



Methoden der MRD-Analyse im Kontext der allogenen Stammzelltransplantation



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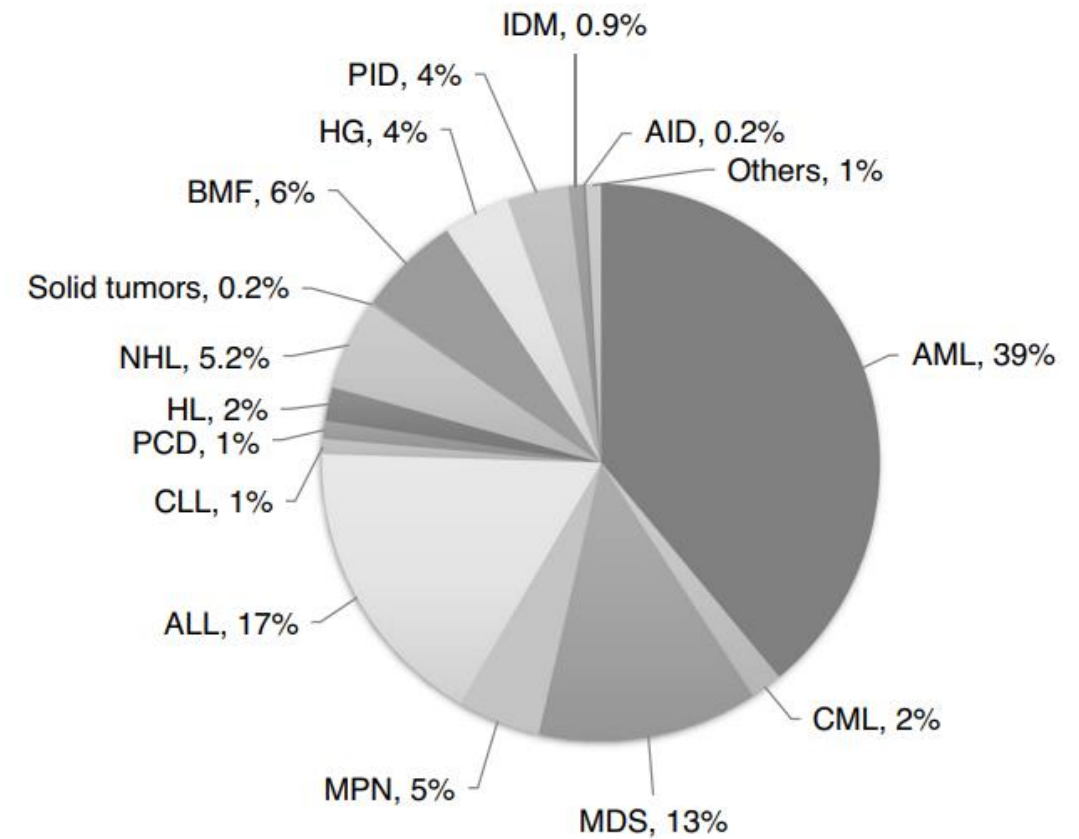
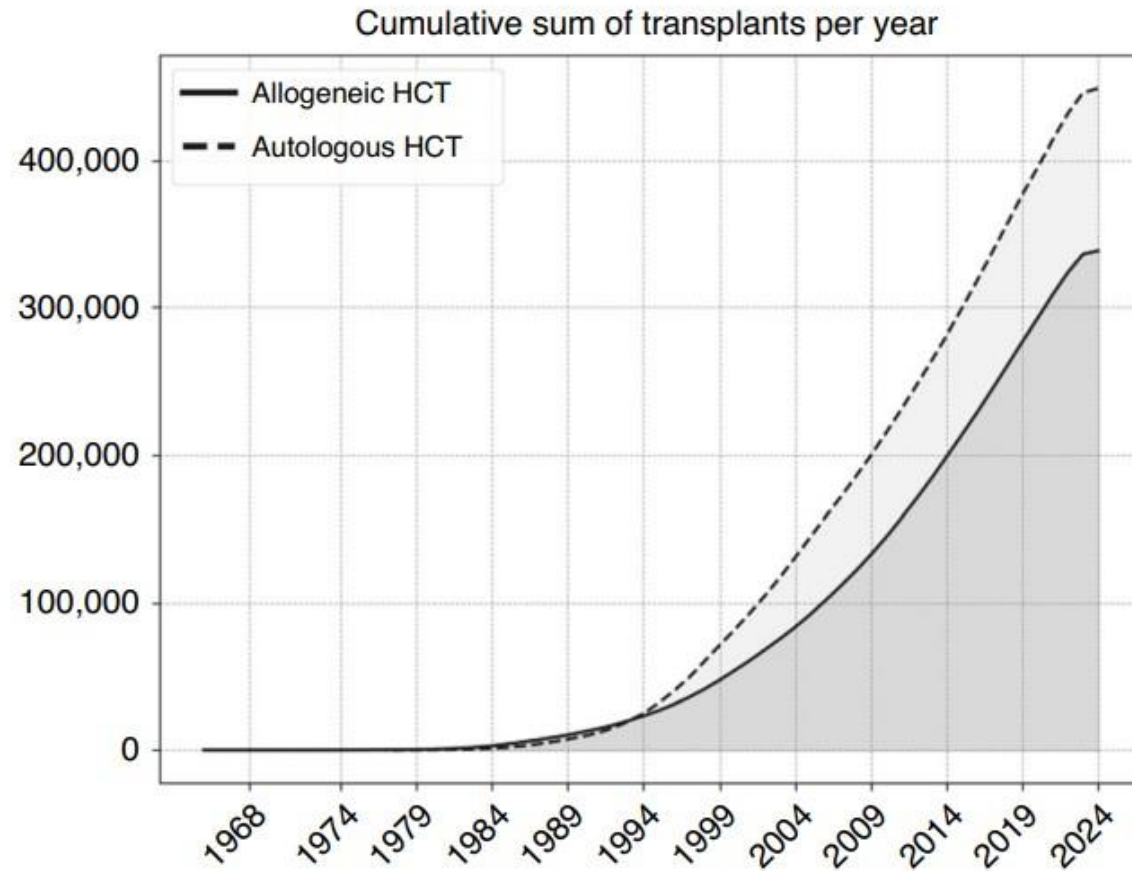
Disclosures

speakers honoraria: Novartis, BMS/Celgene, Incyte, AOP, GSK

advisory board: Novartis, BMS/Celgene, Incyte, Pfizer

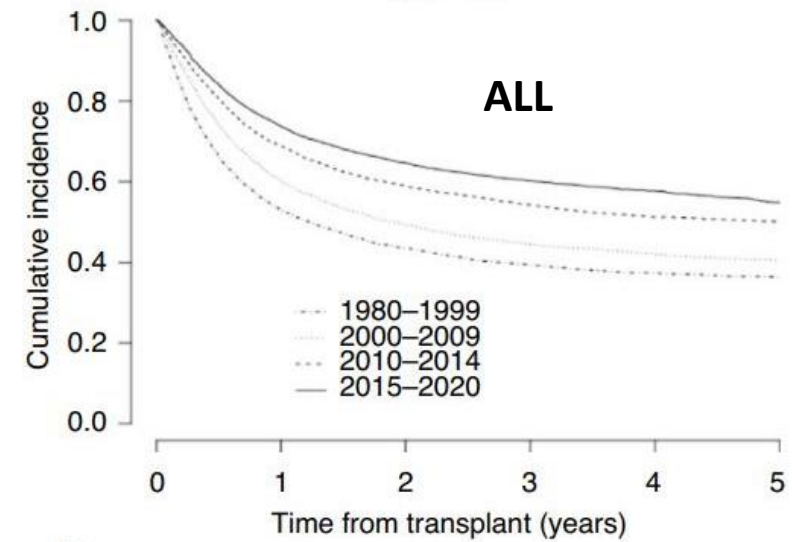
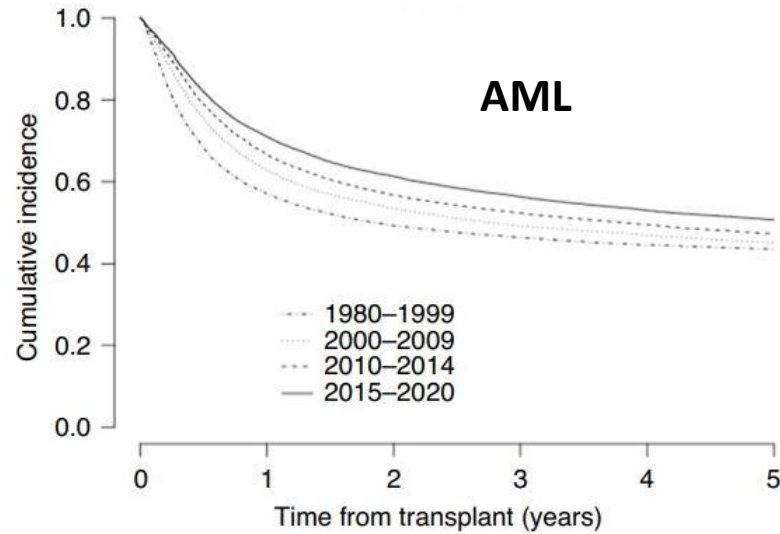
research funding: BD Biosciences

Allogene Stammzelltransplantation in Europa:

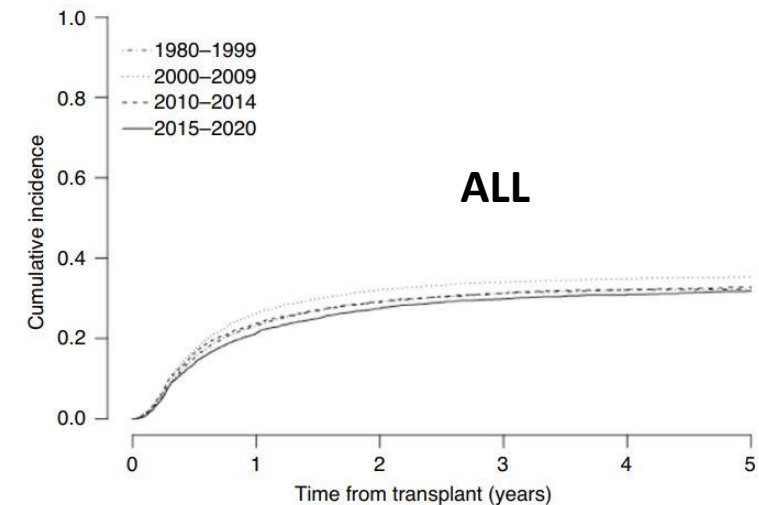
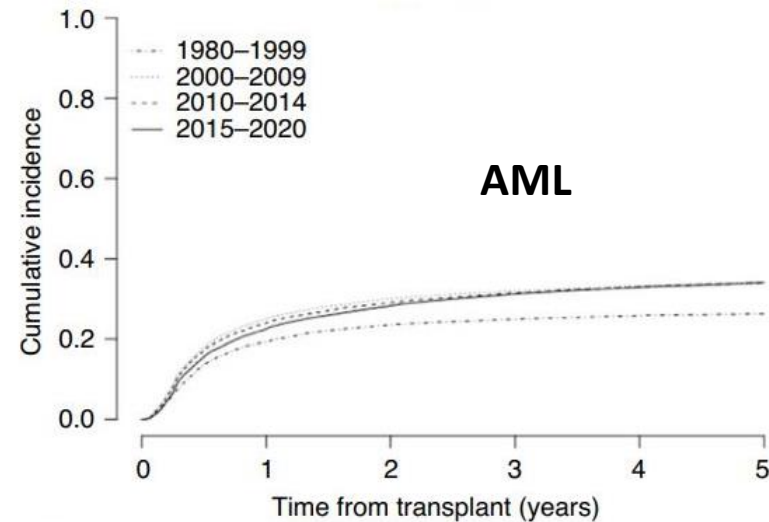


Akute Leukämien - Gesamtüberleben und Relapsinzidenz nach alloSCT

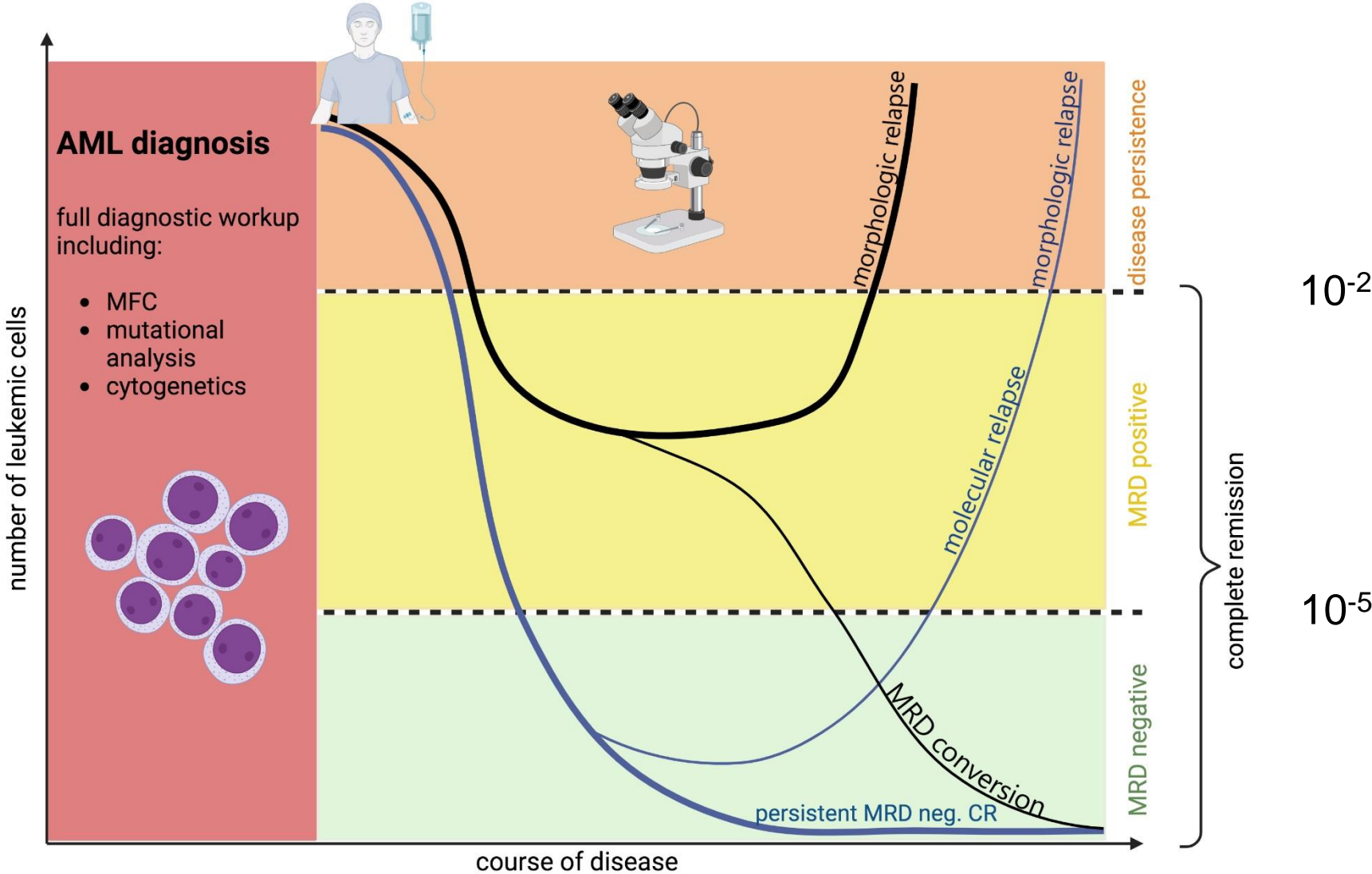
Gesamtüberleben



Relapsinzidenz

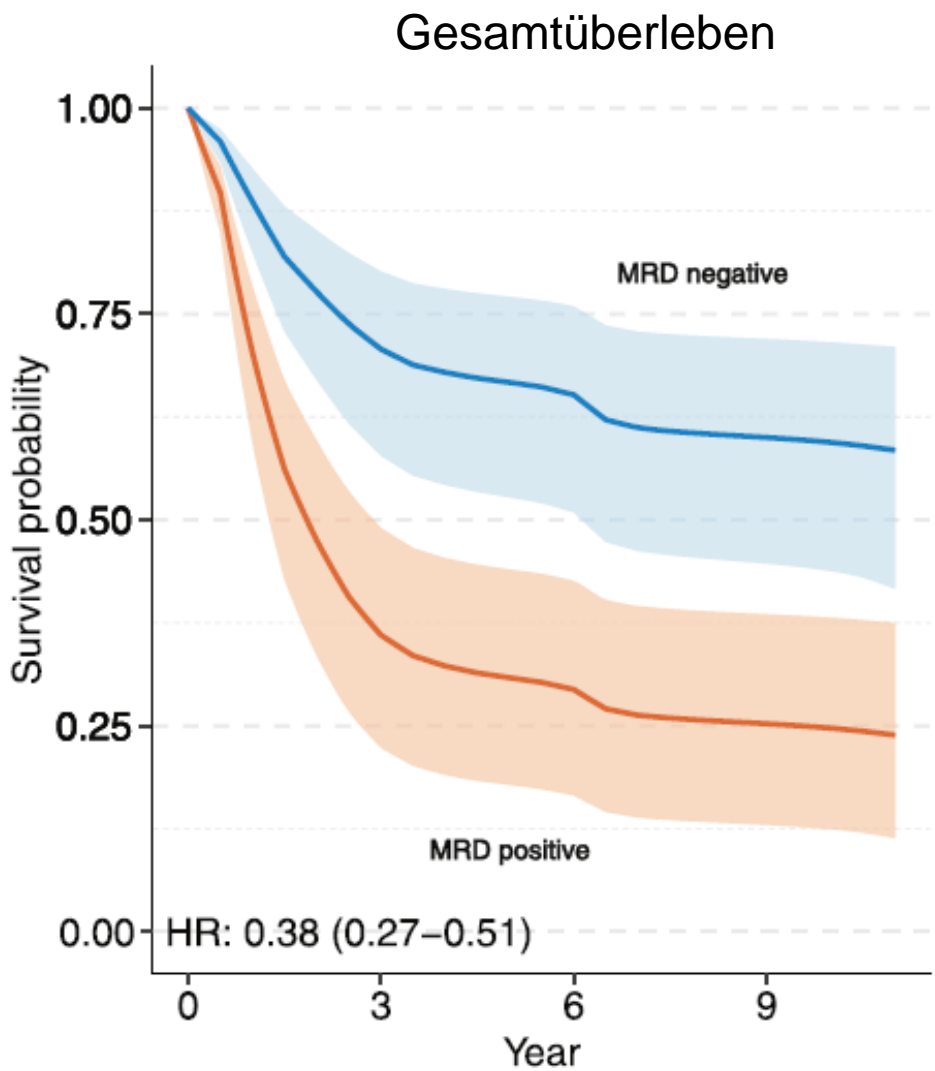


Konzept der messbaren Resterkrankung (MRD) am Beispiel der AML:

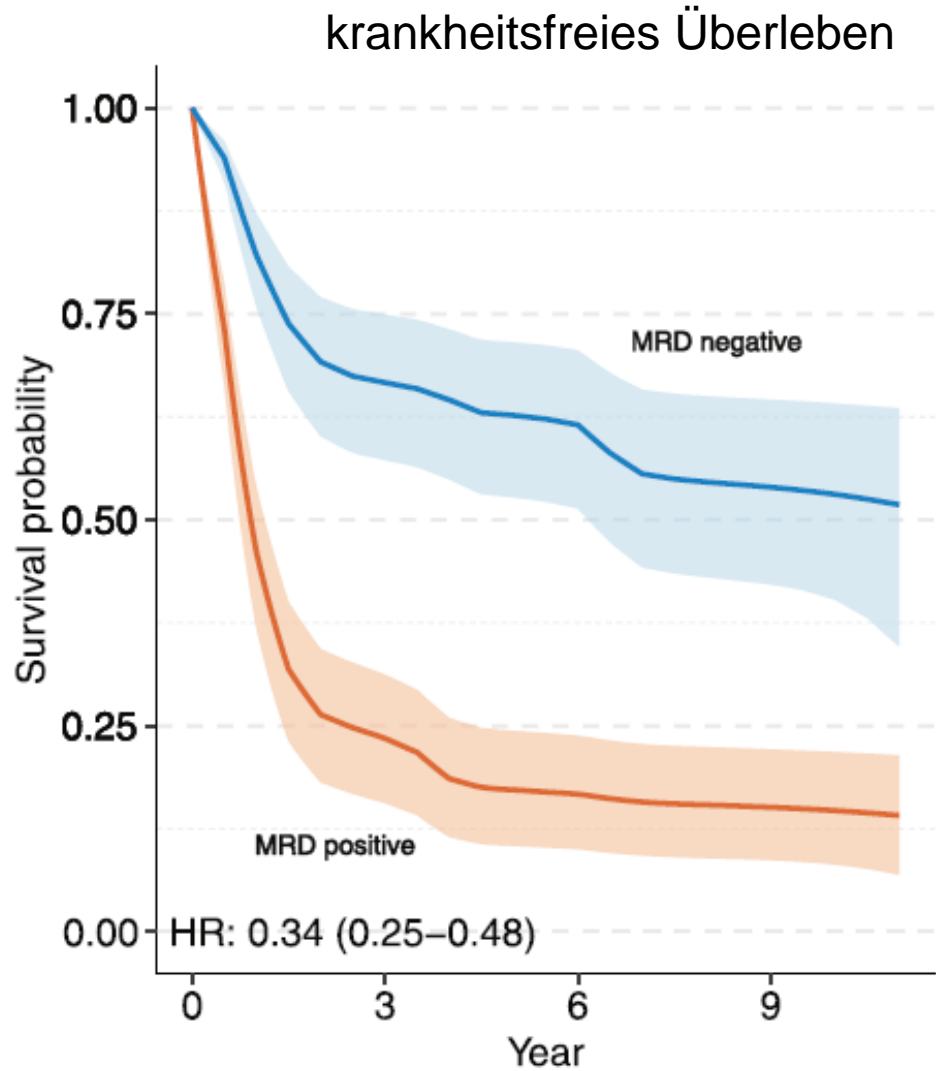


adapted from Moritz et al., Biomedicines 2024

Einfluss der MRD-Positivität auf das Überleben bei AML-Patient:innen:



n=3427 pts

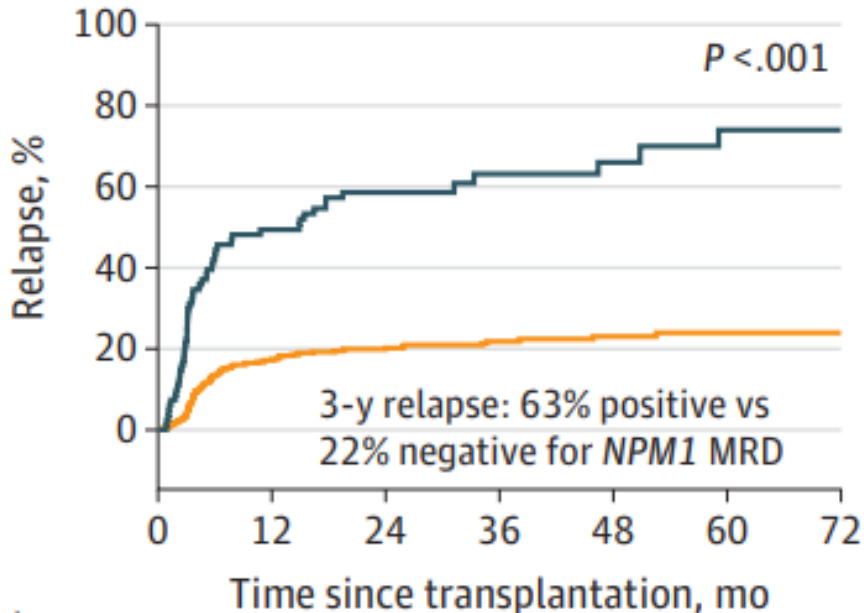


n=2939 pts

Short et al., Leukemia 2022

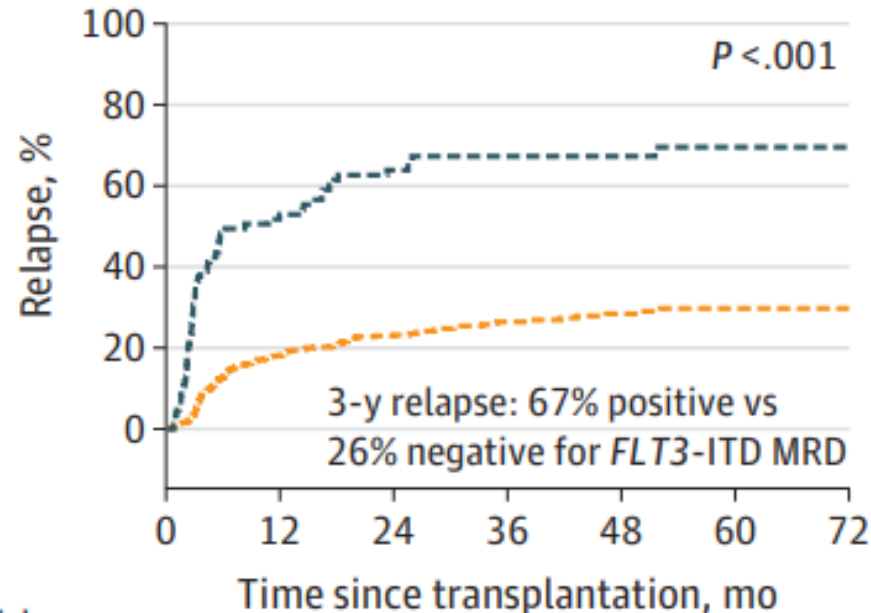
Einfluss der MRD-Positivität vor alloSCT auf das Rezidivrisiko bei AML

NPM1 MRD in patients with *NPM1*-mutated AML



No. at risk								
<i>NPM1</i> MRD								
Negative	450	323	244	138	84	59	20	
Positive	81	33	18	8	7	3	0	

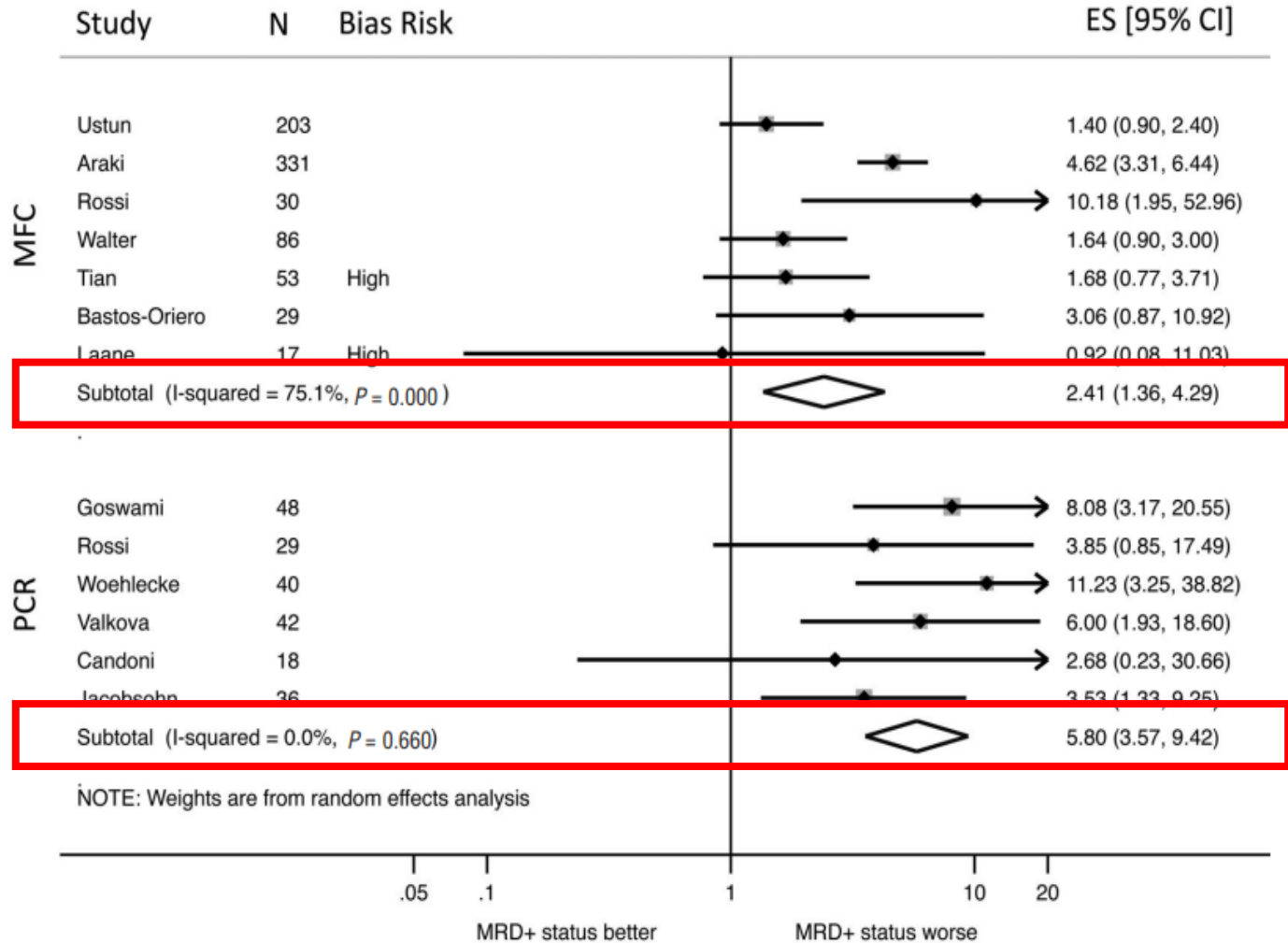
FLT3-ITD MRD in patients with *FLT3*-ITD AML



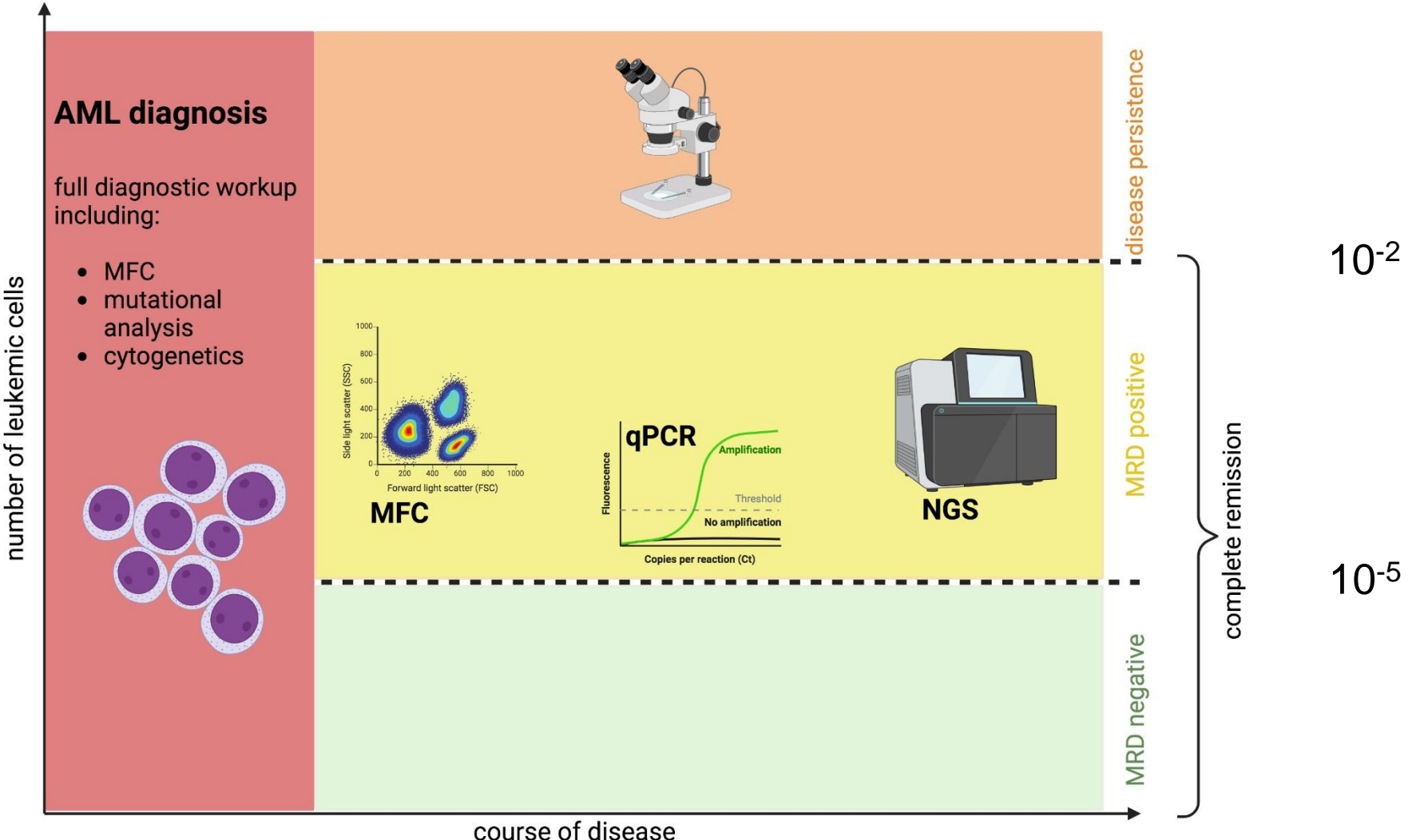
No. at risk								
<i>FLT3</i> -ITD MRD								
Negative	523	372	282	156	95	68	20	
Positive	85	32	20	11	9	8	2	

Einfluss der MRD-Positivität vor alloSCT auf das Rezidivrisiko bei AML

Impact of MRD on Leukemia-Free Survival



Methoden der MRD-Messung (bei der AML):



adapted from Moritz et al., Biomedicines 2024

Methoden der MRD-Messung: Vor- und Nachteile

Method	Sensitivity	Advantages	Disadvantages
qPCR	10^{-5}	<ul style="list-style-type: none"> • High sensitivity • Well standardized • Operator independent 	<ul style="list-style-type: none"> • Only applicable to a subset of patients (about 50%) • Does not cover clonal heterogeneity/evolution • Not directly quantitative
Multicolor flow cytometry (LAIP/DfN)	10^{-3} – 10^{-4}	<ul style="list-style-type: none"> • Applicability to nearly all patients • Rapid availability of results • Ease of quantification • Assessment of hemodilution 	<ul style="list-style-type: none"> • Difficult to standardize • Adequate interpretation requires ample experience • Possible change of immune phenotype during disease course • Requirement of fresh material
NGS	10^{-2} – 10^{-4}	<ul style="list-style-type: none"> • Broad applicability • Multiple genes analyzed at once • May cover clonal evolution during disease course 	<ul style="list-style-type: none"> • Not standardized yet • Low sensitivity due to sequencing errors unless correction methods included • Unable to distinguish true AML-specific from CH mutations in distinct cases

Methoden der MRD-Messung: quantitative PCR (qPCR)

Method	Sensitivity	Advantages	Disadvantages
qPCR	10^{-5}	<ul style="list-style-type: none"> • High sensitivity • Well standardized • Operator independent 	<ul style="list-style-type: none"> • Only applicable to a subset of patients (about 50%) • Does not cover clonal heterogeneity/evolution • Not directly quantitative
Multicolor flow cytometry (LAIP/DfN)	10^{-3} – 10^{-4}	<ul style="list-style-type: none"> • Applicability to nearly all patients • Rapid availability of results • Ease of quantification • Assessment of hemodilution 	<ul style="list-style-type: none"> • Difficult to standardize • Adequate interpretation requires ample experience • Possible change of immune phenotype during disease course • Requirement of fresh material
NGS	10^{-2} – 10^{-4}	<ul style="list-style-type: none"> • Broad applicability • Multiple genes analyzed at once • May cover clonal evolution during disease course 	<ul style="list-style-type: none"> • Not standardized yet • Low sensitivity due to sequencing errors unless correction methods included • Unable to distinguish true AML-specific from CH mutations in distinct cases

Methoden der MRD-Messung: (RT-)qPCR - Beispiele

AML:

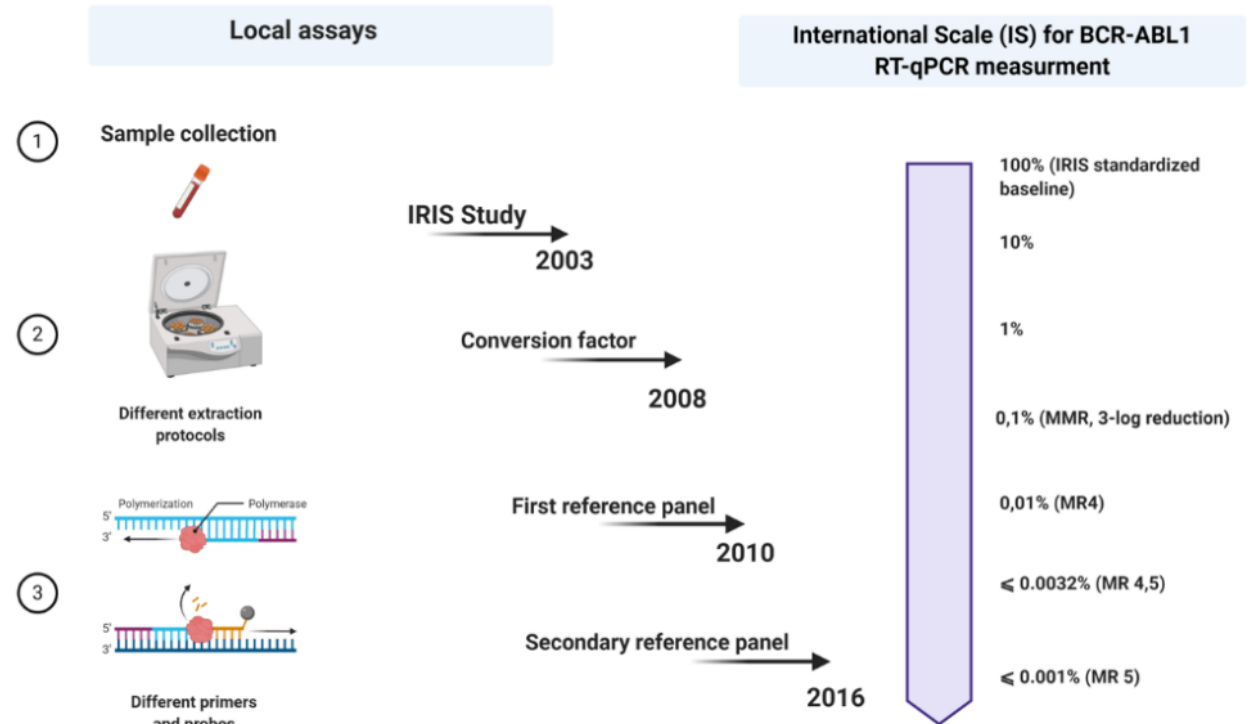
*RUNX1::RUNX1T1, CBFB::MYH11,
PML::RARA, DEK::NUP214,.....
NPM1*

ALL:

*IG/TCR-Gen-Rearrangement,
BCR::ABL1, KMT2A-Rearrangement,
ETV6::RUNX1,*

CML: *BCR::ABL1*

.....



Methoden der MRD-Messung: (RT-)qPCR - Beispiele

AML:

*RUNX1::RUNX1T1, CBFB::MYH11,
PML::RARA, DEK::NUP214,.....
NPM1*

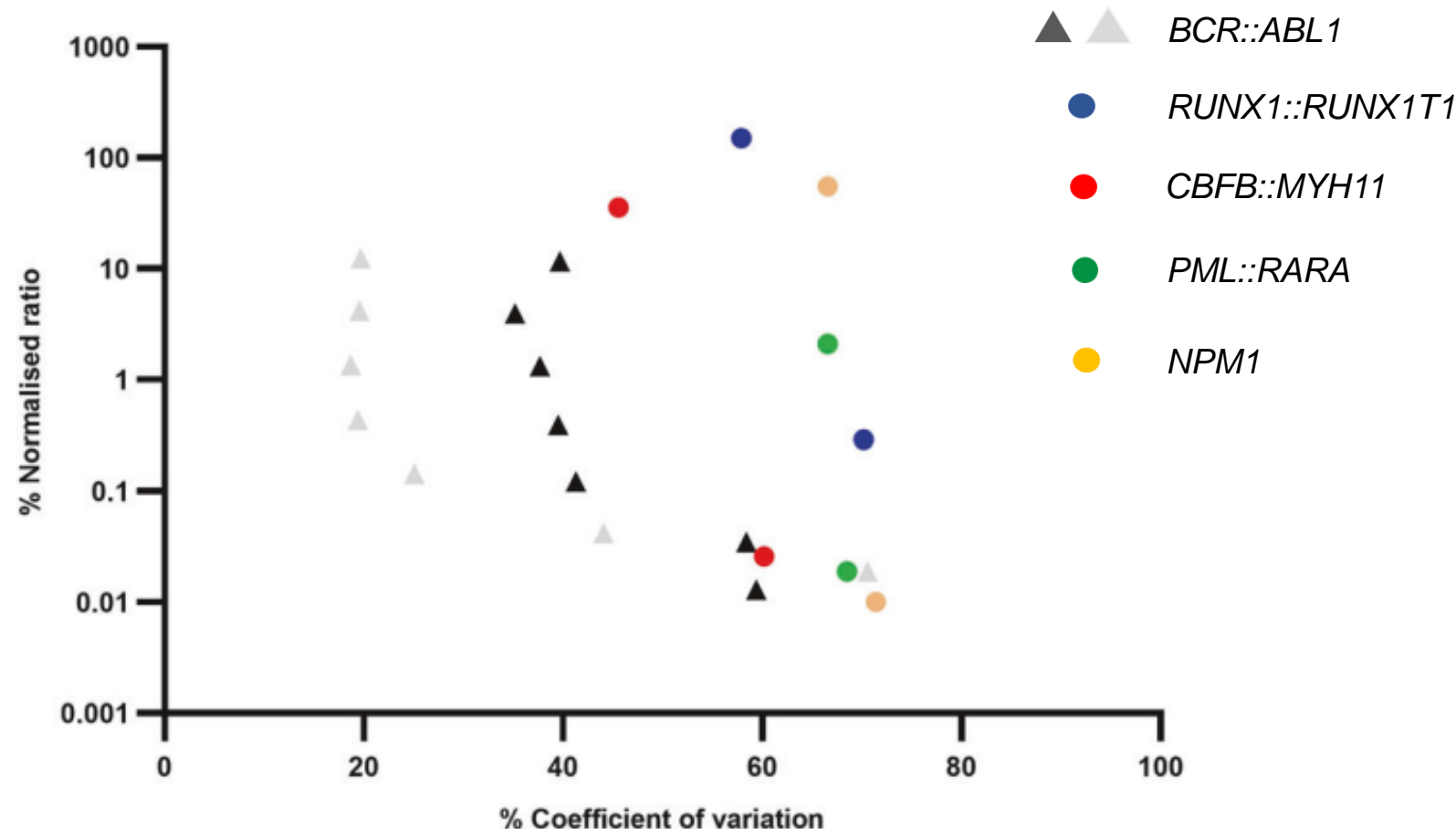
ALL:

*IG/TCR-Gen-Rearrangement,
BCR::ABL1, KMT2A-Rearrangement,
ETV6::RUNX1,*

CML: *BCR::ABL1*

.....

Comparison of interlaboratory variation



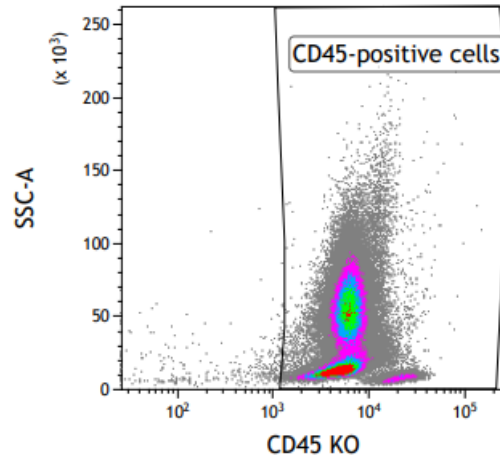
Methoden der MRD-Messung: Durchflusszytometrie (MFC)

Method	Sensitivity	Advantages	Disadvantages
qPCR	10^{-5}	<ul style="list-style-type: none"> • High sensitivity • Well standardized • Operator independent 	<ul style="list-style-type: none"> • Only applicable to a subset of patients (about 50%) • Does not cover clonal heterogeneity/evolution • Not directly quantitative
Multicolor flow cytometry (LAIP/DfN)	10^{-3} – 10^{-4}	<ul style="list-style-type: none"> • Applicability to nearly all patients • Rapid availability of results • Ease of quantification • Assessment of hemodilution 	<ul style="list-style-type: none"> • Difficult to standardize • Adequate interpretation requires ample experience • Possible change of immune phenotype during disease course • Requirement of fresh material
NGS	10^{-2} – 10^{-4}	<ul style="list-style-type: none"> • Broad applicability • Multiple genes analyzed at once • May cover clonal evolution during disease course 	<ul style="list-style-type: none"> • Not standardized yet • Low sensitivity due to sequencing errors unless correction methods included • Unable to distinguish true AML-specific from CH mutations in distinct cases

Durchflusszytometrie (MFC) - Beispiel

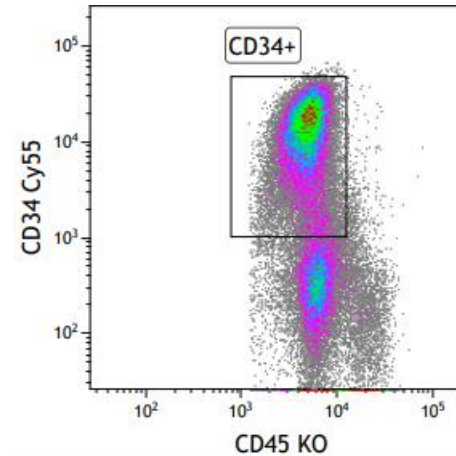
**AML
Diagnose**

single cells



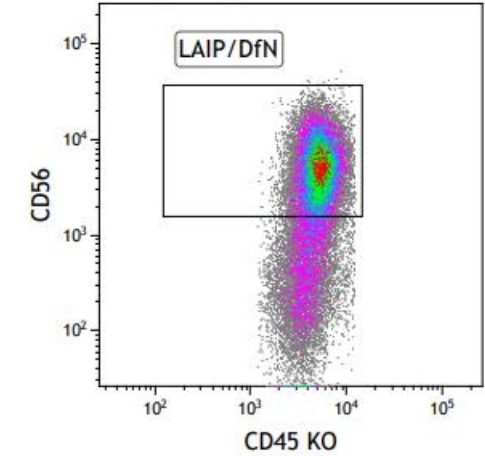
Gate	Number	%Total	%Gated
All	104.028	97,38	100,00
CD45-positive cells	103.647	97,03	99,63

MNCs



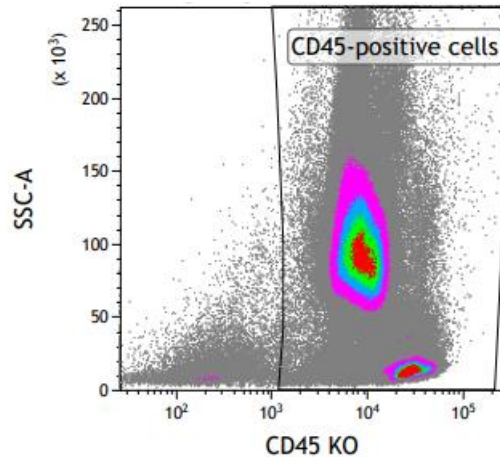
Gate	Number	%Total	%Gated
All	55.871	52,30	100,00
CD34+	33.200	31,08	59,42

CD34⁺ cells

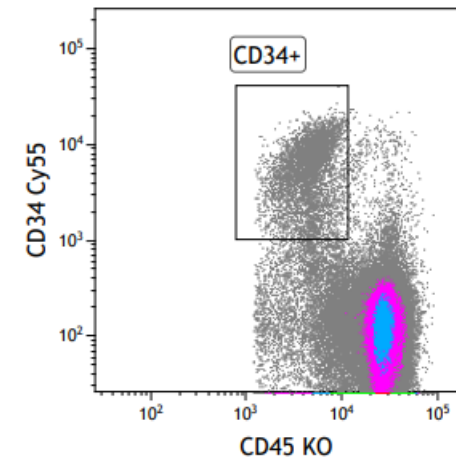


Gate	Number	%Total	%Gated
All	33.200	31,08	100,00
LAIP/DfN	22.930	21,47	69,07

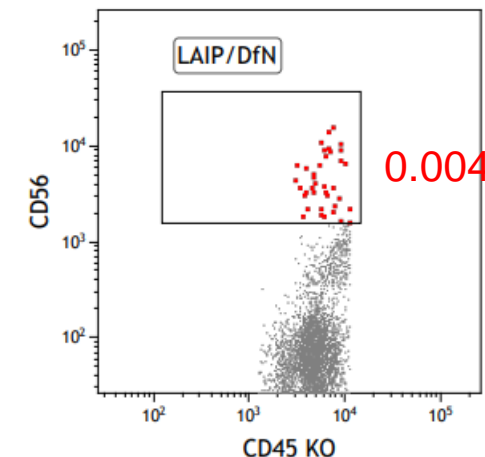
**normales
Knochenmark**



Gate	Number	%Total	%Gated
All	827.396	98,98	100,00
CD45-positive cells	809.136	96,79	97,79



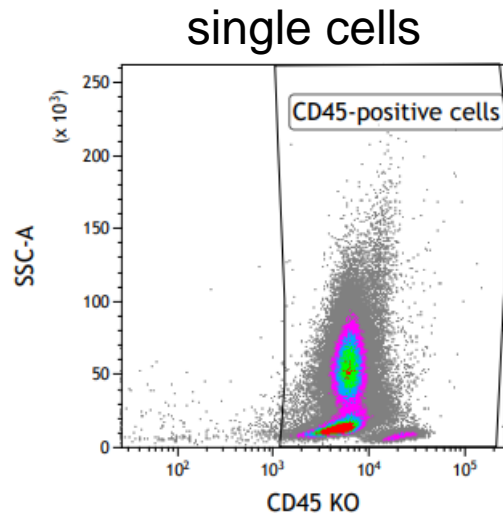
Gate	Number	%Total	%Gated
All	144.741	17,31	100,00
CD34+	6.044	0,72	4,18



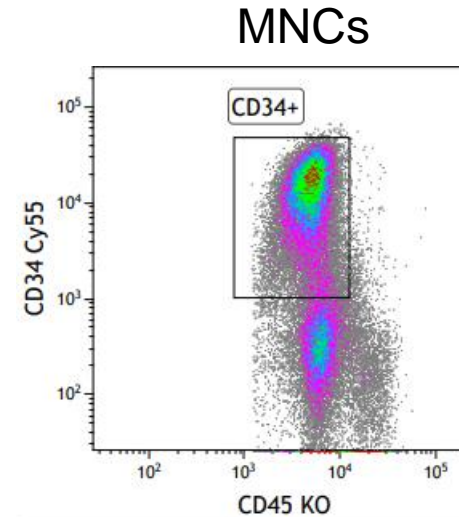
Gate	Number	%Total	%Gated
All	6.044	0,72	100,00
LAIP/DfN	38	0,00	0,63

Durchflusszytometrie (MFC) - Beispiel

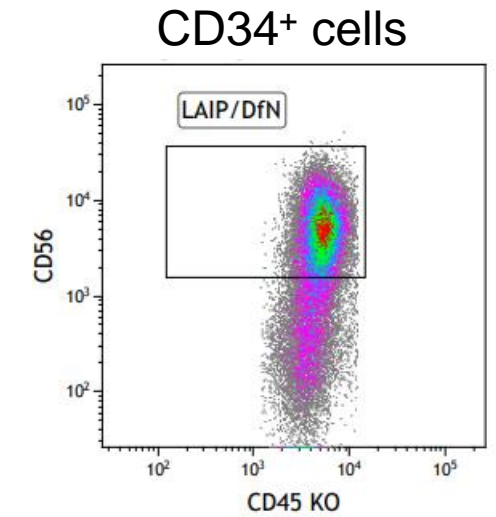
AML Diagnose



Gate	Number	%Total	%Gated
All	104.028	97,38	100,00
CD45-positive cells	103.647	97,03	99,63

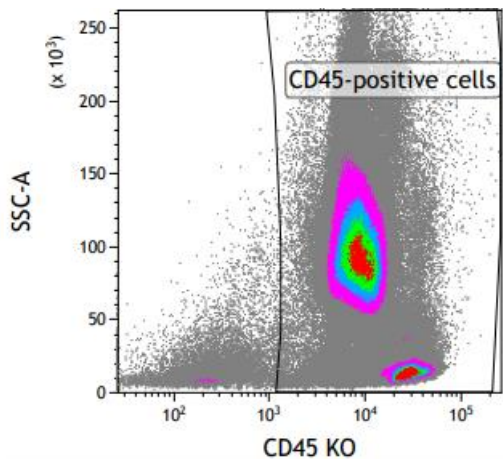


Gate	Number	%Total	%Gated
All	55.871	52,30	100,00
CD34+	33.200	31,08	59,42

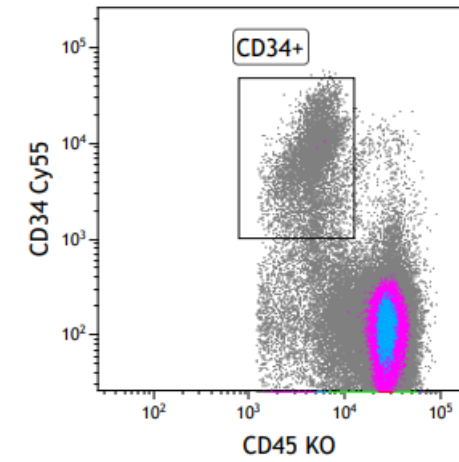


Gate	Number	%Total	%Gated
All	33.200	31,08	100,00
LAIP/DfN	22.930	21,47	69,07

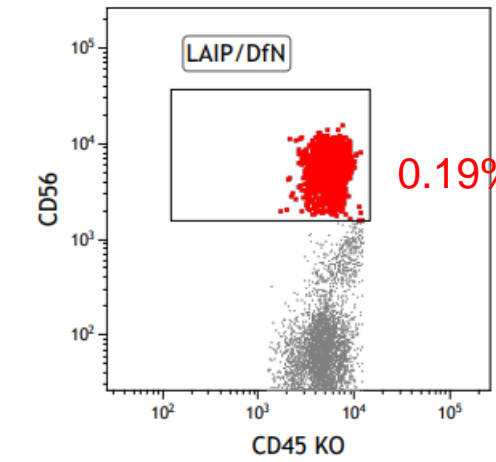
AML Remission



Gate	Number	%Total	%Gated
All	827.279	98,96	100,00
CD45-positive cells	808.892	96,76	97,78

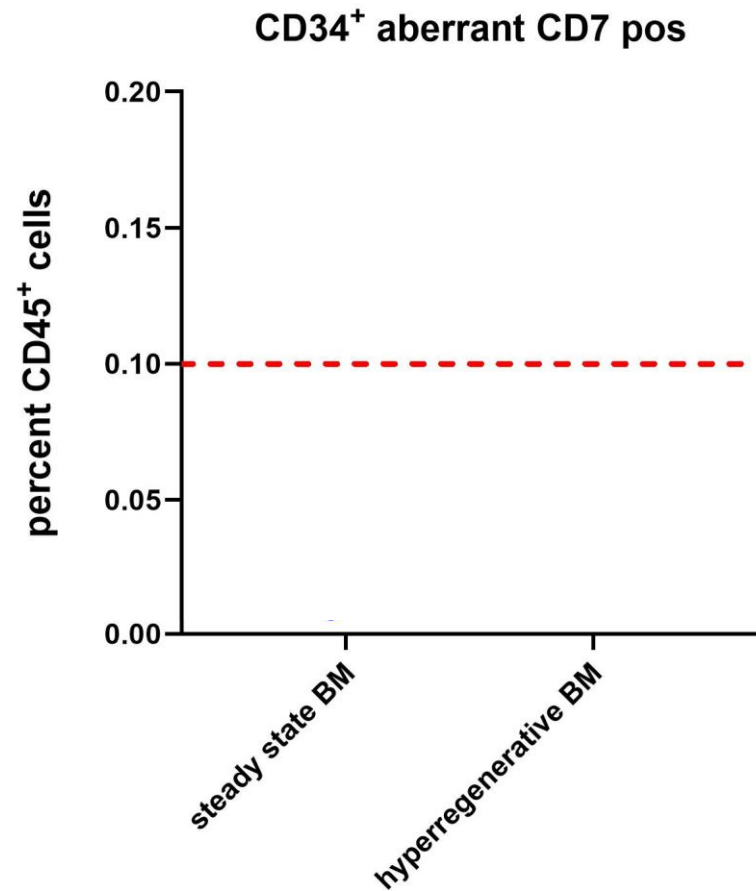


Gate	Number	%Total	%Gated
All	144.678	17,31	100,00
CD34+	7.577	0,91	5,24



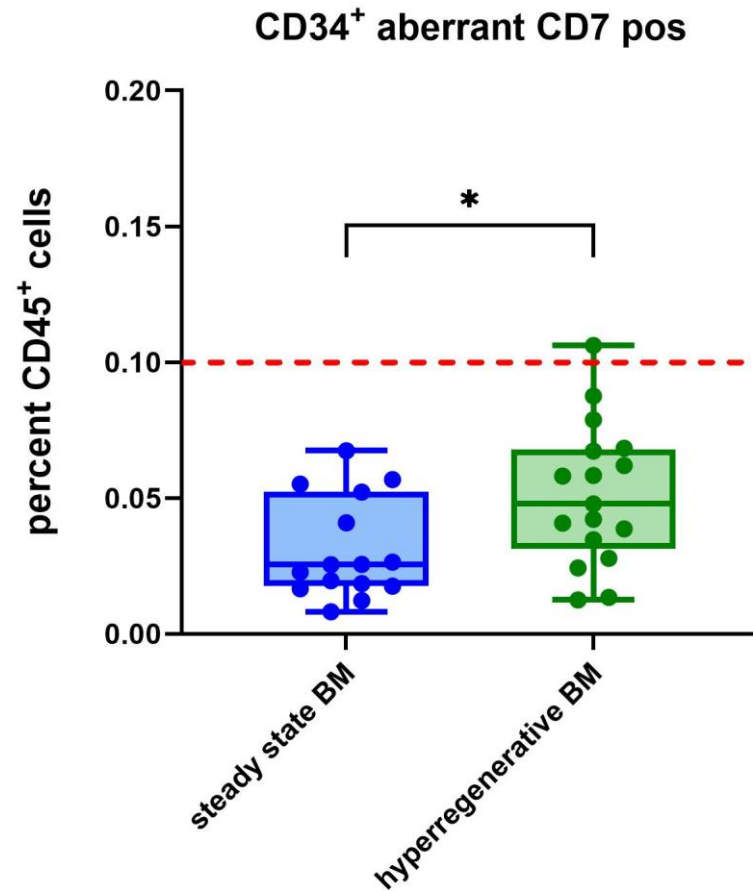
Gate	Number	%Total	%Gated
All	7.577	0,91	100,00
LAIP/DfN	1.538	0,18	20,30

Durchflusszytometrie zur MRD-Messung: Limitationen



Durchflusszytometrie zur MRD-Messung: Limitationen

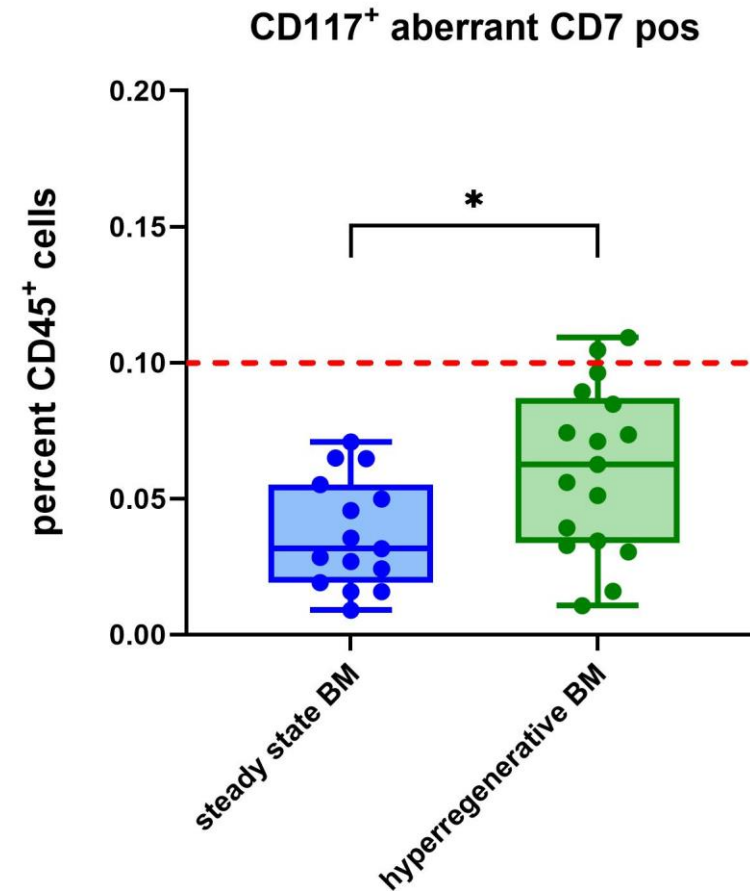
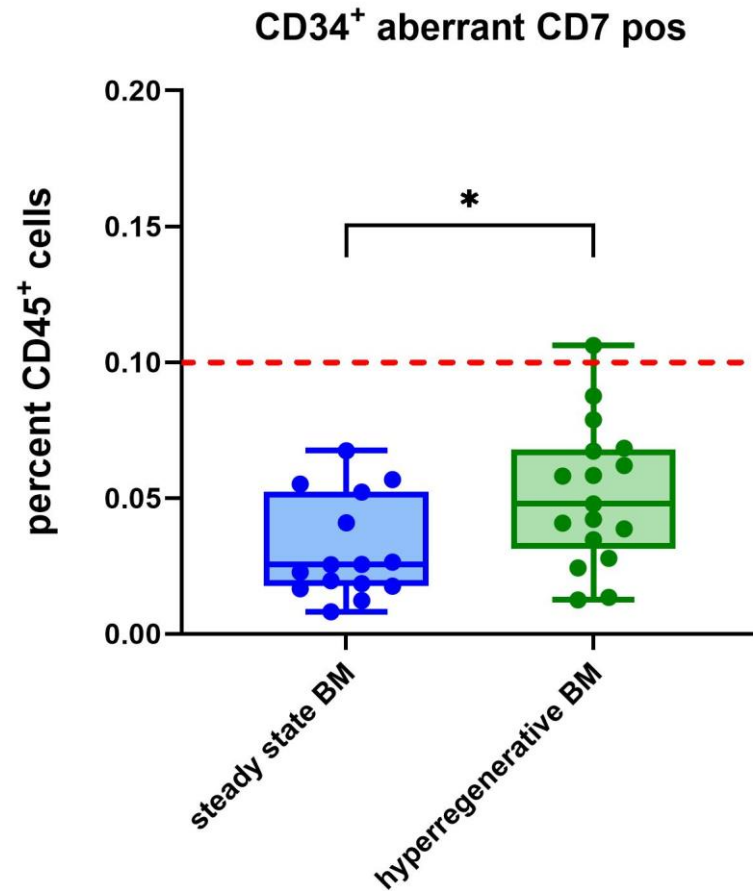
normal bone marrow without evidence of disease



* p<0.05

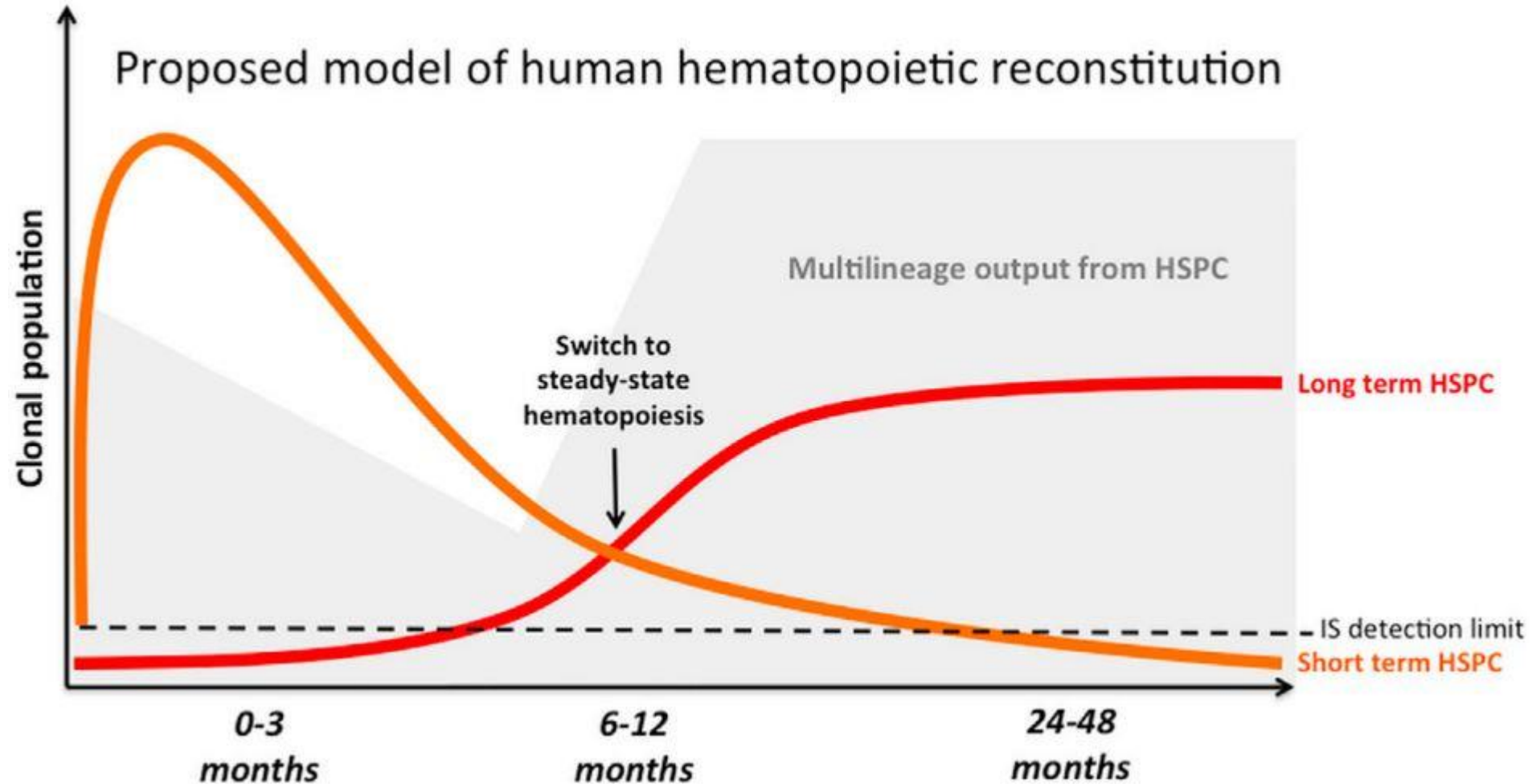
Durchflusszytometrie zur MRD-Messung: Limitationen

normal bone marrow without evidence of disease



* p<0.05

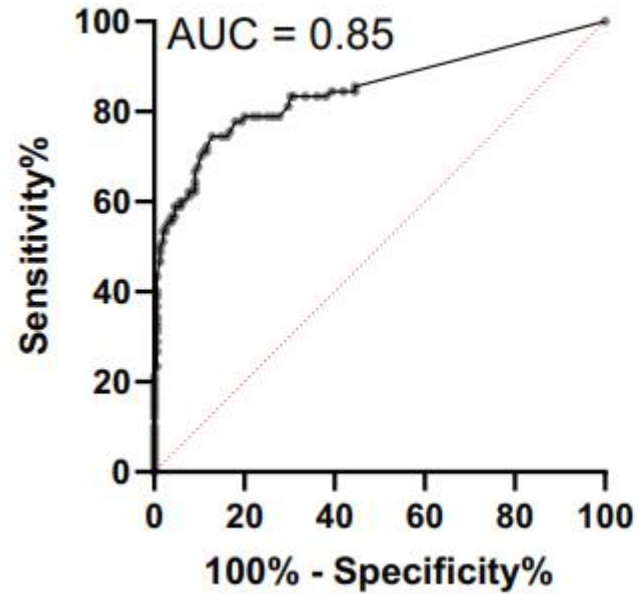
Hämatopoietische Rekonstitution nach Stammzelltransplantation:



Verbessertes MRD-Monitoring: MFC computational unsupervised analysis

true positives: 136 pts (median 2.5 months before relapse)

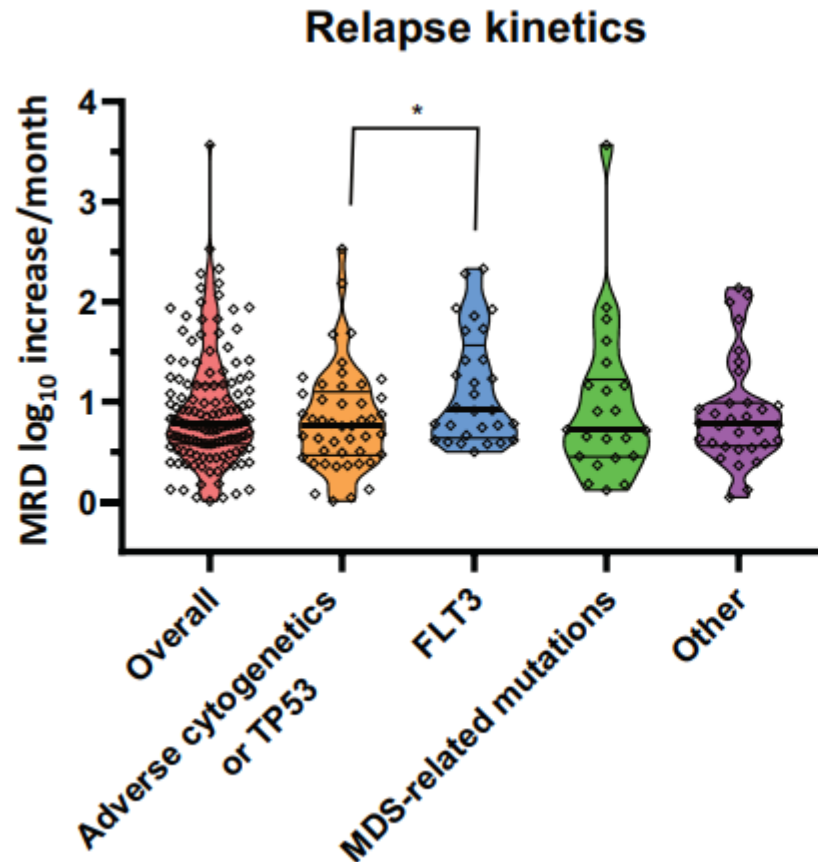
true negatives: 155 pts



Threshold			
Type	Value	Sensitivity	Specificity
MaxStat optimum	0.040%	74%	87%
Standard	0.050%	64%	91%
Standard	0.100%	51%	98%

Verbessertes MRD-Monitoring: MFC computational unsupervised analysis

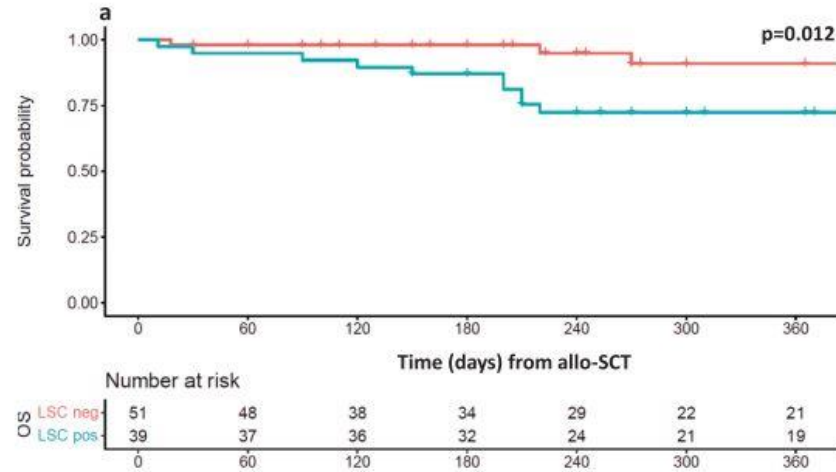
true positives: 136 pts (median 2.5 months before relapse)



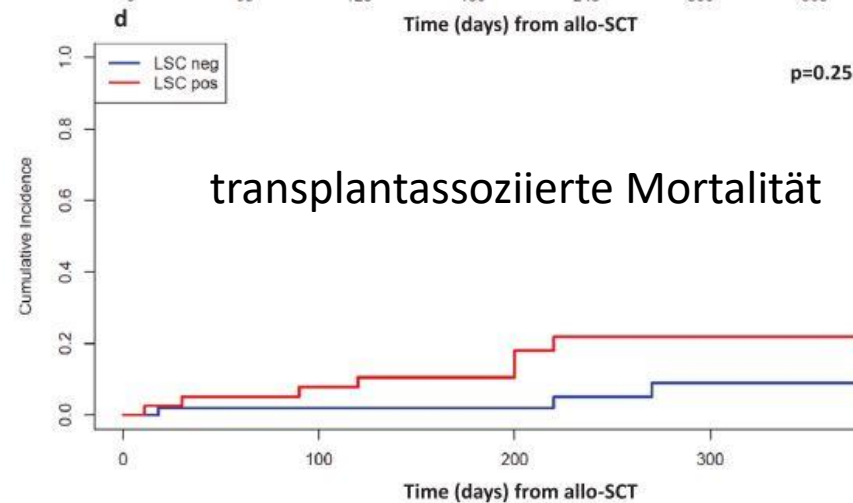
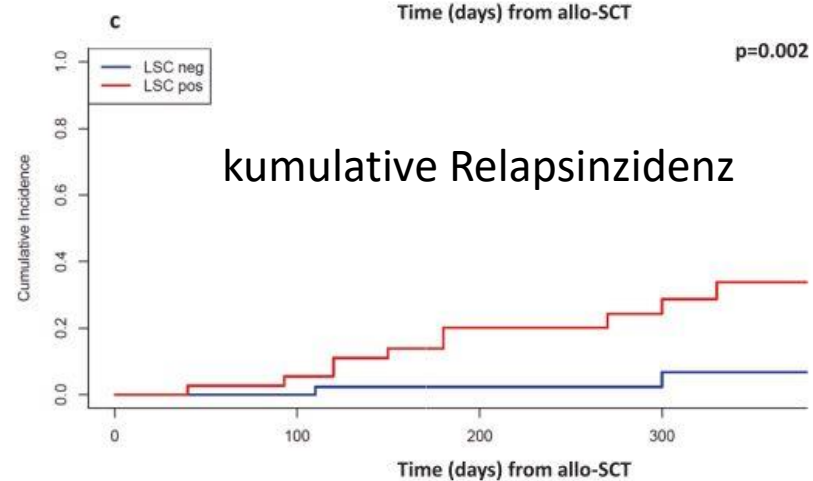
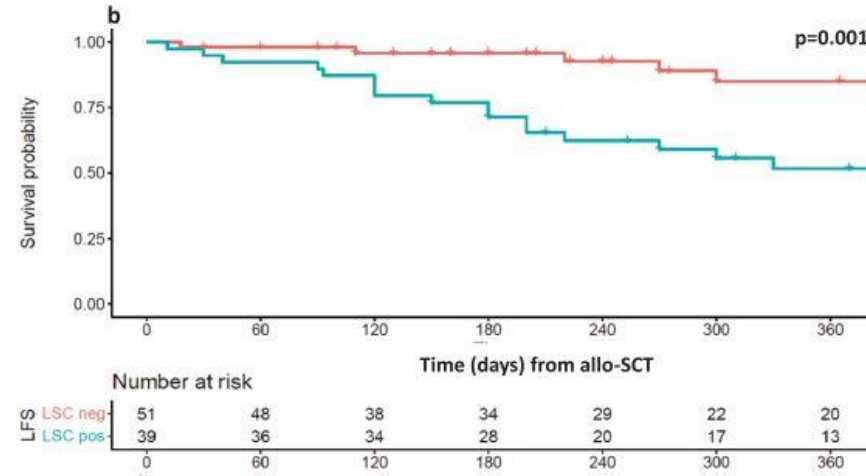
Group	n=	Log ₁₀ increase, median [IQR]
Overall	136	0.78 [0.58 - 1.17]
Adverse cytogenetics or TP53	46	0.77 [0.48 - 1.07]
FLT3	29	0.92 [0.66 - 1.42]
MDS-related mutations	22	0.72 [0.46 - 1.17]
Other	32	0.78 [0.57 - 0.99]

Verbessertes MRD-Monitoring mittels MFC-LSC:

Gesamtüberleben



Leukämiefreies Überleben



Verwendung zusätzlicher Marker in der CD34⁺38⁻-LSC-Population:

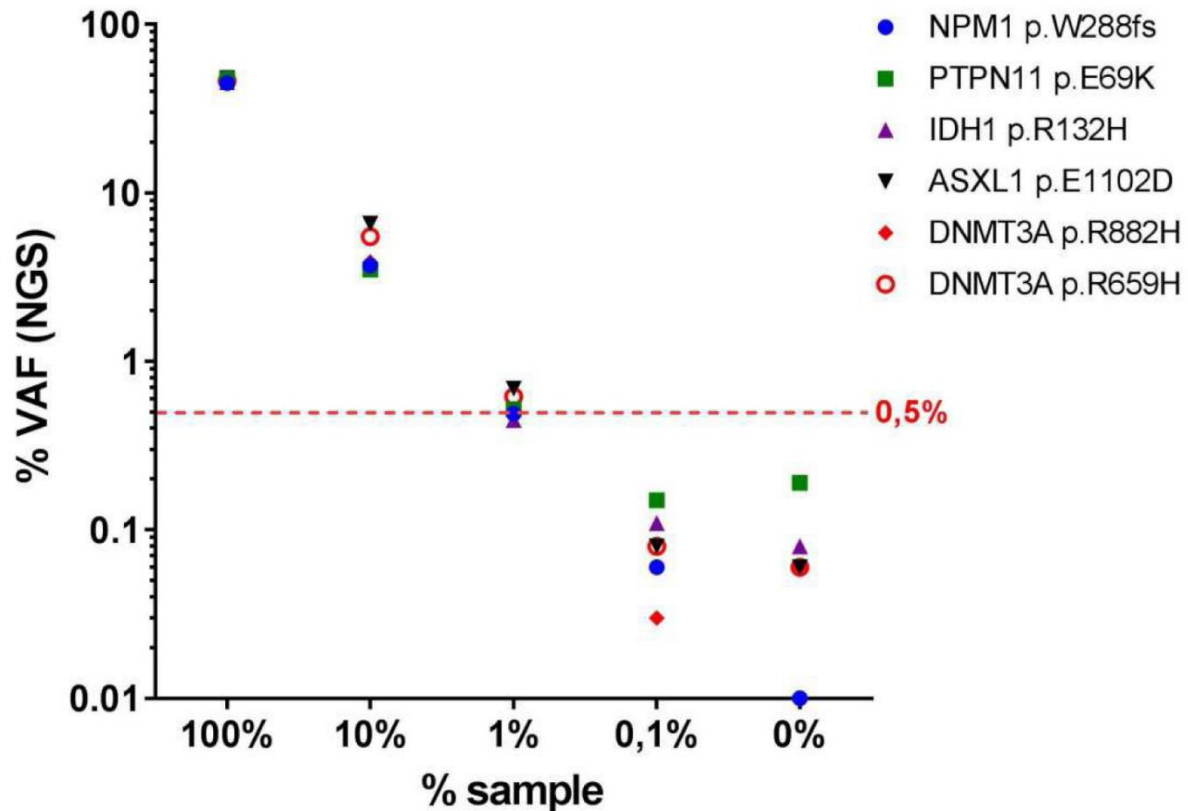
CD366, CD123, CD371, CD7, CD11b, CD22, CD56

MRD detection in AML: current limitations

Method	Sensitivity	Advantages	Disadvantages
qPCR	10^{-5}	<ul style="list-style-type: none"> • High sensitivity • Well standardized • Operator independent 	<ul style="list-style-type: none"> • Only applicable to a subset of patients (about 50%) • Does not cover clonal heterogeneity/evolution • Not directly quantitative
Multicolor flow cytometry (LAIP/DfN)	10^{-3} – 10^{-4}	<ul style="list-style-type: none"> • Applicability to nearly all patients • Rapid availability of results • Ease of quantification • Assessment of hemodilution 	<ul style="list-style-type: none"> • Difficult to standardize • Adequate interpretation requires ample experience • Possible change of immune phenotype during disease course • Requirement of fresh material
NGS	10^{-2} – 10^{-4}	<ul style="list-style-type: none"> • Broad applicability • Multiple genes analyzed at once • May cover clonal evolution during disease course 	<ul style="list-style-type: none"> • Not standardized yet • Low sensitivity due to sequencing errors unless correction methods included • Unable to distinguish true AML-specific from CH mutations in distinct cases

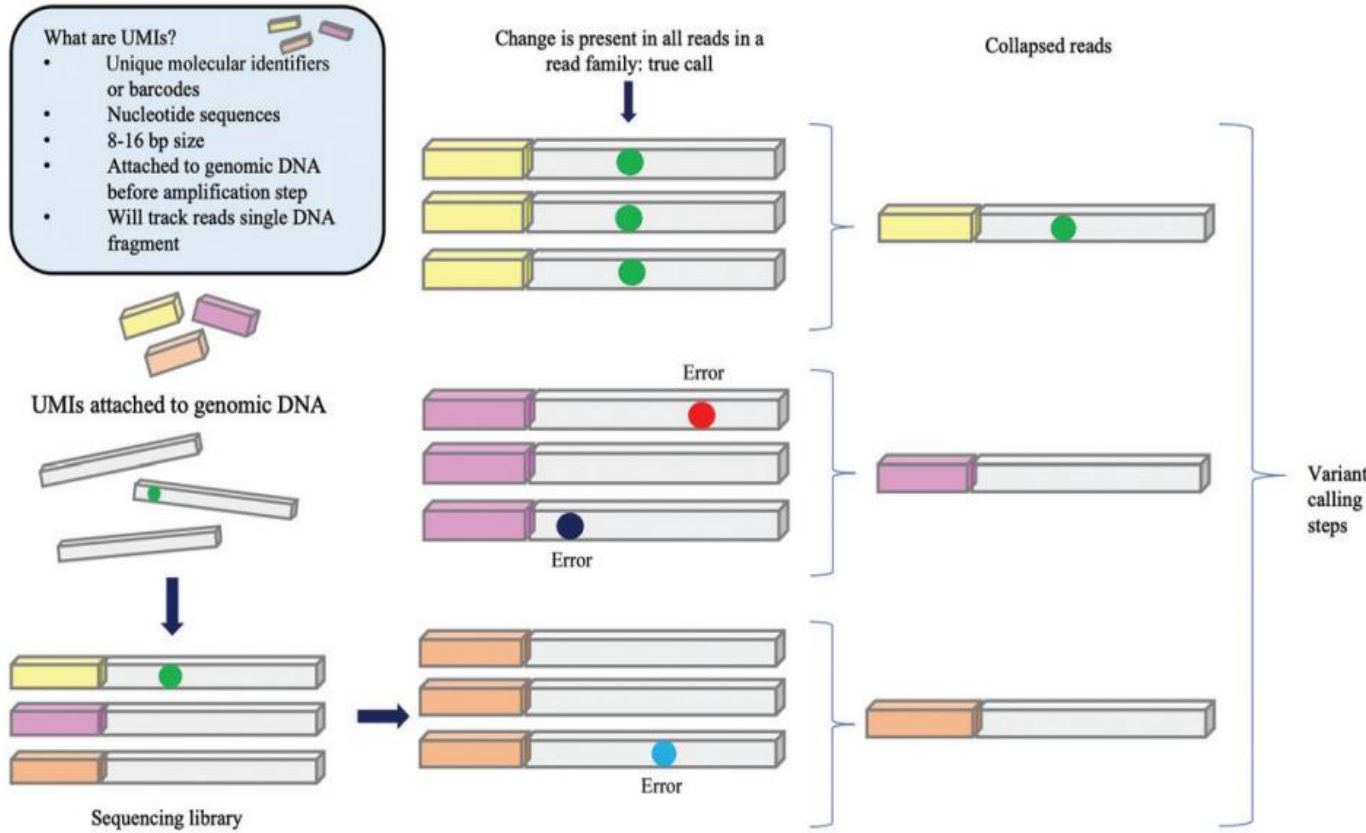
MRD-Messung mittels NGS: Sensitivität und Spezifität

Assessment of analytical sensitivity of NGS



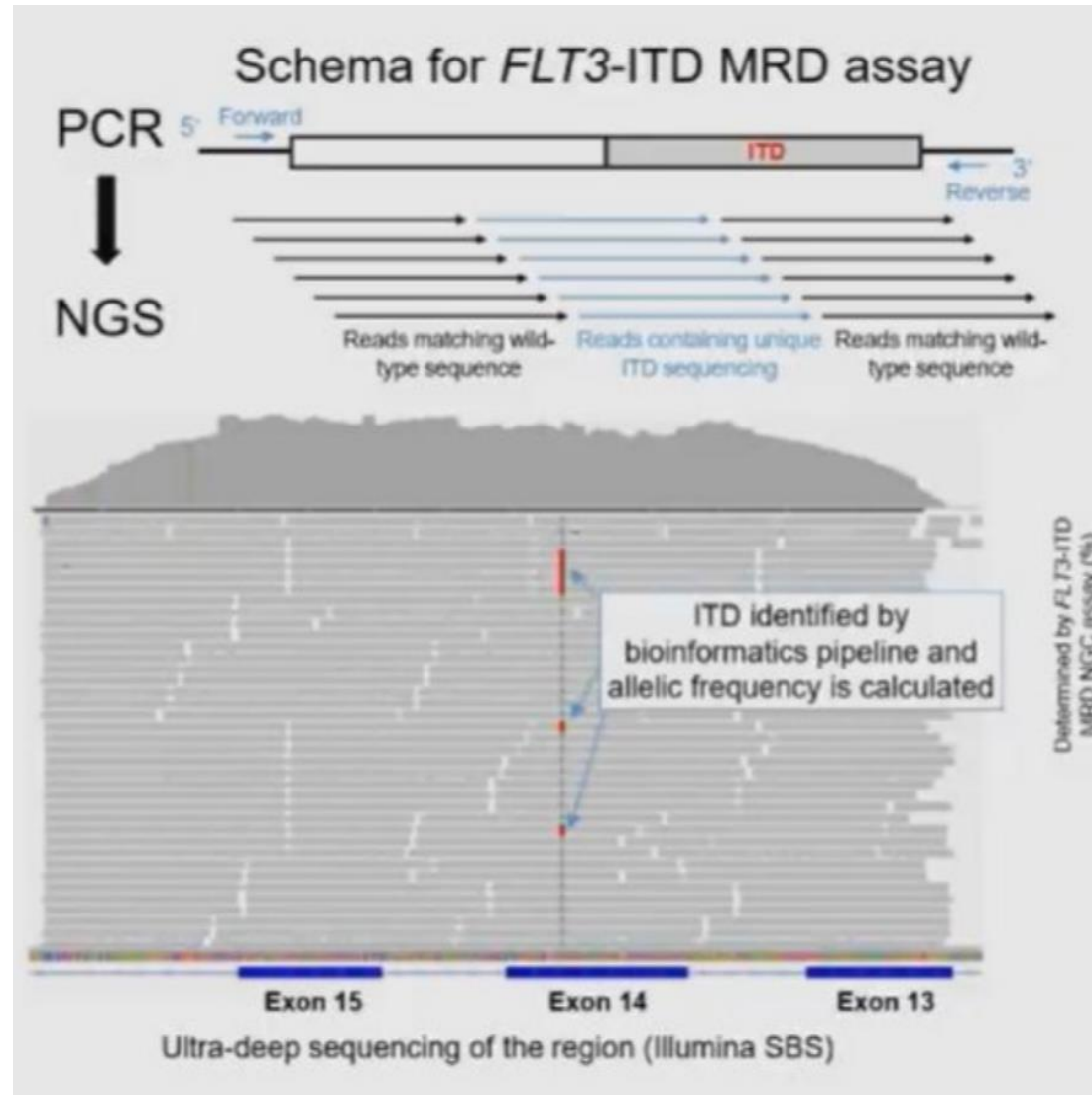
Mutation	Limit of detection* (%)	
	diagnosis	remission
DNMT3A NM_022552, c. 2645G>A, p.R882H (8 pts.)	0,23	0,30
IDH1 NM_001282386, c. 394C>T, p.R132C (1 pt.)	0,24	0,30
IDH2 NM_002168, c. 419 G>A, p.R140Q (1 pt.)	0,63	0,21
RUNX1 NM_001754, c. 485G>A, p.R162K (1 pt.)	0,22	0,45
GATA2 NM_001145661, c.952G>A, p.A318T (1 pt.)	0,36	0,46
FLT3 NM_004119, c. 2503G>T, p.D835Y (2 pts.)	0,40	0,27
WT1 NM_024426, c. 1385G>A, p.R462Q (1 pt.)	0,27	0,79
TP53 NM_000546, c. 266C>G, p.P89R (1 pt.)	0,10	0,08
NRAS NM_002524, c. 34G>T, p.G12C (1 pt.)	0,02	0,13
PTPN11 NM_002834, c. 205G>A, p.E69K (1 pt.)	0,29	0,56
CEBPA NM_004364, c. 928A>G, p.T310A (1 pt.)	0,80	0,55
TET2 NM_001127208, c. 1630C>T, p.R544* (1 pt.)	0,17	0,26
TET2 NM_001127208, c. 3845G>A, p.G1282D (1 pt.)	0,20	0,30
TET2 NM_001127208, c. 3898T>A, p.F1300I (1 pt.)	0,08	0,01
NPM1 NM_002520, c.863_864insTCTG, p.W288fs (12 pts.)	0,10	0,02
WT1 NM_024426, c.1145_1146insCCGA ,p. A382fs (1 pt.)	0,03	0,01
average	0,26	0,29

Verbessertes MRD-Monitoring: Error-corrected NGS



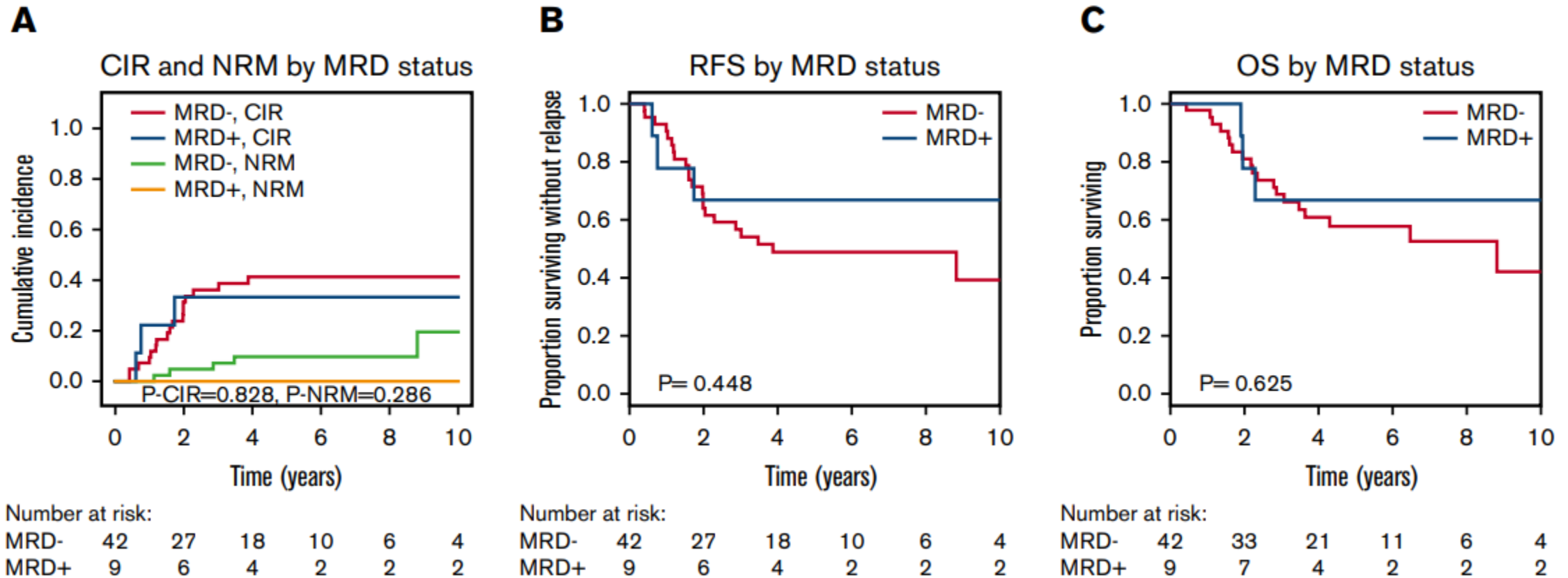
1. Physical/ Biochemical error correction methods
<p>Changes in library preparation</p> <ul style="list-style-type: none"> • Incorporation of unique molecular identifiers (UMI) <ul style="list-style-type: none"> - Vendor vs laboratory- derived - PCR based, smMIPS based - Single UMI vs Duplex UMI • Incorporation of proof-reading polymerase
2. Computational methods
Removal of reads with high base error rates (Q scores <30)
Removal of reads with low mapping scores
Determination of background error rate by sequencing control specimens
Use of algorithms according to specific variants eg for <i>FLT3</i> -ITD
3. Combination of 1 and 2

Verbessertes MRD-Monitoring: PCR-NGS zum Nachweis einer FLT3-ITD



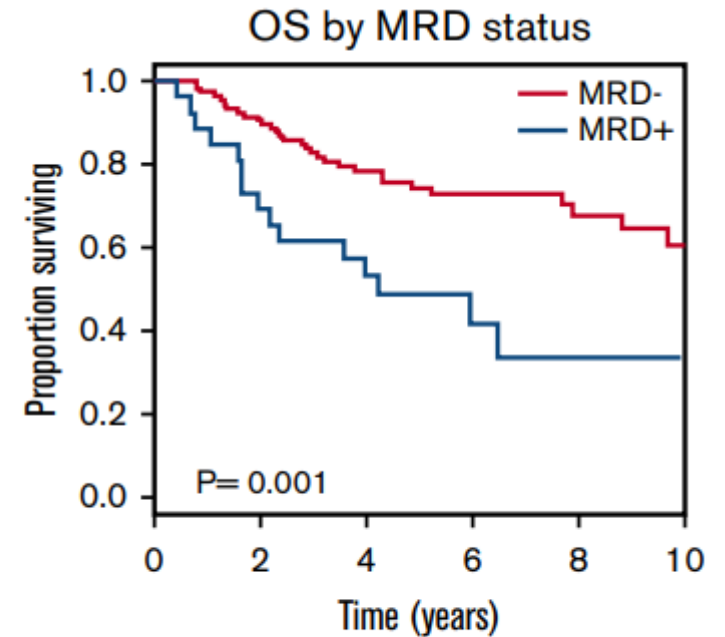
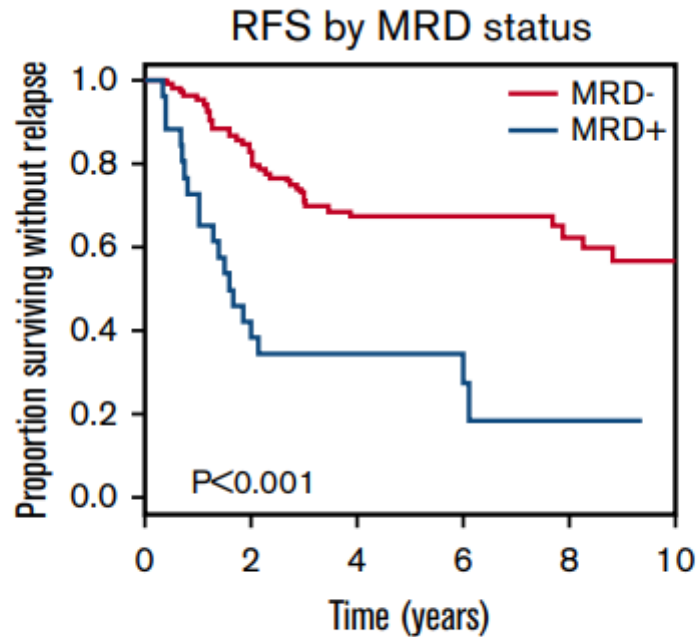
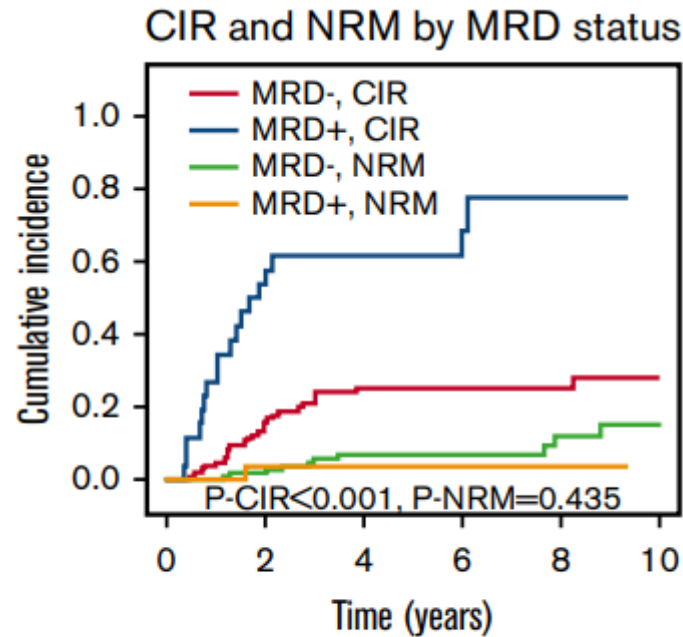
MRD-Monitoring nach alloSCT: Mutationen assoziiert mit klonaler Hämatopoese

DTA-Mutationen (*DNMT3A*, *TET2*, *ASXL1*)

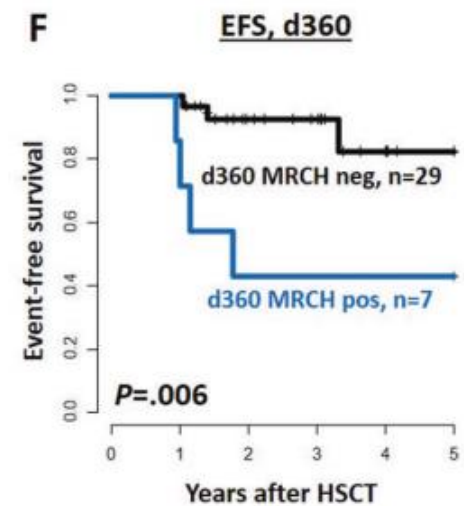
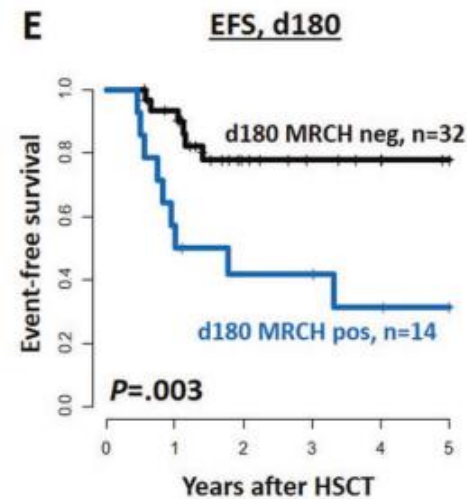
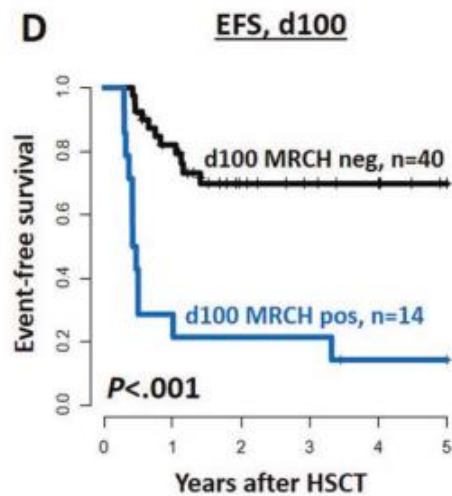
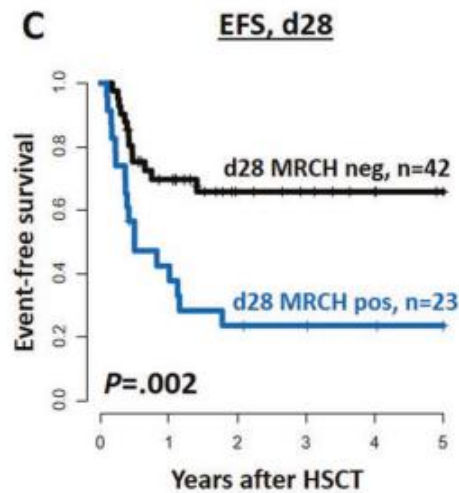
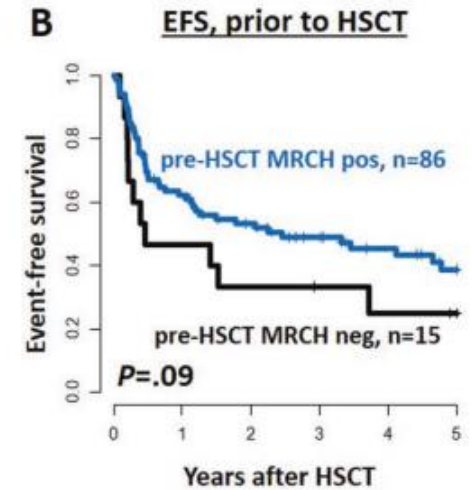
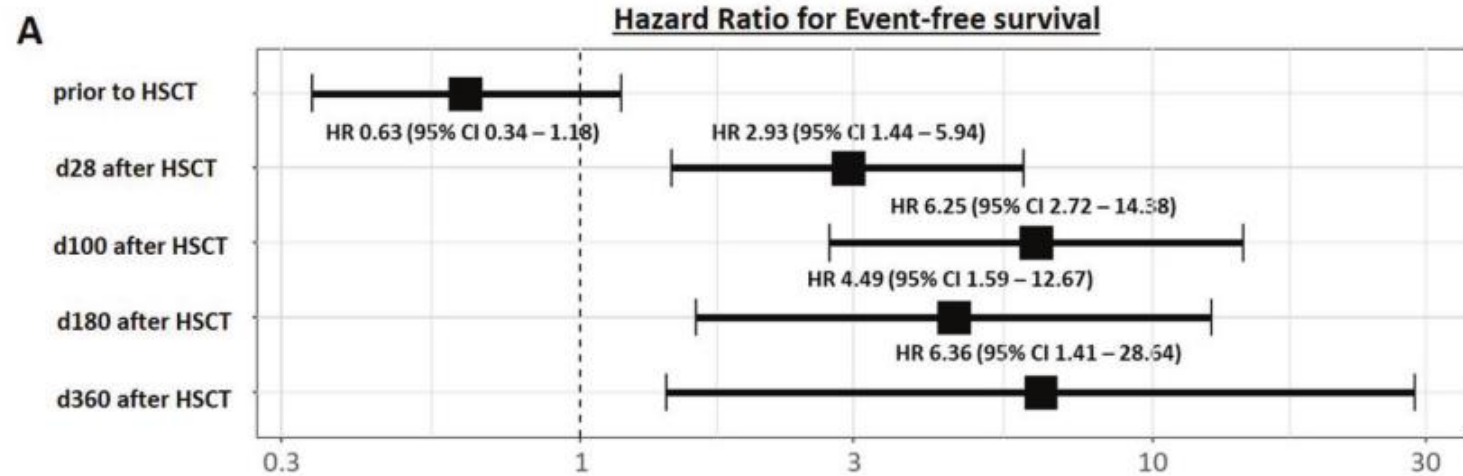


MRD-Monitoring nach alloSCT: Mutationen assoziiert mit klonaler Hämatopoese

Non-DTA-Mutationen



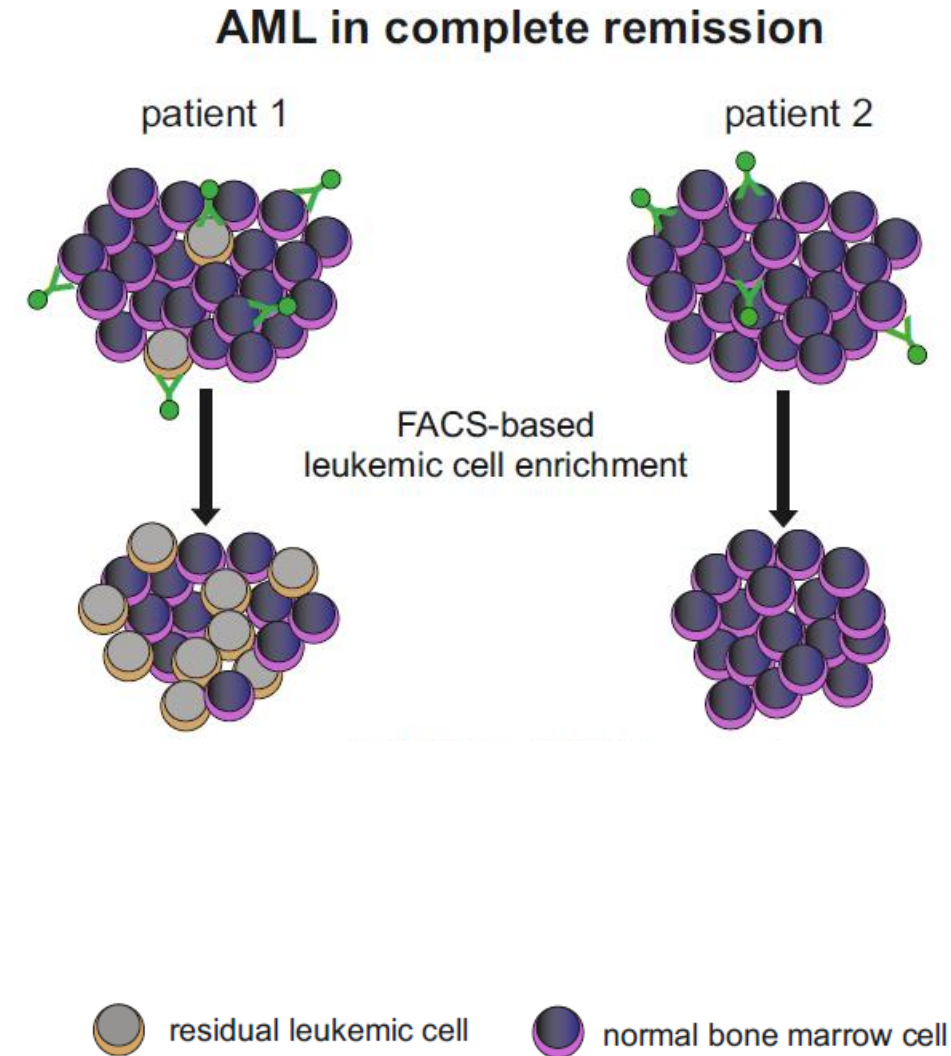
MRD-Monitoring nach alloSCT: Mutationen assoziiert mit klonaler Hämatopoese



Kombination von MFC-basierter Zellenanreicherung und NGS:

two-step process for MRD detection:

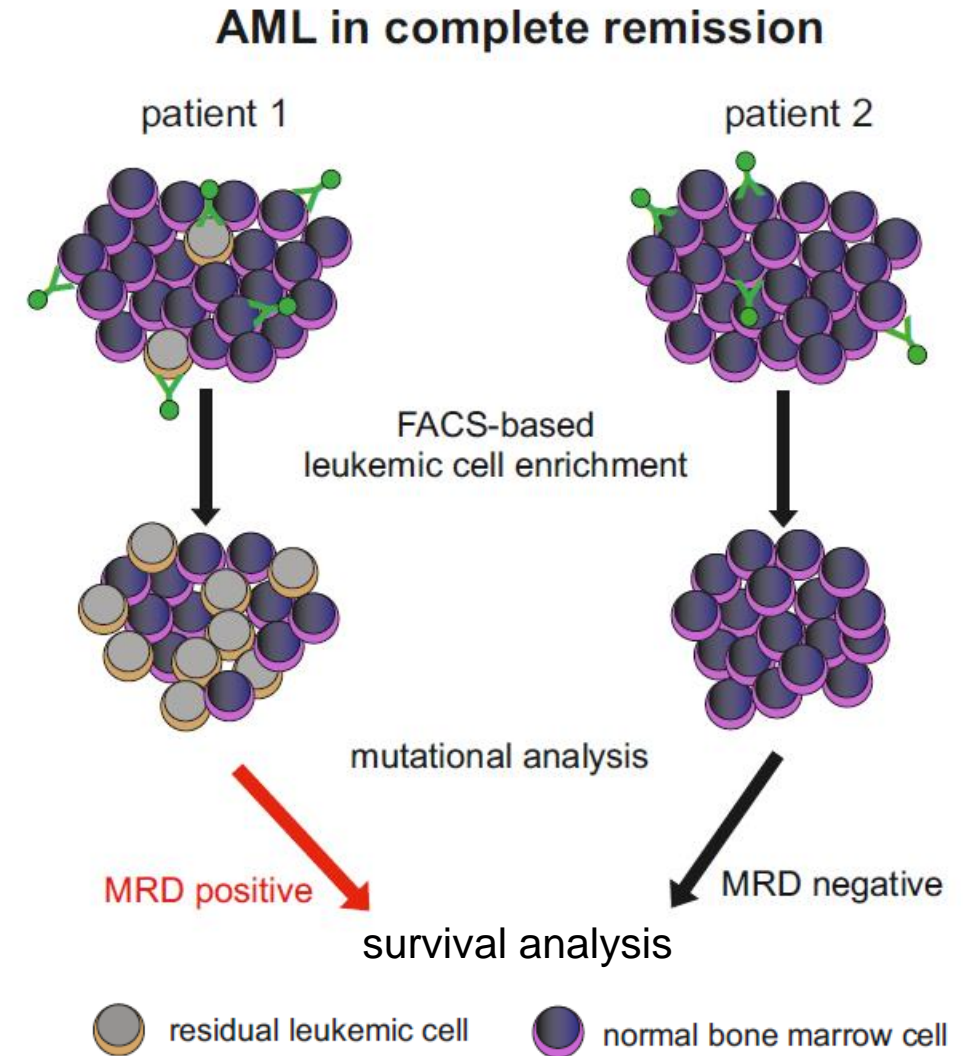
- ◆ enrichment for residual leukemic cells using flow cytometry-based sorting (CD117, CD123, CD366, CD371)



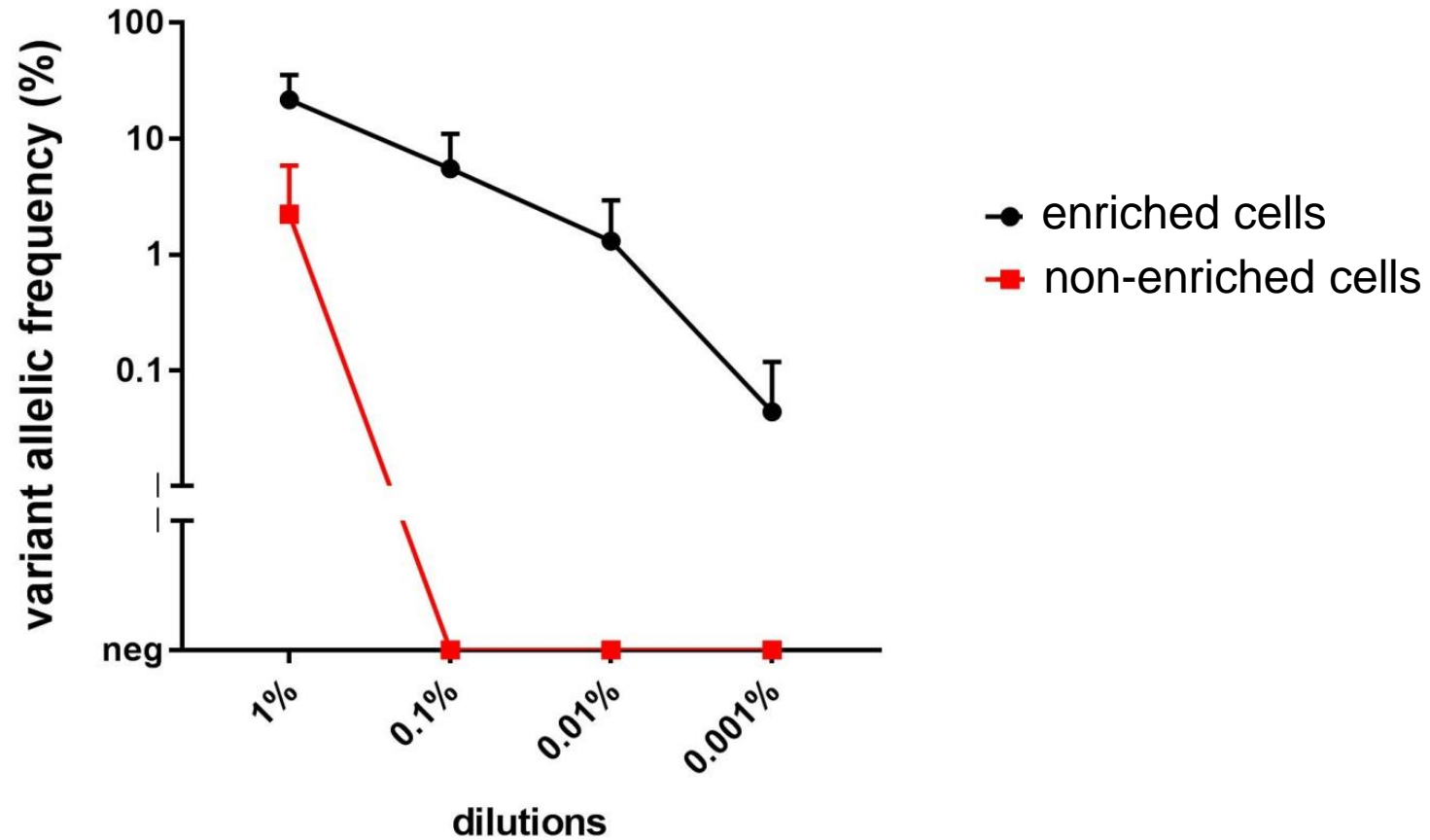
Kombination von MFC-basierter Zellenanreicherung und NGS:

two-step process for MRD detection:

- ◆ enrichment for residual leukemic cells using flow cytometry-based sorting (CD117, CD123, CD366, CD371)
- ◆ applying NGS for detection of leukemia-specific mutations (panel of 39 genes recurrently mutated in AML)

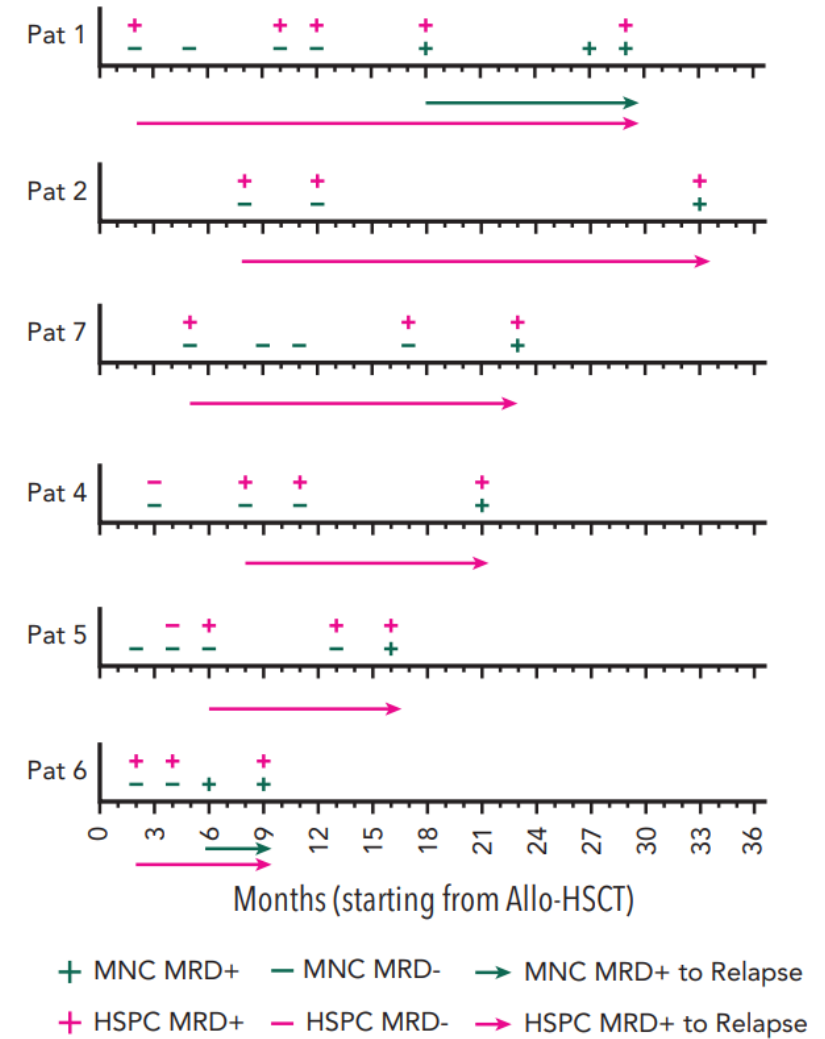
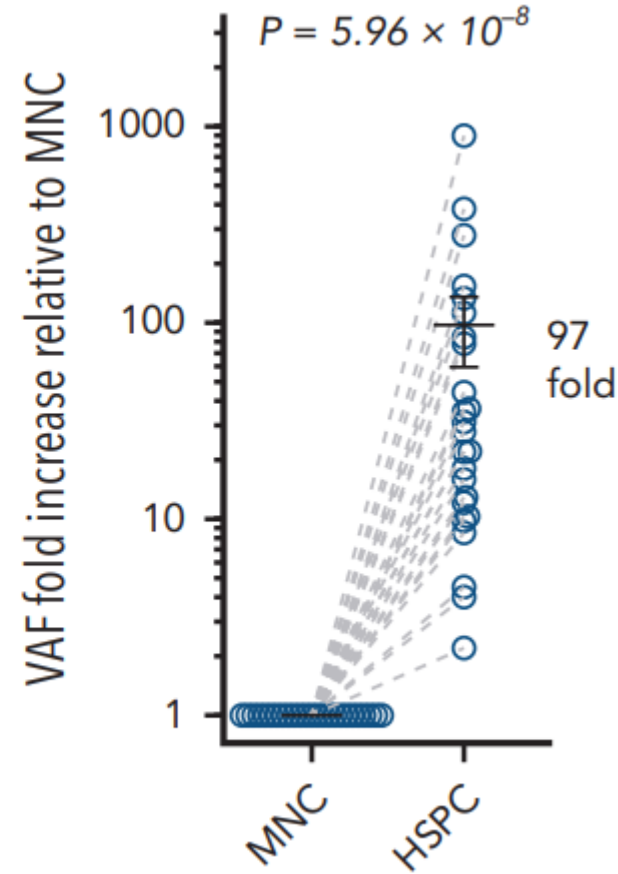
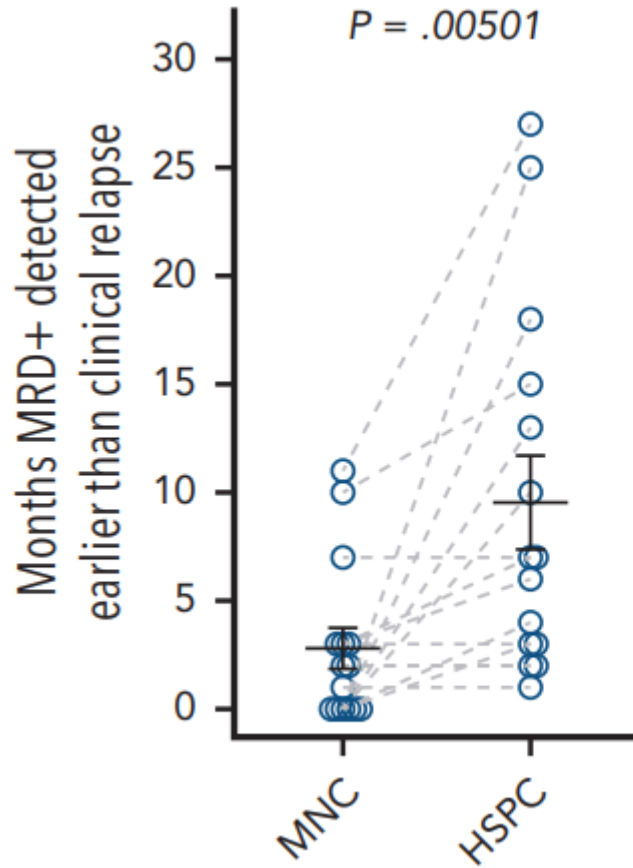


Kombination von MFC-basierter Zellenanreicherung und NGS:

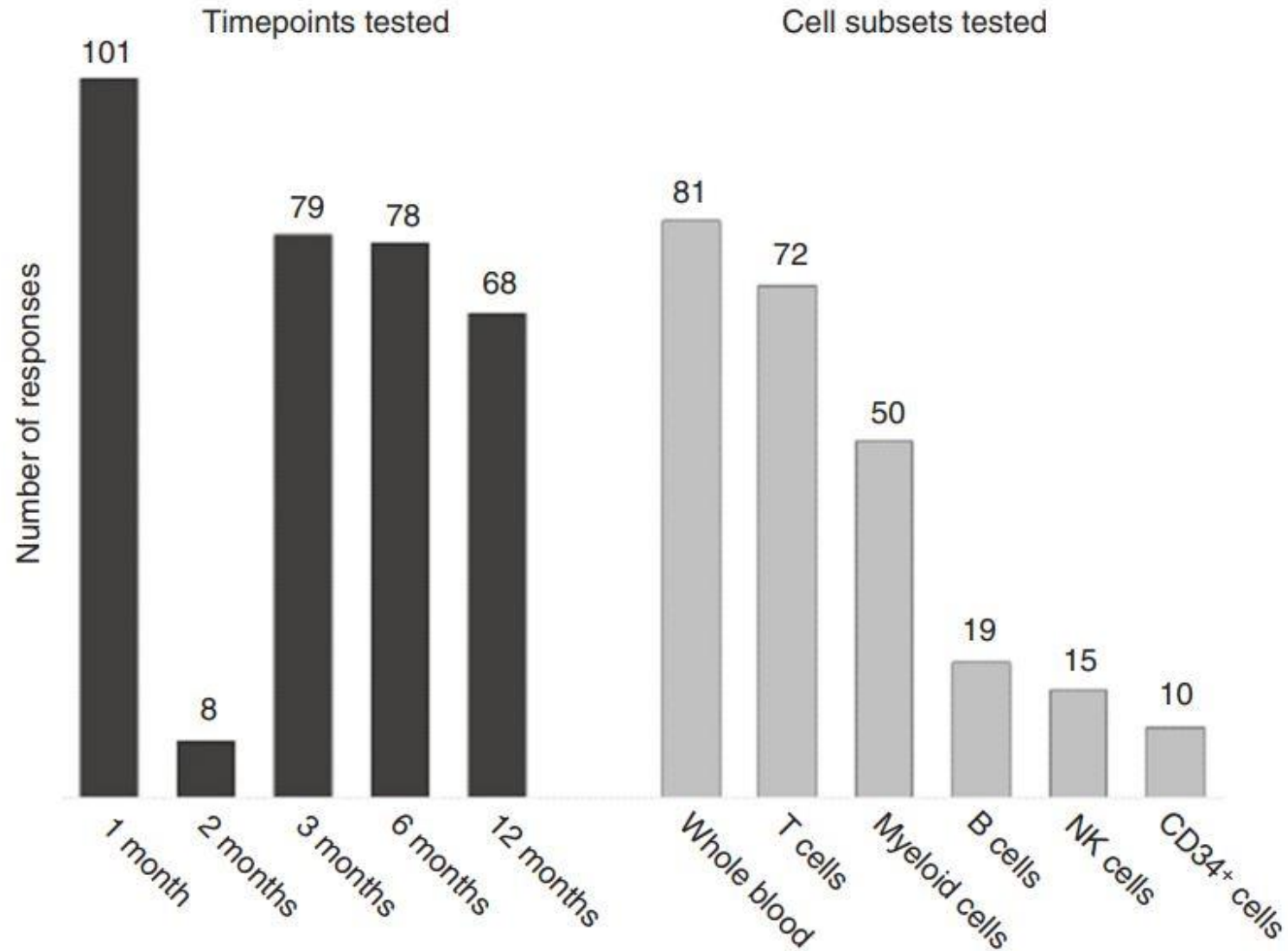


sensitivity for detection of residual disease : 10^{-4} - 10^{-5}

Kombination von MFC-basierter Zellenanreicherung und NGS:

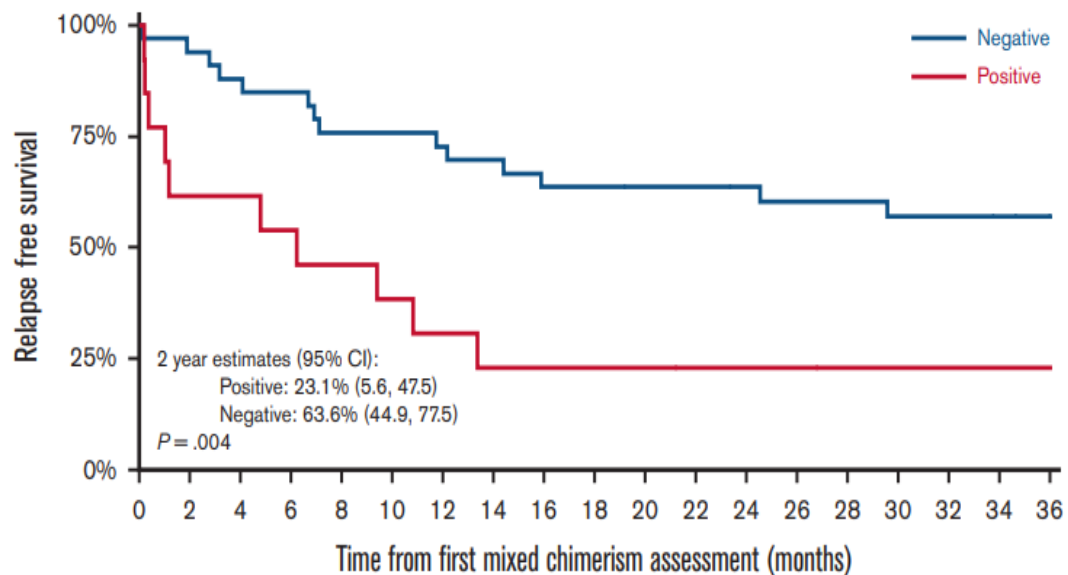


Chimärismus-Analysen nach allo-SCT – Zeitpunkte und Zellpopulationen:



Kombination von Chimärismus-Analyse und MRD-Messung:

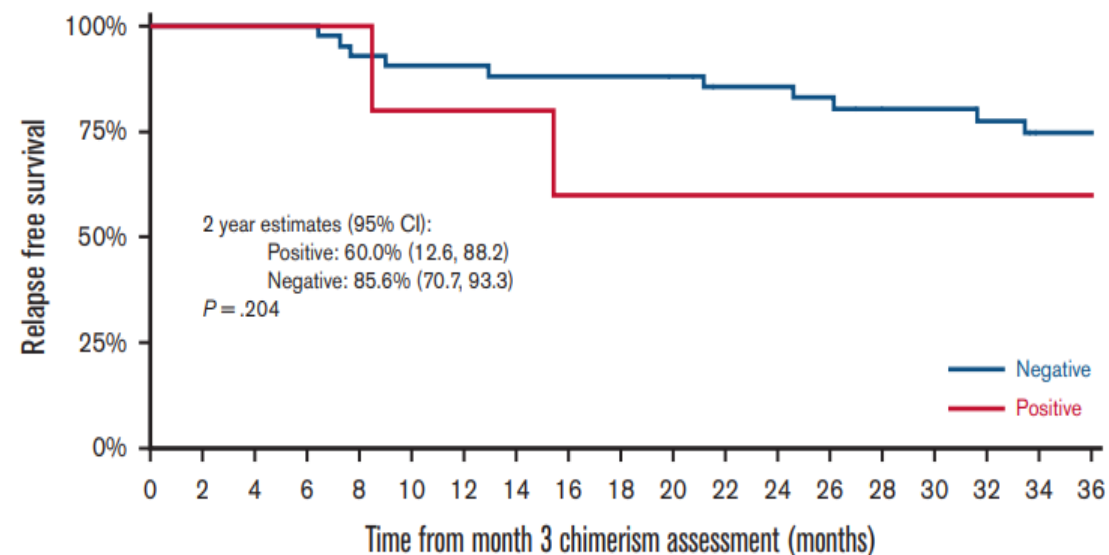
mixed T-cell chimerism (<95%)



At Risk

Negative:	33	31	29	28	25	25	24	23	21	21	20	20	19	18	18	17	17	16	14
Positive:	14	8	8	7	6	5	4	3	3	3	3	2	2	2	1	1	1	1	1

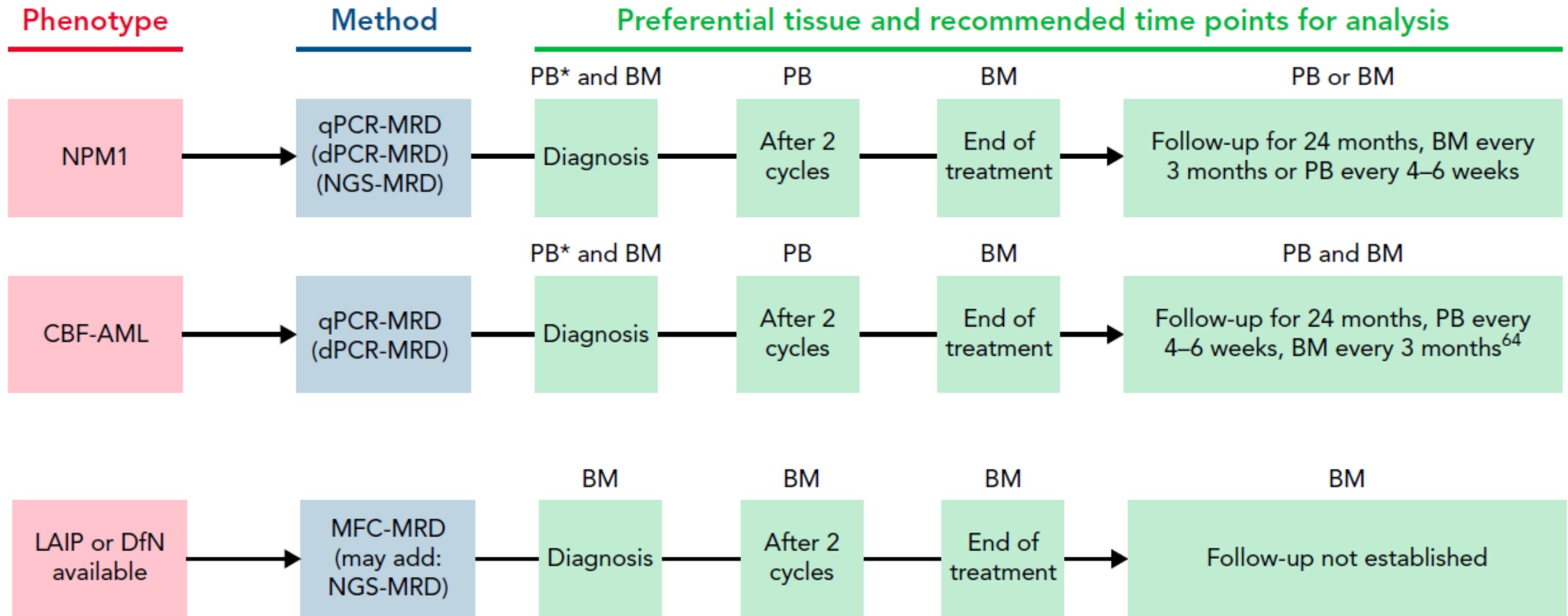
full T-cell chimerism (>95%)



At Risk

Negative:	42	42	42	42	39	38	38	37	37	37	36	33	33	32	29	29	27	24	24
Positive:	5	5	5	5	5	4	4	4	3	3	3	3	3	3	3	3	3	3	3

ELN-Guidelines 2021: MRD-Monitoring AML post alloSCT:



Zusammenfassung

- Bei Patient:innen mit *NPM1*-mutierter AML oder CBF-AML steht durch die (RT-)qPCR eine sensitive und gut etablierte Methode zum MRD-Monitoring nach alloSCT zur Verfügung.
- NGS-basierte Methoden erlauben einen hochsensitiven Nachweis von FLT3-ITD und anderer Leukämie-spezifischer Mutationen. Die Bedeutung von CH-assoziierten Mutationen nach alloSCT im Rahmen des MRD-Monitorings ist noch nicht eindeutig geklärt.
- Bei fehlenden molekularen MRD-Markern hat die MFC (ev. in Zusammenschau mit der Chimärismus-Analyse) eine zunehmende Bedeutung im MRD-Monitoring nach alloSCT.
- Der Stellenwert von Kombinationsmethoden (MFC-basierte Zellanreicherung kombiniert mit genetischer Analyse) muss in weiteren Studien geprüft werden.