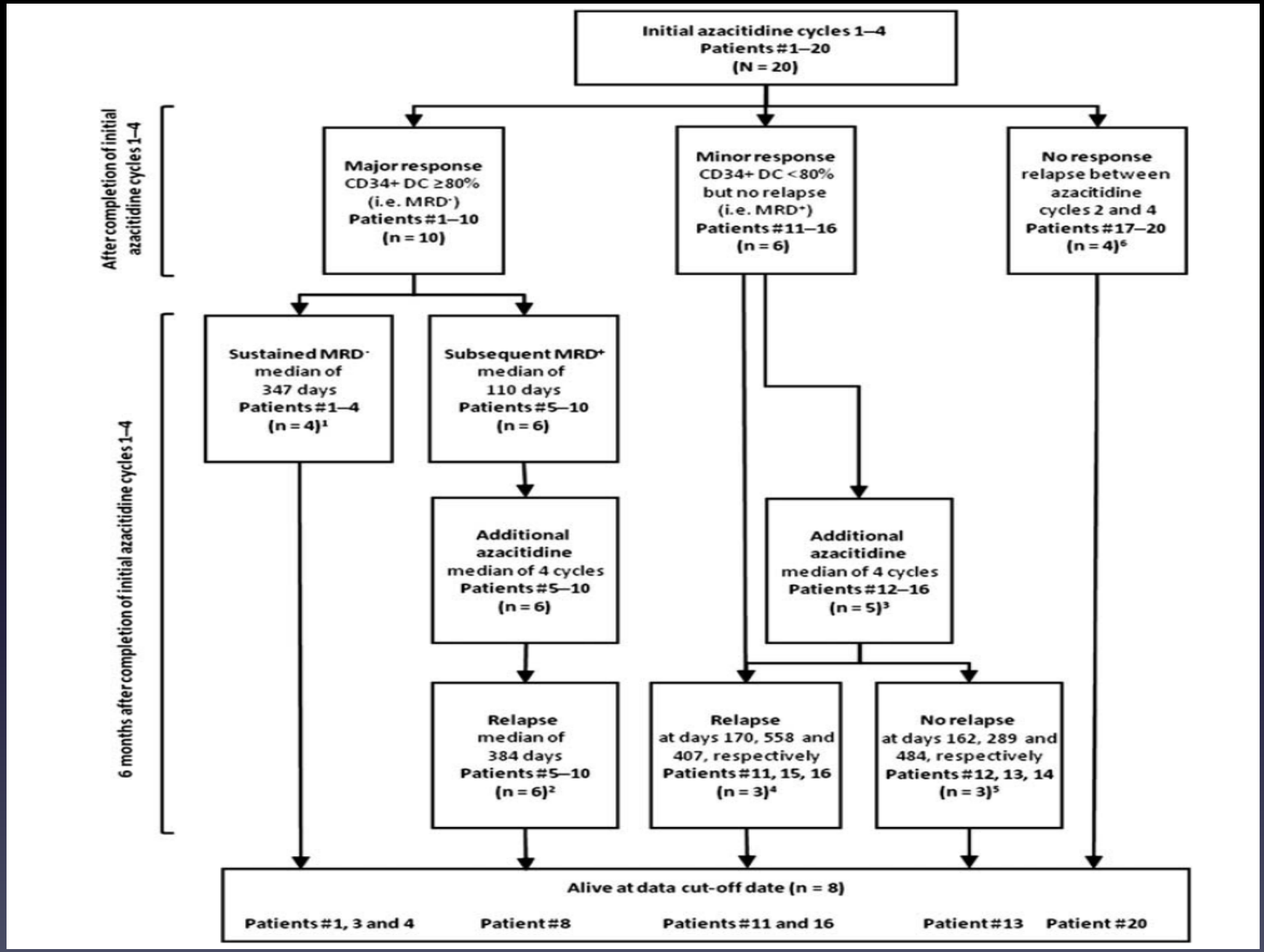

Platzbecker U et al. Leukemia 2012;26 (3): 381-89