

Dalla biopsia liquida al paziente: il monitoraggio della malattia

...In corso di Leucemia

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Convegno Regionale SIES
Delegazione Emilia Romagna

Biopsia liquida:

**CHE TRAFFICO
IN PERIFERIA!**

Bologna

28 Febbraio – 1 Marzo 2025

Aula 1 – Complesso UniOne, Università di Bologna

Disclosures of Maria Ilaria Del Principe

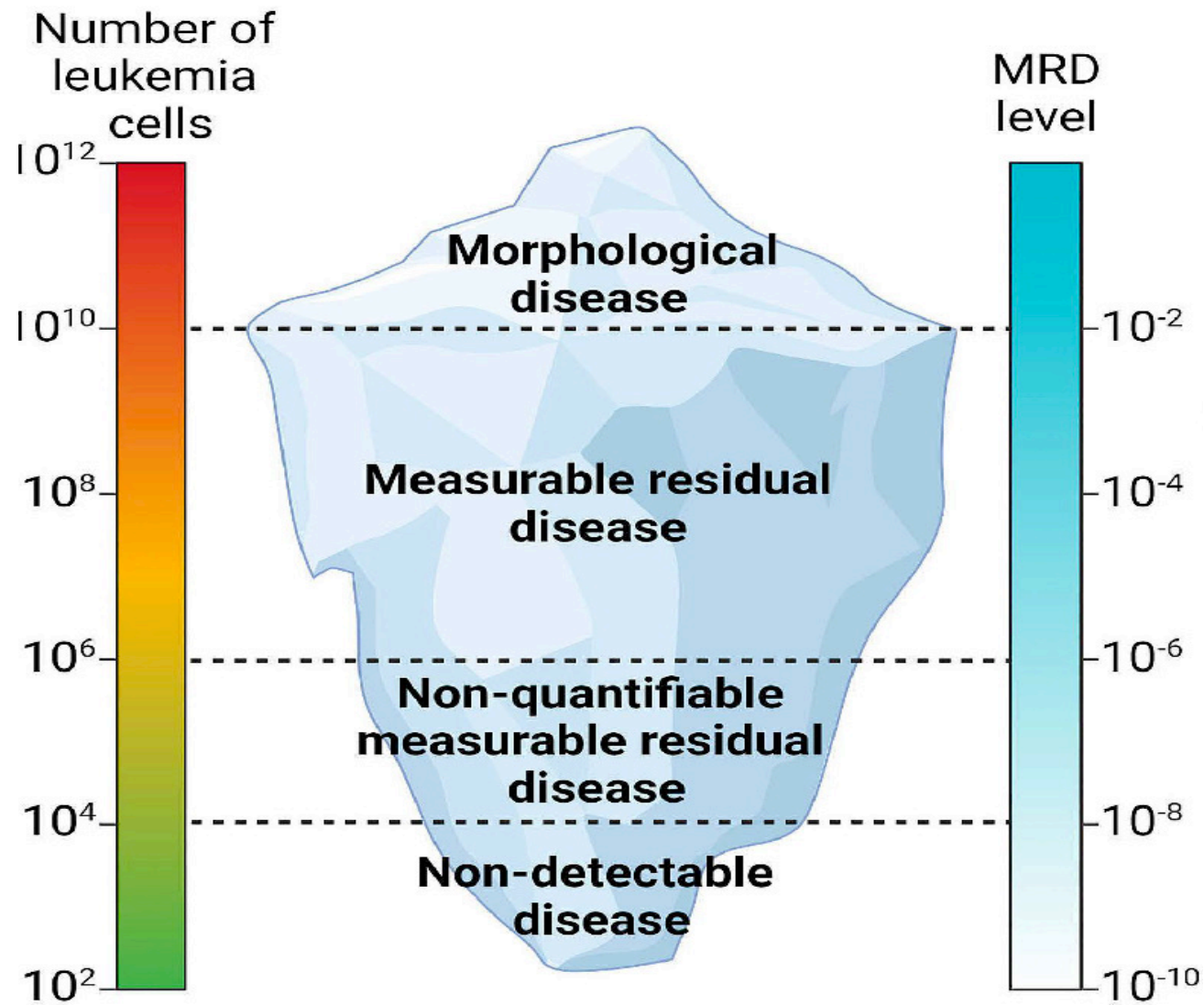
Company name	Research support	Employee	Consultant	Stockholder	Speakers bureau	Advisory board	Other
Gilead Sciences	x					x	
Amgen						x	
Jhonson & Jhonson							x
Incyte							x
Abbvie							x



MY AGENDA

- *Minimal residual disease (MRD)*
- ✓ Acute myeloid leukemia (AML)
- ✓ Acute lymphoblastic leukemia (ALL)
- *CNS involvement*

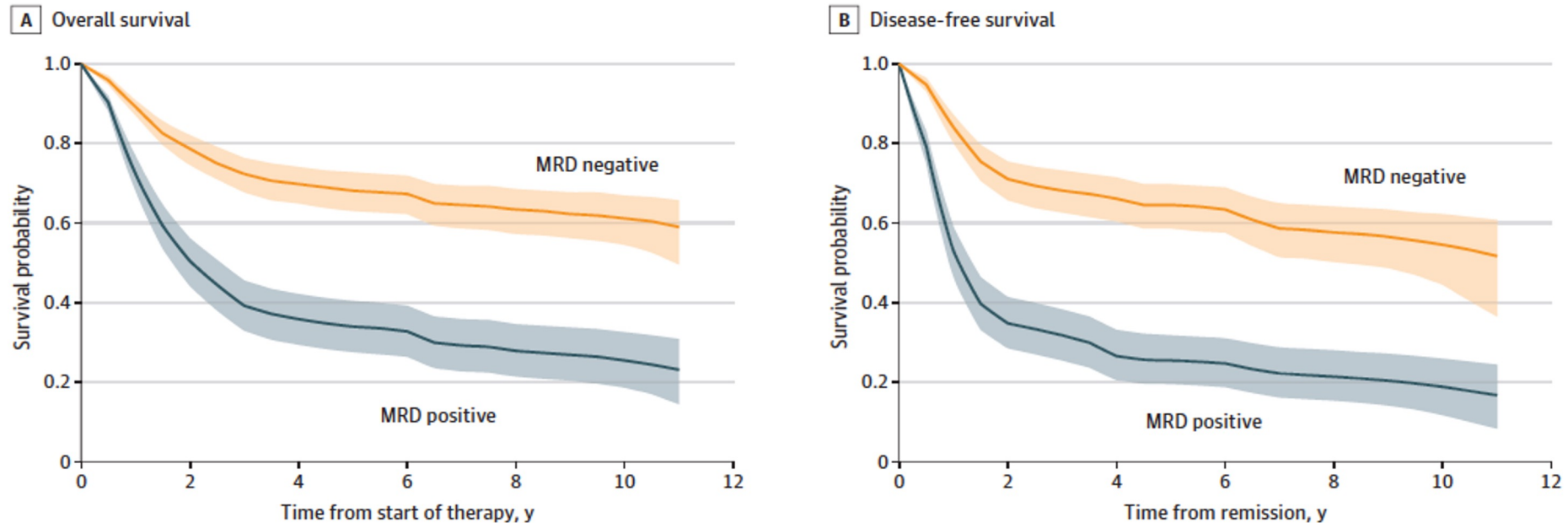




Saygin C et al., Haematologica 2022



Association of MRD with survival outcomes in patients with AML



- Systematic review and meta-analysis of 81 publications reporting on 11'151 patients
- Estimated 5-year DFS was 64% for patients MRD^{NEG} and 25% for those MRD^{POS}
- Estimated OS was 68% for patients MRD^{NEG} and 34% for those MRD^{POS}
- The difference of 5-year survival of the MRD^{NEG} and MRD^{POS} groups was 15.4 months for OS and 19.6 months for DFS.

Short N, et al. JAMA Oncology. 2020





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. Ossenkoppele¹



Special Report

2021 Update on MRD in acute myeloid leukemia: a consensus document from the European LeukemiaNet MRD Working Party

Michael Heuser,¹ Sylvie D. Freeman,² Gert J. Ossenkoppele,³ Francesco Buccisano,⁴ Christopher S. Hourigan,⁵ Lok Lam Ngai,³
Jesse M. Tetters,³ Costa Bachas,³ Constance Baer,⁶ Marie-Christine Béné,⁷ Veit Bücklein,⁸ Anna Czyz,⁹ Barbara Denys,¹⁰ Richard Dillon,¹¹
Michaela Feuring-Buske,¹² Monica L. Guzman,¹³ Torsten Haferlach,⁶ Lina Han,¹⁴ Julia K. Herzig,¹² Jeffrey L. Jorgensen,¹⁵ Wolfgang Kern,⁶
Marina Y. Konopleva,¹⁴ Francis Lacombe,¹⁶ Marta Libura,¹⁷ Agata Majchrzak,¹⁸ Luca Maurillo,⁴ Yishai Ofran,¹⁹ Jan Philippe,¹⁰
Adriana Plesa,²⁰ Claude Preudhomme,²¹ Farhad Ravandi,¹⁴ Christophe Roumier,²¹ Marion Subklewe,⁸ Felicitas Thol,¹
Arjan A. van de Loosdrecht,³ Bert A. van der Reijden,²² Adriano Venditti,⁴ Agnieszka Wierzbowska,²³ Peter J. M. Valk,²⁴ Brent L. Wood,²⁵
Roland B. Walter,²⁶ Christian Thiede,^{27,28} Konstanze Döhner,¹² Gail J. Roboz,¹³ and Jacqueline Cloos³



Special Report

Diagnosis and management of AML in adults: 2022 recommendations from an international expert panel on behalf of the ELN

Hartmut Döhner,¹ Andrew H. Wei,² Frederick R. Appelbaum,³ Charles Craddock,⁴ Courtney D. DiNardo,⁵ Hervé Dombret,⁶
Benjamin L. Ebert,⁷ Pierre Fenaux,⁸ Lucy A. Godley,⁹ Robert P. Hasserjian,¹⁰ Richard A. Larson,¹¹ Ross L. Levine,¹² Yasushi Miyazaki,¹³
Dietger Niederwieser,¹⁴ Gert Ossenkoppele,¹⁵ Christoph Röllig,¹⁶ Jorge Sierra,¹⁷ Eytan M. Stein,¹⁸ Martin S. Tallman,¹⁸
Hwei-Fang Tien,¹⁹ Jianxiang Wang,²⁰ Agnieszka Wierzbowska,²¹ and Bob Löwenberg²²

“Initial risk assignment may change
during the treatment course based on
the results from analyses of
measurable residual disease”

- The points discussed below are relevant to intensive approaches (induction chemotherapy) but have not been validated for other modalities of treatment.
- For patients with favorable-risk disease, if MRD is persistently positive after induction and/or consolidation, consider a clinical trial or alternative therapies, including allogeneic HCT.
- **Timing of MRD assessment: Upon completion of initial induction.⁴⁻⁶ Before allogeneic HCT.⁸ Additional time points should be guided by the regimen used.^{2,3}**
- The most frequently employed methods for MRD assessment include **real-time quantitative PCR (RQ-PCR) assays** (ie, NPM^{1,2} CBFβ::MYH11, RUNX1::RUNX1T13) and **multicolor flow cytometry (MFC) assays** specifically designed to detect abnormal MRD immunophenotypes.¹
- The threshold to define MRD+ and MRD- samples depends on the technique and subgroup of AML. **NGS-based assays to detect mutated genes (targeted sequencing, 20–50 genes per panel)^{4,5} is not routinely used**, as the sensitivity of PCR-based assays and flow cytometry is superior to what is achieved by conventional NGS. Mutations associated with clonal hematopoiesis of indeterminate potential (CHIP) and aging (ie, DNMT3A, TET2, potentially ASXL1) are also not considered reliable markers for MRD.⁴⁻⁶
- Based on the techniques, the optimal sample for MRD assessment is either peripheral blood (NPM1 PCR-based techniques) or an early, dedicated pull of the BM aspirate (ie, other PCR, flow cytometry, NGS). The quality of the sample is of paramount importance to have reliable evaluation.
- MRD positivity is not proof of relapse. However, a persistently positive MRD result after induction, which depends on the technique used and the study, is associated with an increased risk of relapse.



¹ Schuurhuis GJ, Heuser M, Freeman S, et al. Minimal/measurable residual disease in AML: consensus document from ELN MRD Working Party. *Blood* 2018;131:1275-1291.

² Ivey A, Hills RK, Simpson MA, et al. Assessment of minimal residual disease in standard-risk AML. *N Engl J Med* 2016;374:422-433.

³ Jourdan E, Boissel N, Chevret S, et al. Prospective evaluation of gene mutations and minimal residual disease in patients with core binding factor acute myeloid leukemia. *Blood* 2013;121:2213-2223.

⁴ Jongen-Lavrencic M, Grob T, Hanekamp D, et al. Molecular minimal residual disease in acute myeloid leukemia. *N Engl J Med* 2018;378:1189-1199.

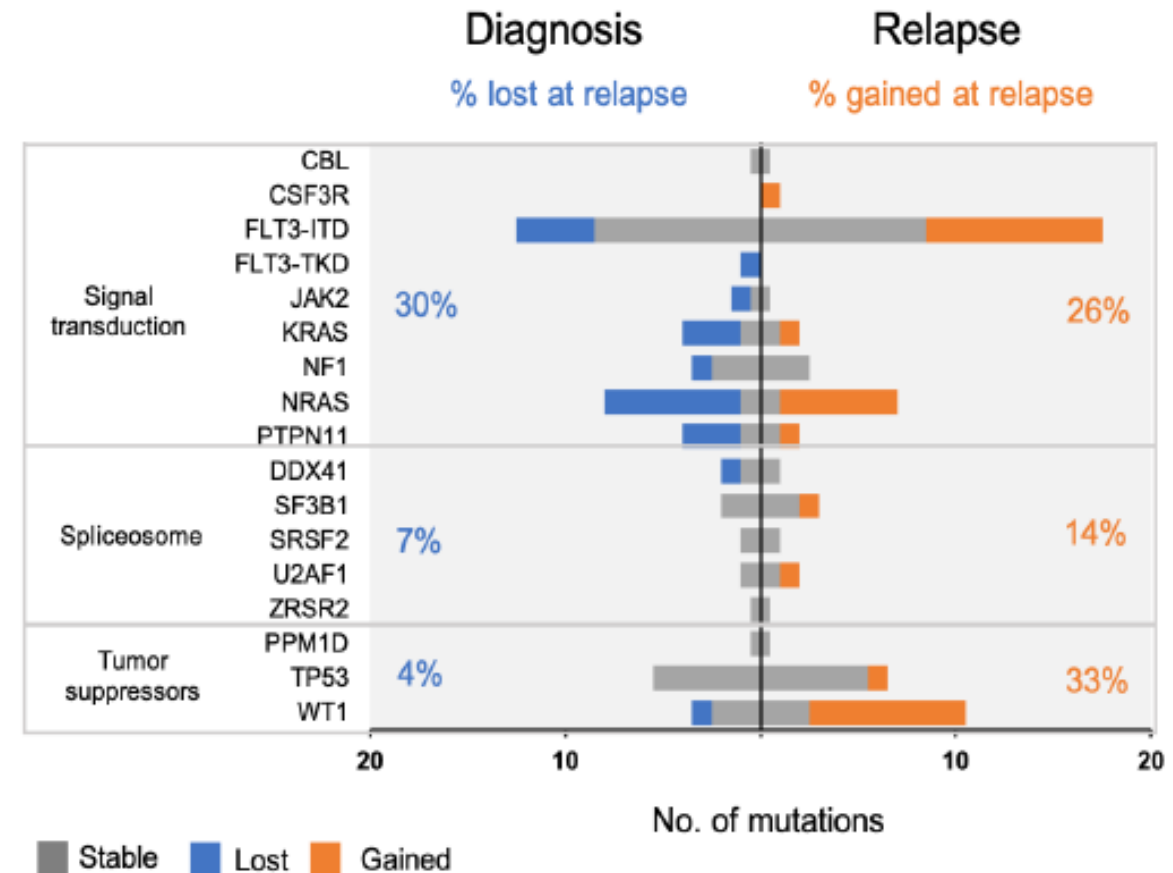
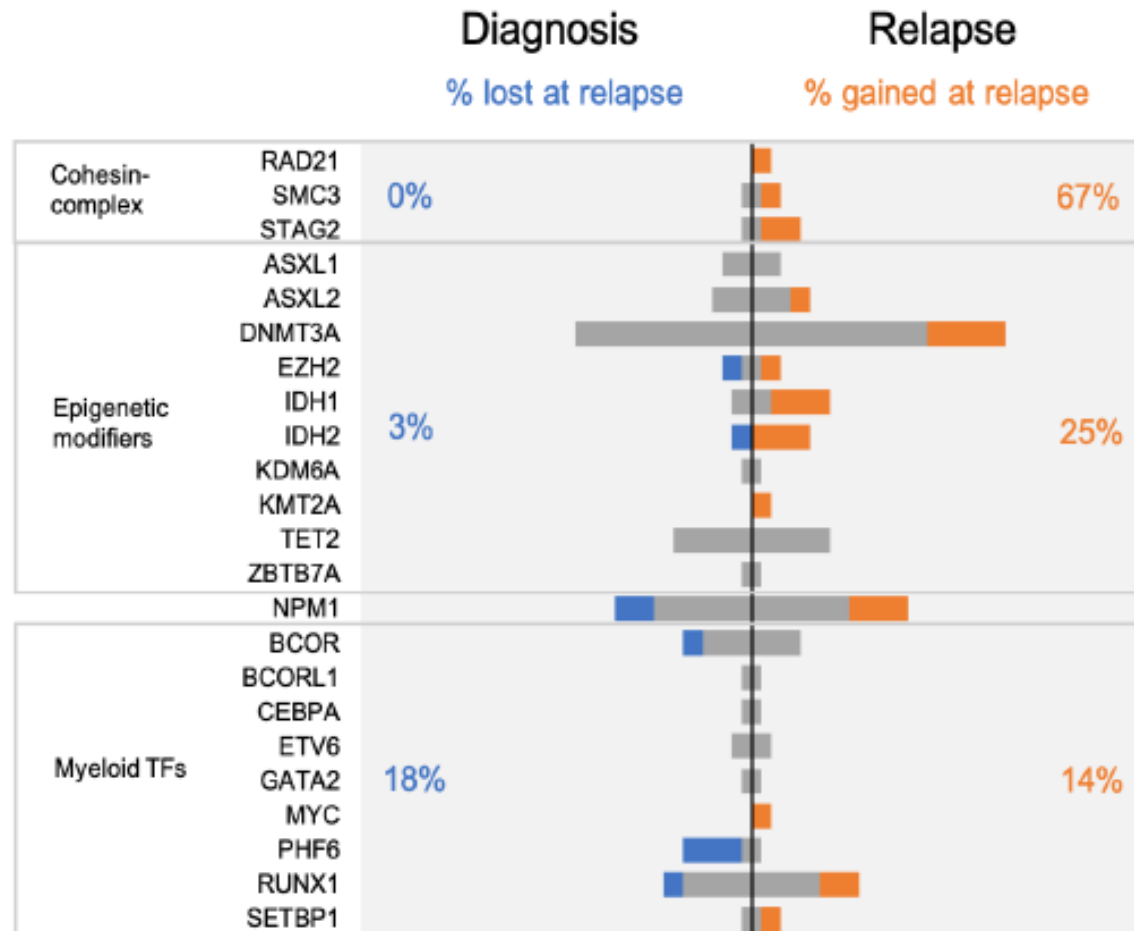
⁵ Kico JM, Miller CA, Griffith M, et al. Association between mutation clearance after induction therapy and outcomes in acute myeloid leukemia. *JAMA* 2015;314:811-822.

⁶ Morita K, Kantarjian H, Wang F, et al. Clearance of somatic mutations at remission and the risk of relapse in acute myeloid leukemia. *J Clin Oncol* 2018;36:1788-1797.

⁷ Short NJ, et al. Association of measurable residual disease with survival outcomes in patients with acute myeloid leukemia: A systematic review and meta-analysis. *JAMA Oncol* 2020;6:1890-1899.

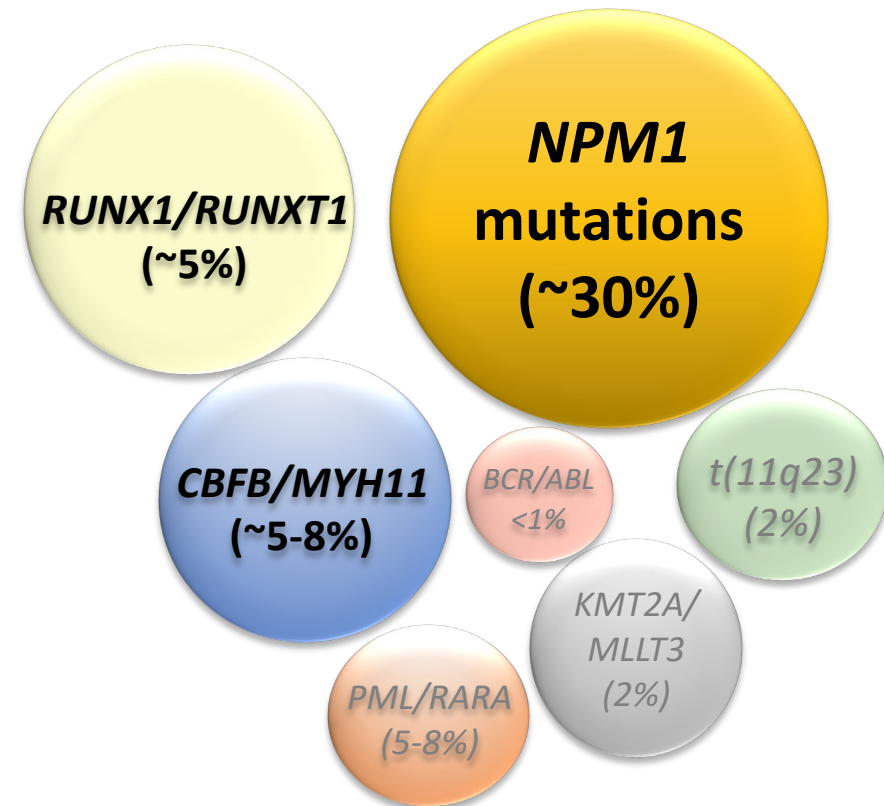
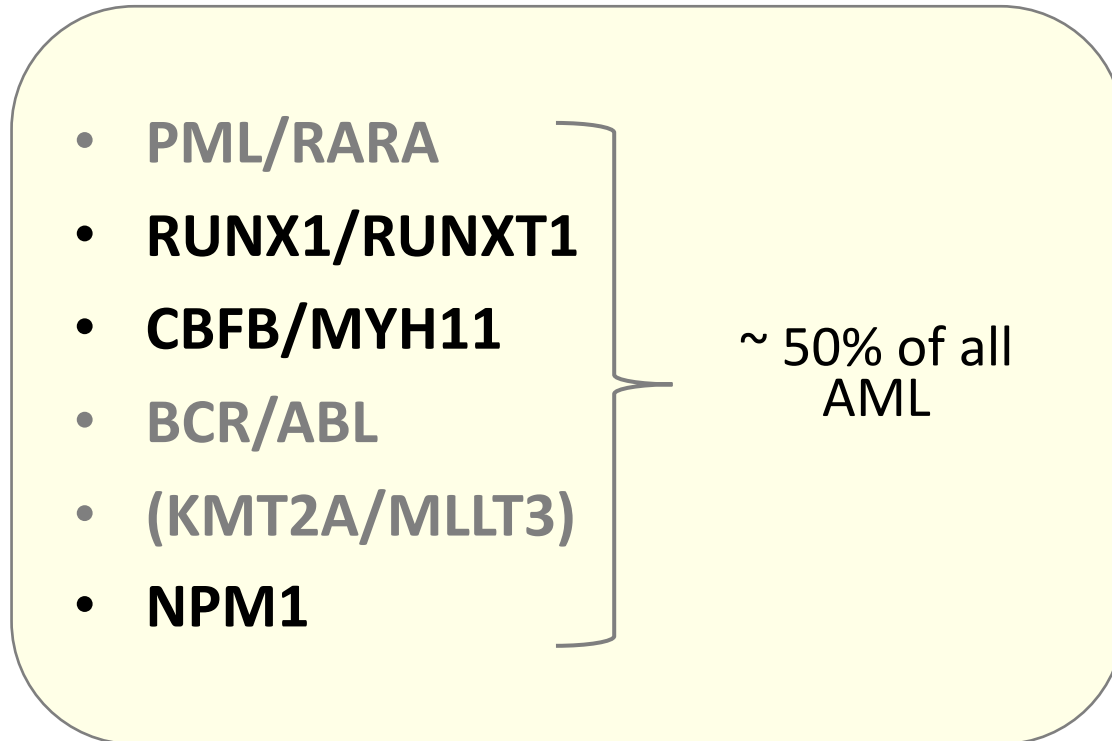
⁸ Thol F, Gabdoulline R, Liebich A, et al. Measurable residual disease monitoring by NGS before allogeneic hematopoietic cell transplantation in AML. *Blood* 2018;132:1703-1713.

Heterogeneity of AML through clonal evolution

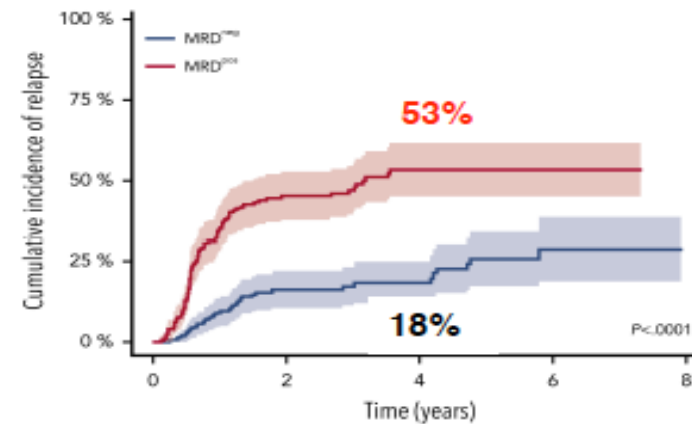
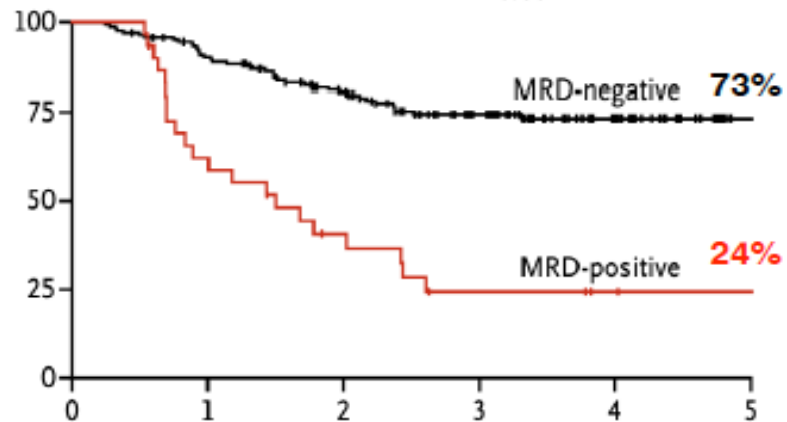
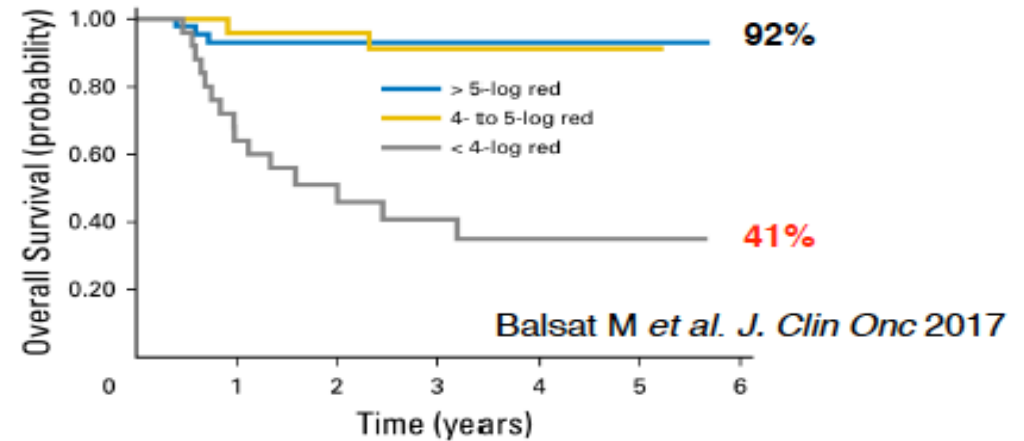
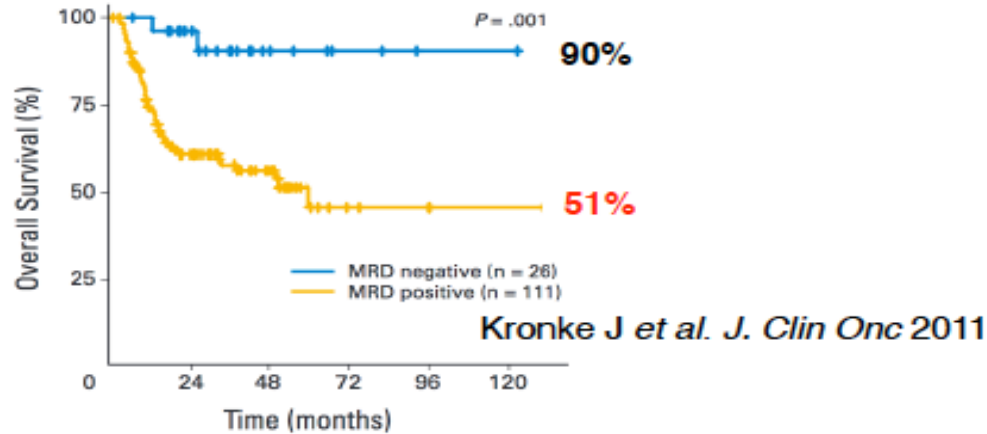


Molecular markers used for RT-qPCR based MRD monitoring

- MRD monitoring by TR-qPCR has been restricted to AML subtypes characterized by gene fusions resulting from translocations/inversions or by hot spot mutations



Post-Induction NPM1 MRD Predicts Relapse and Death



Ivey, A. *et al. NEJM* 2015

Kapp-Schworer, S. *et al. Blood* 2020

Aberrancies detection by flow

Leukemia-associated immunophenotypes (LAIPs)

MRD identical to the leukemic phenotype at diagnosis

Cross-lineage expression

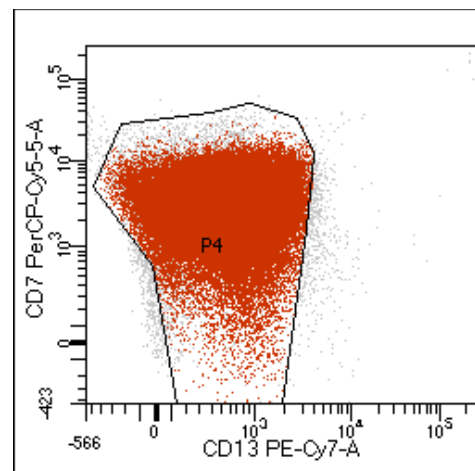
Highly dependent on individually selected antibody combination

Asynchronous expression

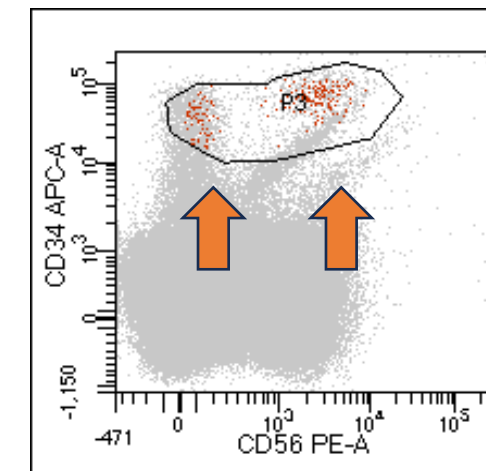
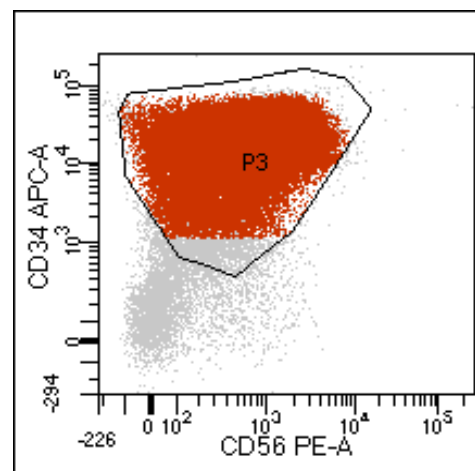
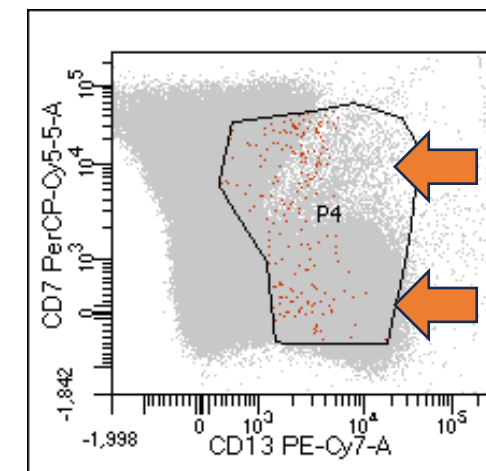
Lack/overexpression

Heuser M et al., Blood 2021

DIAGNOSIS



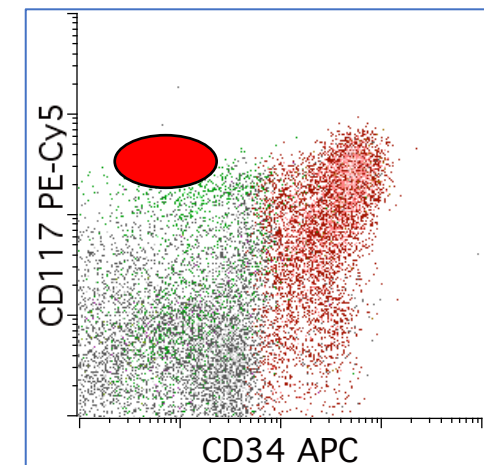
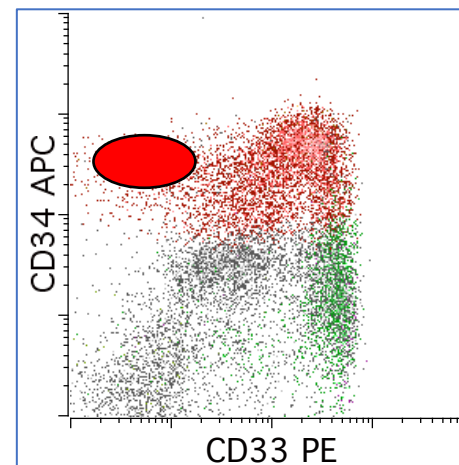
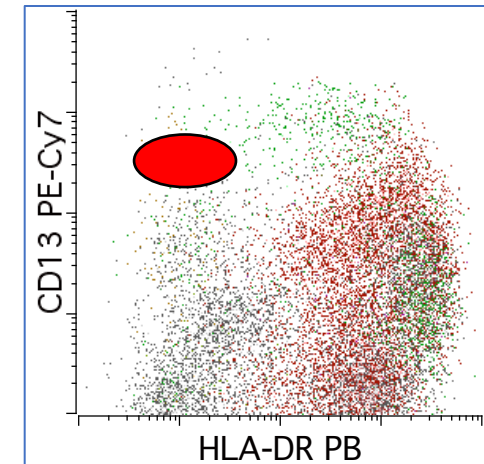
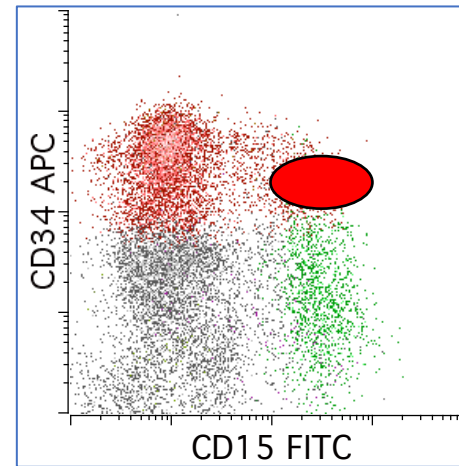
Post-CYCLE 2



Aberrancies detection by flow

Different from normal (DfN)

- Harmonized panel of antibodies for all specimens and distinguishes abnormal residual leukemic cells from normal ones with established immunophenotypic profiles,
- Does not require knowledge of the phenotype at diagnosis for the MRD detection
- Phenotypical abnormalities associated with CHIP*



*Jevremovic, American J Clin Patol 2022; W Kern et al, Cytometry Part B
Clinical Cytometry 2023

Heuser M et al., Blood 2021



Choosing the right MRD assay

ELN risk group	Genetic subgroup	MRD assay in non-transplanted patients	MRD assay after alloHCT
Favorable	NPM1 mut	qPCR	qPCR
	RUNX1/RUNX1T1 or CBFB/MYH11	qPCR	qPCR
	CEBPA bZIP inframe	Not established	Not established
Intermediate	FLT3-ITD NPM1wt	FLT3-NGS or MFC	FLT3-NGS or MFC
	FLT3-ITD NPM1mut	FLT3-NGS or qPCR	FLT3-NGS or qPCR
	MLLT3::KMT2A	MFC or qPCR	qPCR (MFC, NGS)
	Other	MFC	MFC or NGS
Adverse	Fusion genes	qPCR or MFC	qPCR or MFC or NGS
	-5 or del(5q); -7; -17/abn(17p)	MFC	MFC or NGS
	Complex karyotype	MFC	MFC or NGS
	Myelodysplasia-related gene mutations	MFC	MFC or NGS
	TP53	MFC	MFC or NGS

Modified from Heuser et al. Blood. 2021



ELN2022 clinical recommendations for AML treatment

Favorable

- $t(8;21)(q22;q22.1)/RUNX1::RUNX1T1$
- $inv(16)(p13.1q22)$ or $t(16;16)(p13.1;q22)/CBFB-MYH11$
- Mutated NPM1 without FLT3-ITD
- *bZIP in-frame mutated CEBPA*

Intermediate

- Mutated NPM1 with FLT3-ITD
- Wild-type NPM1 with FLT3-ITD
- $t(9;11)(p21.3;q23.3)/MLL3::KMT2A$
- Cytogenetic and/or molecular abnormalities not classified as favorable or adverse

Adverse

- $t(6;9)(p23;q34.1)/DEK::NUP214$
- $t(v;11q23.3)/KMT2A$ -rearranged
- $t(9;22)(q34.1;q11.2)/BCR::ABL1$
- $t(8;16)(p11;p13)/KAT6A::CREBBP$
- $inv(3)(q21.3q26.2)$ or $t(3;3)(q21.3;q26.2)/GATA2, MECOM(EVI1)$
- $t(3q26.2;v)/MECOM(EVI1)$ -rearranged
- -5 or del(5q); -7; -17/abn(17p)
- Complex karyotype, monosomal karyotype
- Mutated ASXL1, BCOR, EZH2, RUNX1, SF3B1, SRSF2, STAG2, U2AF1, or ZRSR2
- Mutated TP53

Patients with non-adverse risk AML with MRD persistence should be considered for SCT

MRD for driving treatment

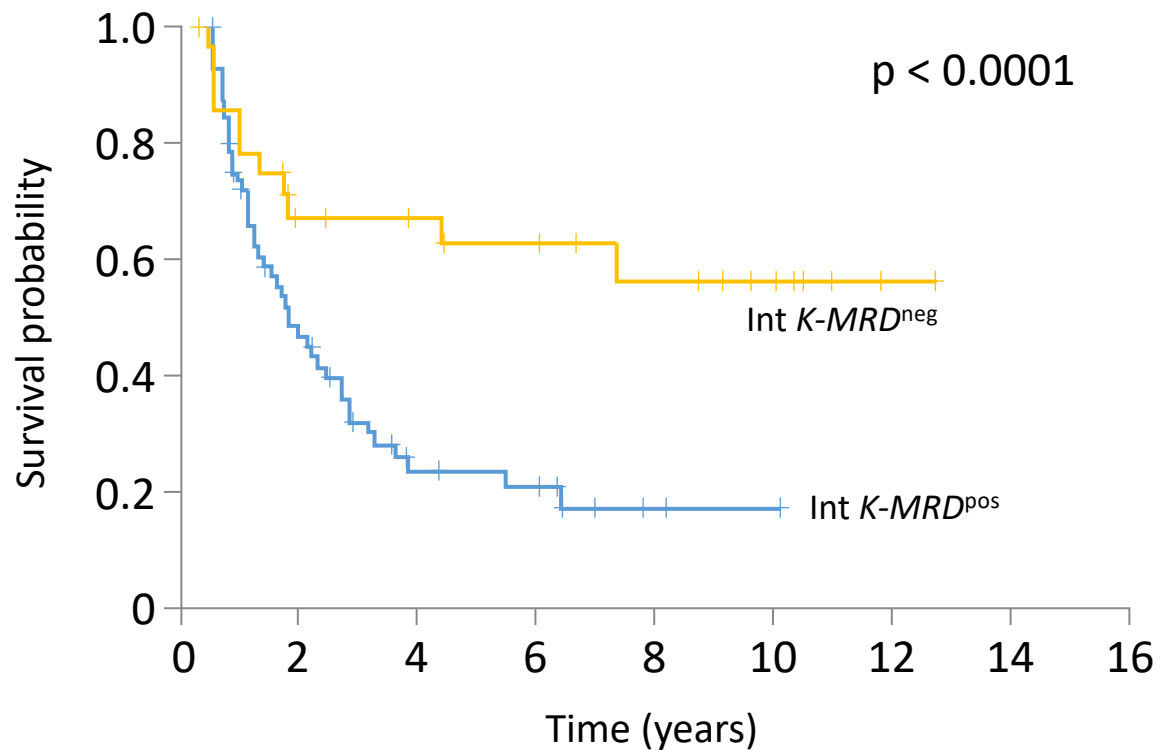
Early intensification in CR1

MRD for selecting type of SCT or preemptive therapy

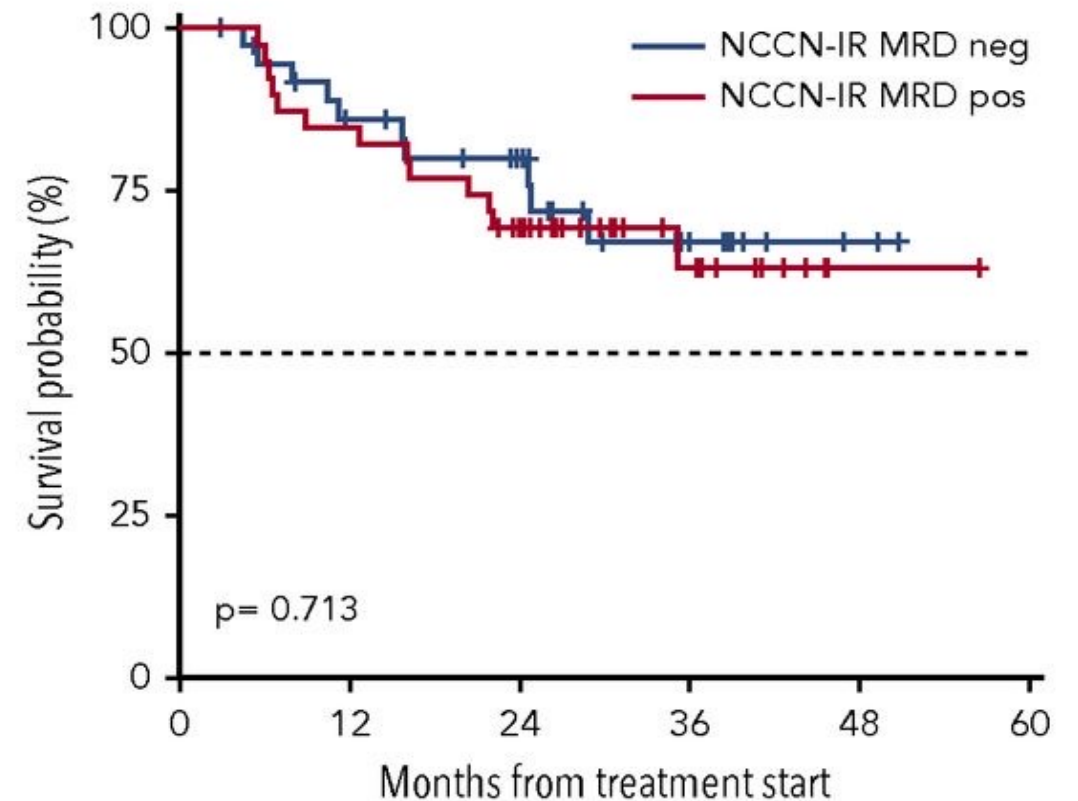


MRD by MFC in Intermediate-risk patients

Retrospective *in house* observation
“donor vs. no donor”



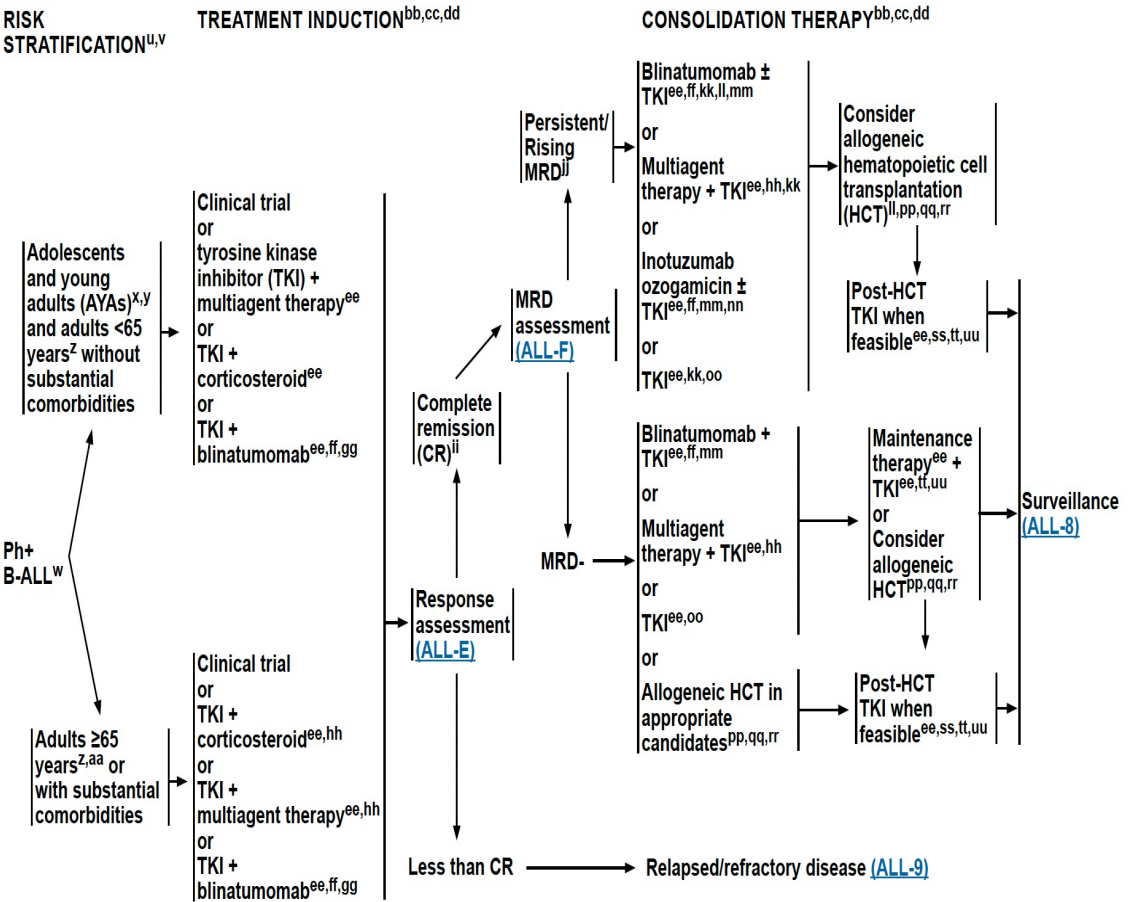
GIMEMA AML1310 protocol
“transplant vs. no transplant”





NCCN Guidelines Version 3.2024 Acute Lymphoblastic Leukemia

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[Footnotes on ALL-5A](#)

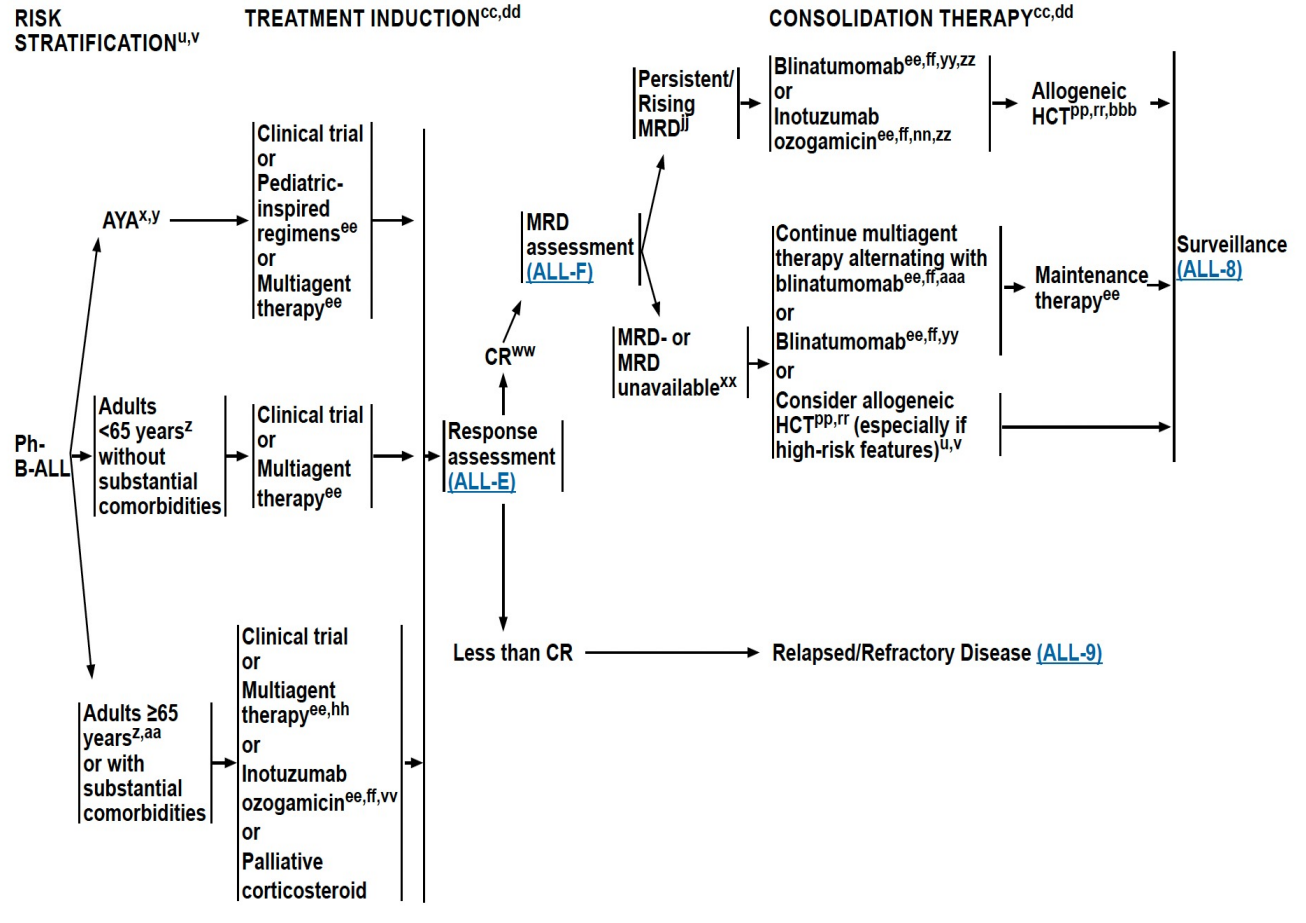
Note: All recommendations are category 2A unless otherwise indicated.

ALL-5



NCCN Guidelines Version 3.2024 Acute Lymphoblastic Leukemia

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[Footnotes on ALL-6A](#)

Note: All recommendations are category 2A unless otherwise indicated.

ALL-6



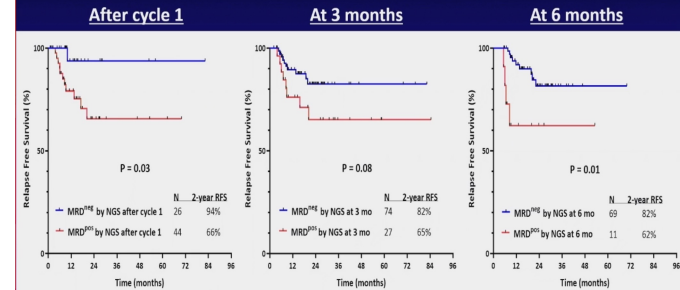
Early Achievement of Deep MRD Negativity IN B-Cell ALL

NGS MRD in B-cell ALL: Patient Characteristics

Characteristic - N (%) / median [range]	Category	Patients (N=161)
Age (years)		46 [18-87]
WBC ($\times 10^9/L$)		7.6 [0.3-698.9]
ALL Subtype	<i>Ph-positive ALL</i>	51 (32)
	<i>Ph-negative ALL</i>	110 (68)
Cytomolecular features of Ph-negative ALL		
		Patients (N=110)
	<i>Diploid</i>	30 (27)
	<i>High hyperdiploidy</i>	3 (3)
	<i>Low hypodiploidy / near triploidy</i>	11 (10)
	<i>KMT2A rearranged</i>	6 (5)
	<i>Complex</i>	8 (7)
	<i>Miscellaneous</i>	31 (28)
	<i>Insufficient metaphases</i>	20 (19)
Cytogenetics		
Ph-like ALL	<i>CRLF2 overexpression by flow and/or CRLF2r</i>	17/107 (16)
	<i>Non-CRLF2 Ph-like ALL</i>	6/107 (6)
<i>TP53</i> -mutated		28/109 (25)
Poor-risk cytomolecular features*		59 (54)

*Low hypodiploidy/near triploidy, complex, *KMT2A* rearranged, Ph-like, and/or *TP53*-mutated

NGS MRD in B-cell ALL: RFS by NGS MRD Response (Entire Cohort)

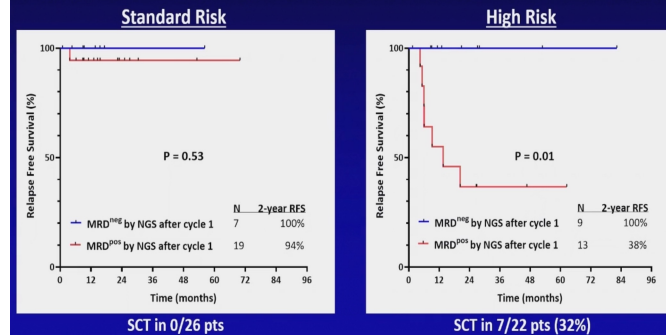


NGS MRD in B-cell ALL: Rate of NGS MRD Negativity by Subgroup

Rate of NGS MRD Negativity by Subgroup n/N (%)	After cycle 1	At 3 months (+/- 1.5 months)	At 6 months (+/- 1.5 months)
Entire Cohort	26/79 (33)	74/113 (65)	69/83 (83)
Ph-Negative B-cell ALL	16/53 (30)	51/81 (63)	42/51 (82)
<i>High Risk</i>	9/25 (36)	26/42 (62)	23/27 (85)
<i>Standard Risk</i>	7/28 (25)	25/39 (64)	19/24 (79)
Ph-Positive B-cell ALL	10/26 (38)	23/32 (72)	27/32 (84)

NGS MRD negativity = 0 residual sequences on with sensitivity of 10^{-6}

NGS MRD in B-cell ALL: RFS by NGS MRD Response after Cycle 1 (Ph-Negative B-cell ALL)

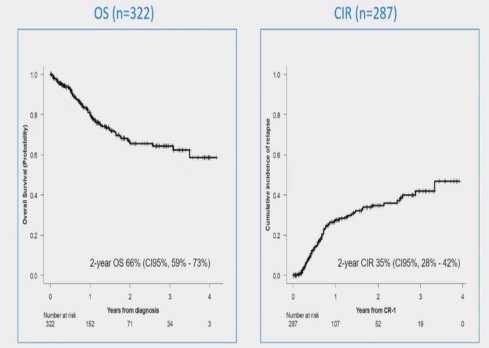


Preliminary Results of Pethema LAL19 Trial

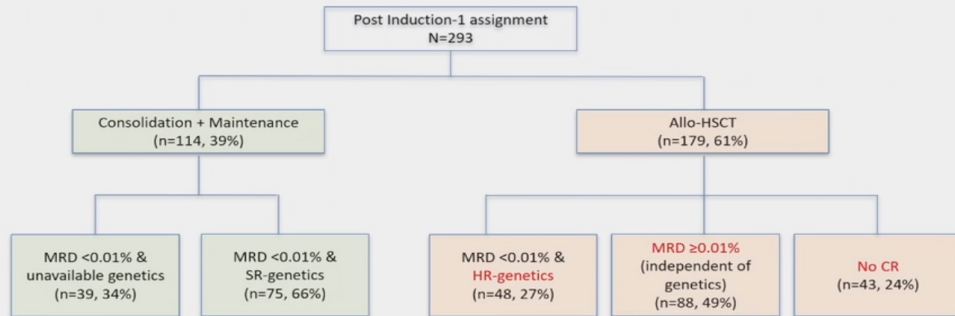
Patient characteristics

	All patients (n=452)	BCP ALL (n=371, 82%)	T-ALL (n=81, 18%)	P
Male, n(%)	245/433 (57)	192/354 (54)	53/79 (67)	0.037
Age, median [min;max]	40 [18 ; 60]	40 [18 ; 60]	34 [18 ; 60]	0.010
AYA (18-40 yr.)	227/429 (53)	178/350 (51)	49/79 (62)	0.072
WBC, median [min; max]	13.2 [0.4 – 740]	9.8 [0.4 – 720]	28.6 [2.2 – 740]	<0.001
WBC>30x10e9/L, n (%)	95/319 (30)	61/250 (24)	34/69 (49)	<0.001
CNS involvement, n (%)	40/295 (14)	28/229 (12)	12/66 (18)	0.213
Genetics, n(%)				0.009
Standard risk	209/346 (60)	179/281 (64)	30/65 (46)	
High risk	137/346 (40)	102/281 (36)	35/65 (54)	

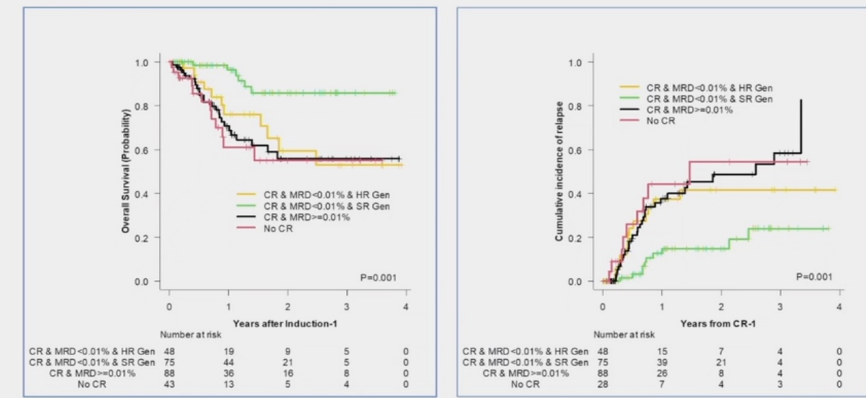
2-year Overall Survival and Cumulative Incidence of Relapse



Post induction-1 allocation



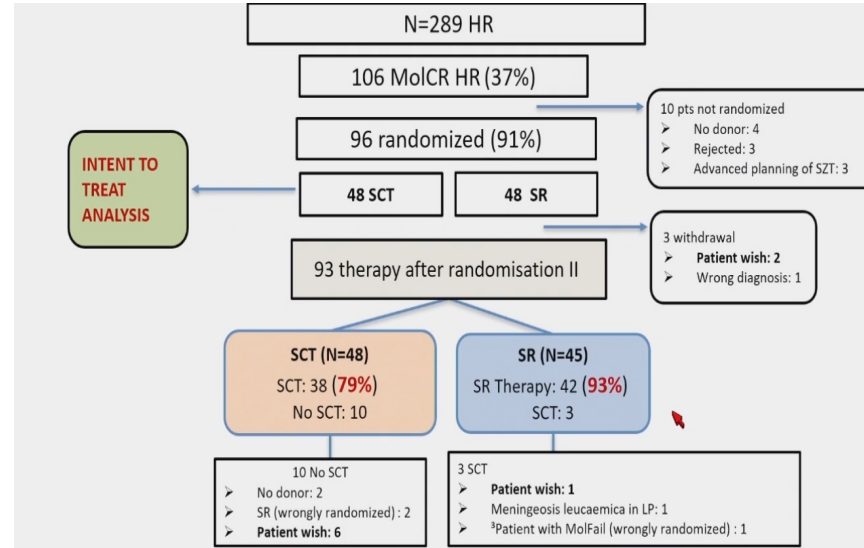
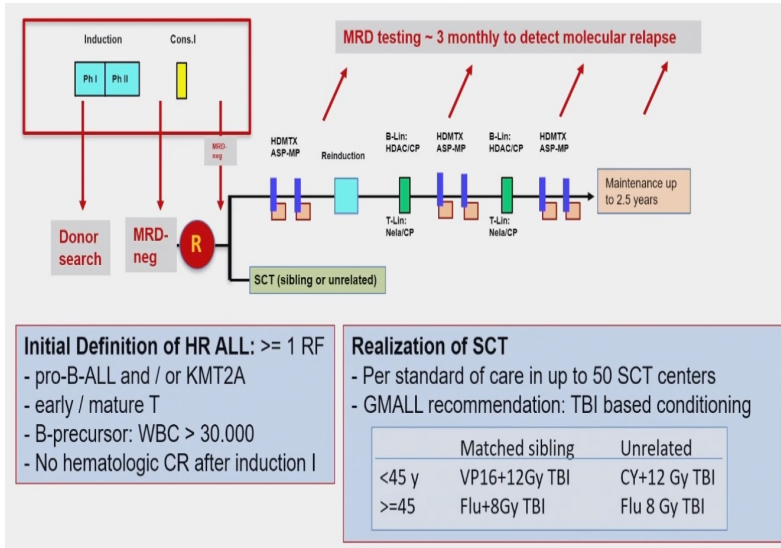
2-year OS and CIR by ITT



Torrent A et al, Abs 962

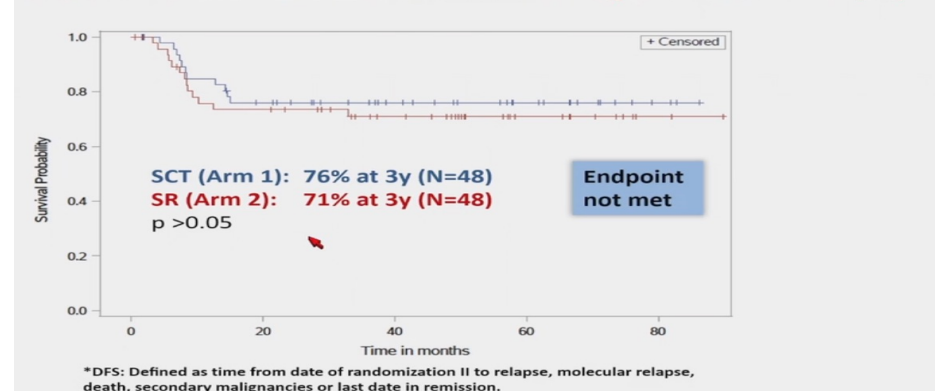


Results of the Randomized GMALL Trial 08/2013



		SCT (Arm 1) N=48	SR (Arm 2) N=48
Relapse	(Cytologic/Molecular)	5 (10%)	9 (19%)
	Cytologic	5	7
	- Prior Mol Rel	4/5	5/7
	- No prior Mol Rel	1/5	2/7
	Molecular	0	2
Relapse localisation	BM +/- other; no CNS	4 (80.0%)	7 (100%)
	Other; no BM; no CNS	1 (20.0%)	0
Death in CR		6 (13%)	4 (8%)

GMALL 08: Disease Free Survival* in HR ALL According to Randomization II (ITT)

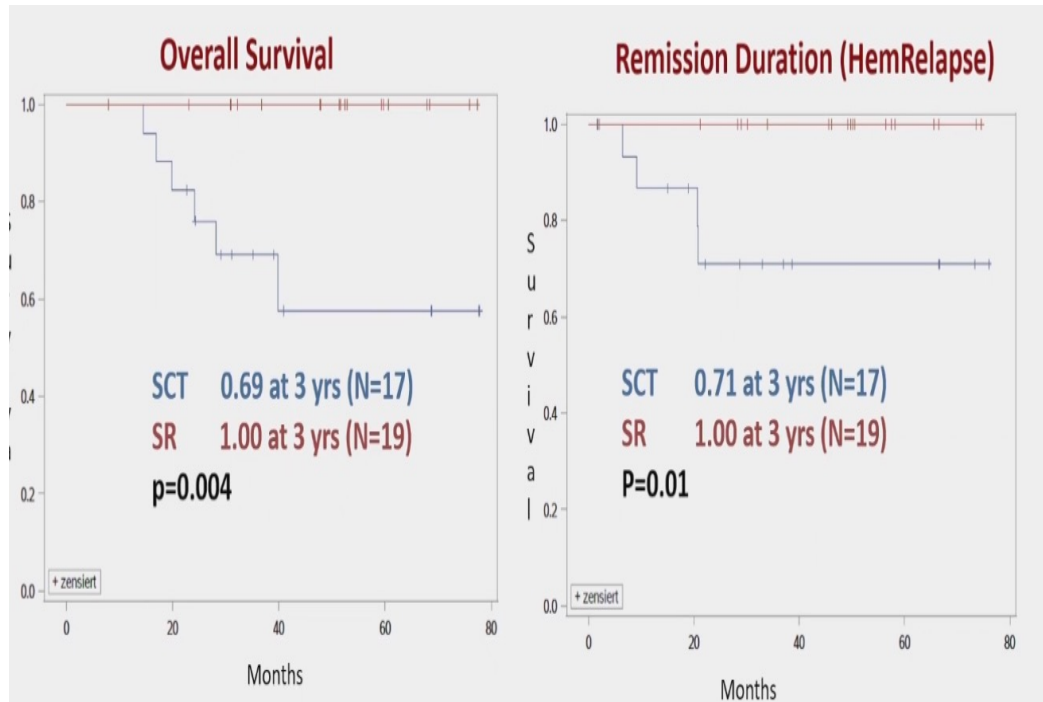


Goekbuget N et al, Abs 961

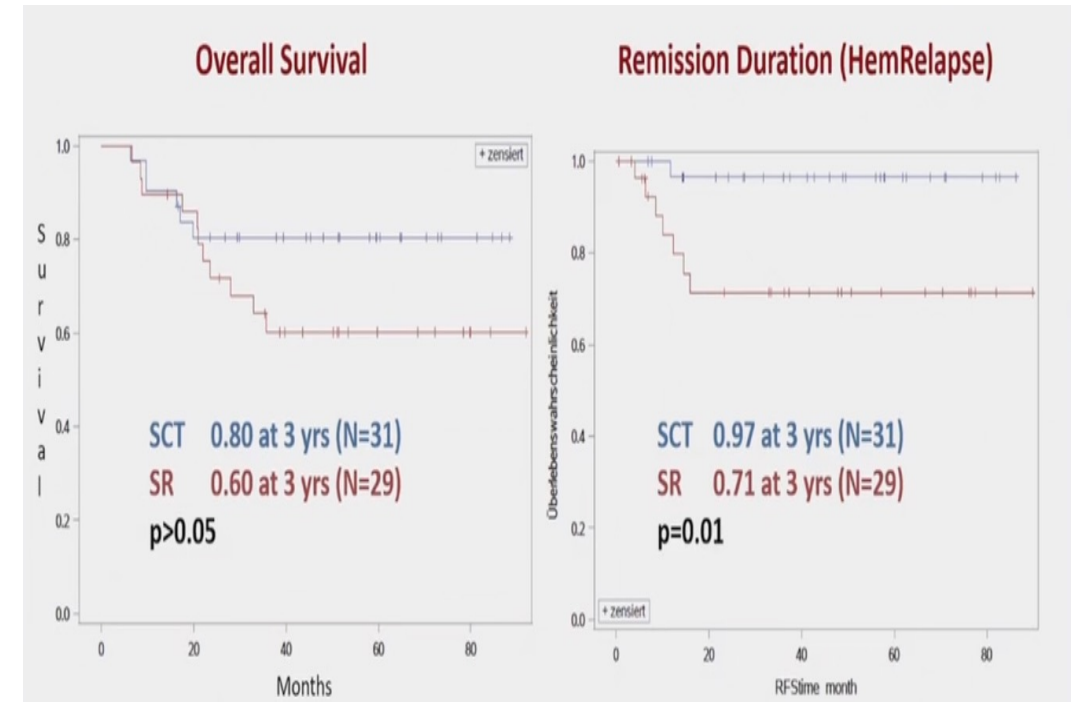


Results of the Randomized GMALL Trial 08/2013

- HR T- ALL according II randomisation



- HR B- ALL according II randomisation



Goekbuget N et al, Abs 961



CNS involvement

#730, Results in Pediatric T-ALL Patients Treated in Trial AIEOP-BFM ALL 2009: Exploring Prognostic Factors in the Context of Modern Risk Adapted Therapy

Variable	N	Hazard ratio	p
AGE			
1-5 yrs	187	Reference	
6-9 yrs	220	0.84 (0.51, 1.37)	0.48
10-17 yrs	303	1.22 (0.79, 1.87)	0.37
WBC			
<100	398	Reference	
100-300	180	0.84 (0.54, 1.32)	0.46
≥ 300	132	1.06 (0.67, 1.67)	0.81
CNS involvement			
CNS1/2	573	Reference	
CNS3	62	2.30 (1.40, 3.78)	<0.001
not known	75	1.01 (0.56, 1.84)	0.97
PDN response			
Good	469	Reference	
Poor	241	1.74 (1.10, 2.75)	0.02
FCM MRD day +15			
<0.1%	192	Reference	
≥ 0.1-10%	305	1.17 (0.67, 2.05)	0.58
≥ 10%	213	0.84 (0.41, 1.73)	0.64
PCR MRD at EOI			
Negative	125	Reference	
Positive NQ or < 5x10 ⁻⁴	238	1.69 (0.82, 3.44)	0.15
≥ 5x10 ⁻⁴ < 5x10 ⁻³	157	2.03 (0.94, 4.37)	0.07
≥ 5x10 ⁻³ < 5x10 ⁻²	109	2.71 (1.21, 6.06)	0.02
≥ 5x10 ⁻²	81	4.71 (2.00, 11.10)	<0.001

Cario G et al, Abs 730

731 Determinants of Isolated CNS Relapse in Adults with Ph-Negative ALL from Graall-2005 to -2014 Trials

	Overall 1530	GRAALL-2005 ¹ 787	GRAALL-2014 ² 743	P value
Median follow-up, years (95% CI)	4.1 (4.0-4.3)	5.2 (5.0-5.4)	3.2 (3.0-3.3)	<0.001
CR rate (%)	93%	92%	93%	0.33
Estimated 3y-DFS, % (95% CI)	61% (58-64)	62% (58-65)	59% (55-63)	0.29
Estimated 3y-OS, % (95% CI)	67% (65-70)	64% (60-67)	71% (67-74)	0.002
Relapse characteristics (among CR)				
	Total N=1416	GRAALL-2005 N=723	GRAALL-2014 N=693	P value
Relapse (%)	445 (31.4)	216 (29.9)	229 (33.0)	0.21
iBM relapse	310 (21.9)	159 (22.0)	151 (21.8)	0.95
BM+CNS relapse	31 (2.2)	19 (2.6)	12 (1.7)	0.28
iCNS relapse	57 (4.0)	19 (2.6)	38 (5.5)	0.007
Other EM	66 (4.7)	38 (5.3)	28 (4.0)	0.31

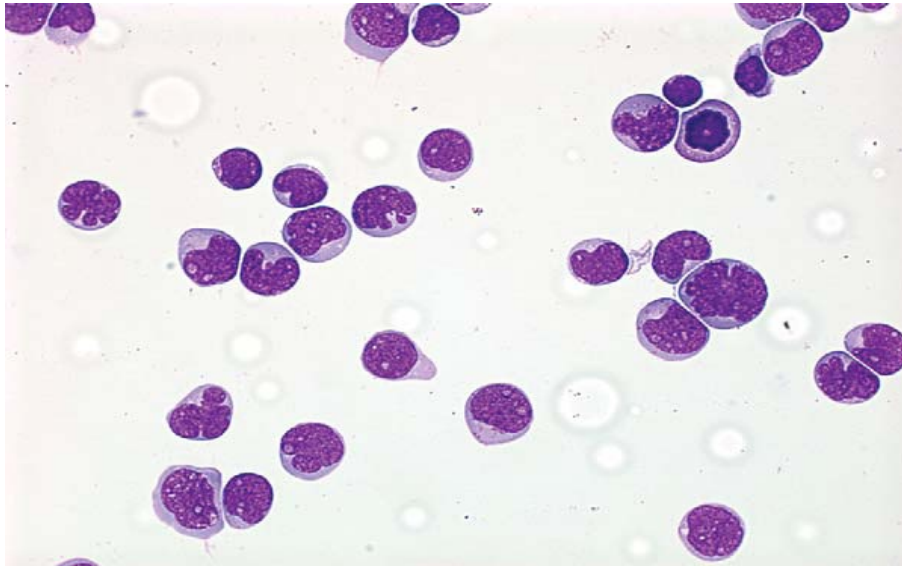
CIR, cumulative incidence of relapse; CITRM, cumulative incidence of treatment-related mortality
OS, overall survival.

- Implications for GRAALL-2024
- ✓ Reinstate prophylactic cranial RT
- ✓ Avoid cranial boost for patients eligible to HSCT post TBI
- Opportunity for more tailored strategies?

Boissel N et al, Abs 731

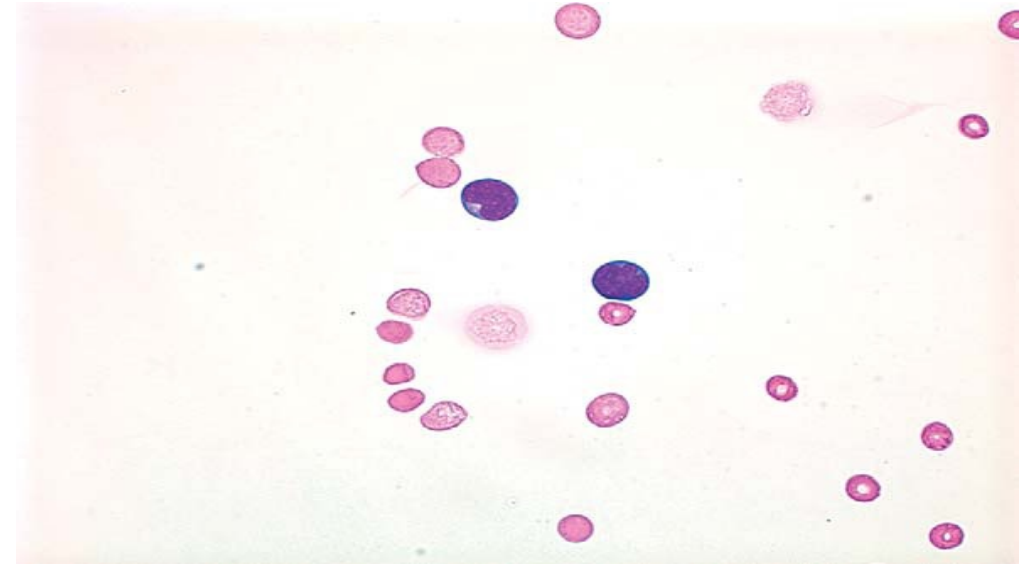


Conventional Cytology of CSF



CSF with lymphoblasts and red blood cells due to traumatic lumbar puncture (ie, at least ten red blood cells per μL of CSF) at diagnosis

Cytocentrifuged CSF containing five or more white blood cells per μL with blasts



MFC and CC for detection of leukemic cells in CSF

STUDY	Disease	N°	N°of MoAb	MFC+	CC+	MFC+CC+	MFC+CC-	MFC-CC+
Nückel et al.2006 (25)	HM	45	3	12(26%)	12(26%)	12(26%)	3(7%)	3(7%)
Di Noto et al. 2008(21)	NHL	42	6	11(26%)	4(9%)	4(9%)	7(16%)	0
Quijano et al.2009 (22)	NHL	123	6	27(22%)	7(6%)	7(6%)	17(14%)	1(1%)
Benevolo et al. 2012(23)	NHL	174	3-4	18(10%)	7(4%)	7(4%)	11(6%)	0
Martínez-Laperche et al. 2013(44)	ALL	108	6	30(28%)	3(3%)	3(3%)	27(25%)	0
Wilson et al. 2014 (24)	246 DLBCL, 80 BL	326	3-8	55(17%)	16(5%)	13(4%)	42(13%)	3(1%)
Mitri et al. 2014 (30)	ALL	80	4	1/66*(1.5%)	1(1,2%)	1	0	0
Ranta et al.2015 (41)	Children ALL	214	4-8	37(17%)	21(9%)	20(9%)	17(7%)	1(0,4%)
Del Principe et al. 2014 (33)	adult ALL/LL	38	6-8	14(24%)	5(13%)	5	9	0
Del Principe et al. 2018 (32)	AML	95	6-8	33(34%)	11(11%)	11(10%)	21(22%)	0
Gong et al. 2018 (57)	adult ALL	357	8	41(11%)	15(4.2%)	15(4.2%)	26(7.3%)	0
Popov et al. 2019 (29)	children ALL	155	6	58(37%)	28(18%)	28(18%)	32(20%)	0
Del Principe et al.2019 (34)	adult ALL	240	6-8	61(25%)	18(7%)	18(7%)	43(18%)	0
Thastrup et al.2020 (35)	children ALL	673	8-9	171(25%)	90(13%)	195(29%)	n.d.	24(3%)
Shalabi et al. 2020 (31)	ALL	352	8	59(6%)	25(6.5%)		34(9.7%)	0

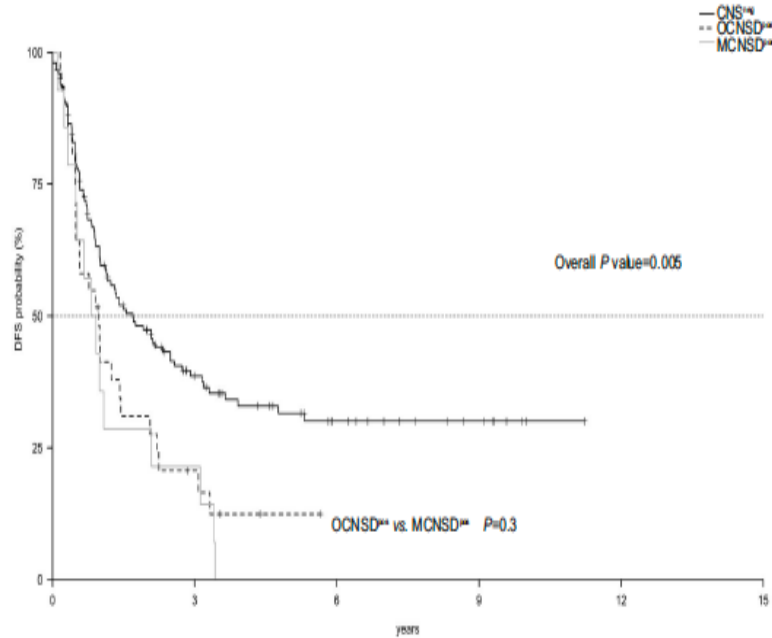
HM, hematologic malignancies; NHL, non Hodgkin Lymphoma; ALL, acute lymphoblastic leukemia; DLBCL, Diffuse Large B cell Lymphoma, BL, Burkitt lymphoma

Del Principe MI et al. Cytometry B Clin Cytom. 2021 ;100(3):269-281

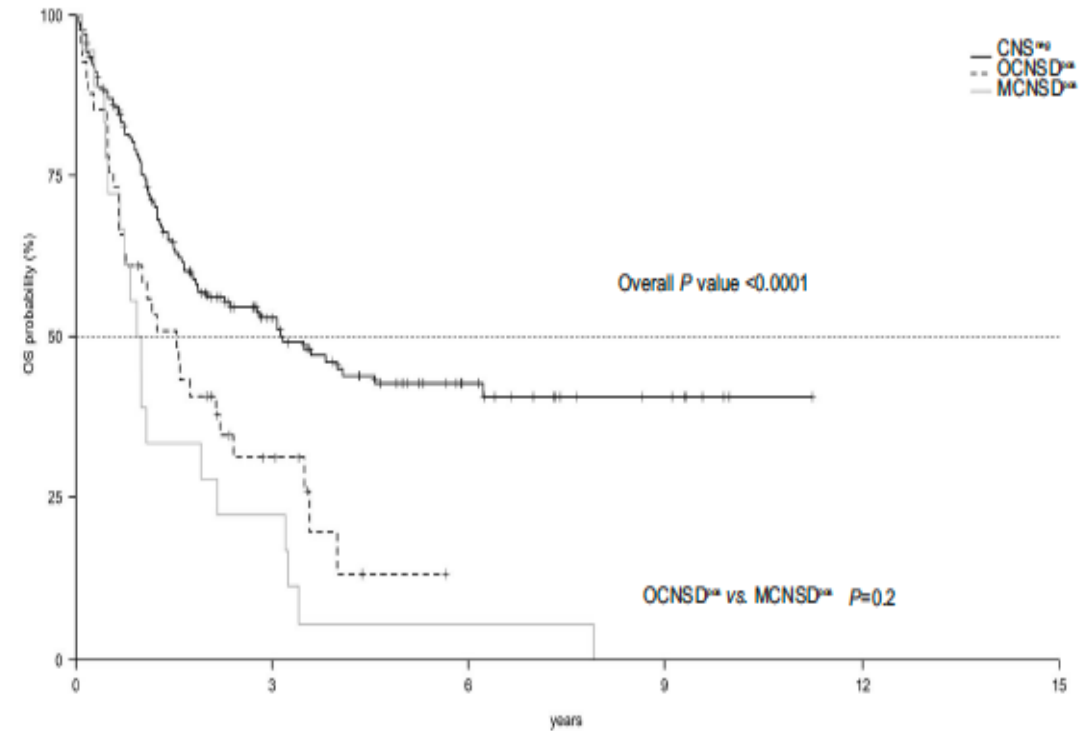


Correlation between CNS status and outcome. A CAMPUS ALL study

Disease free survival



Overall survival



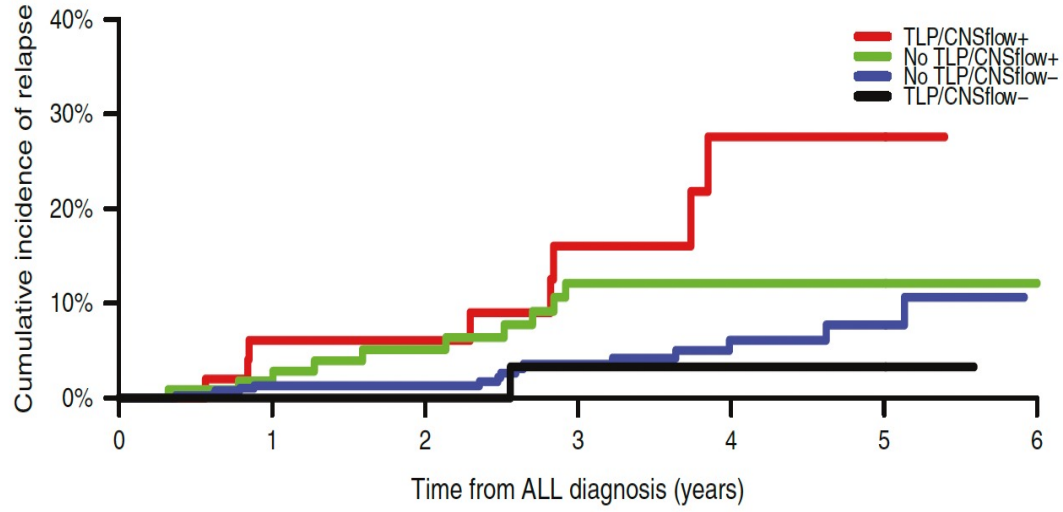
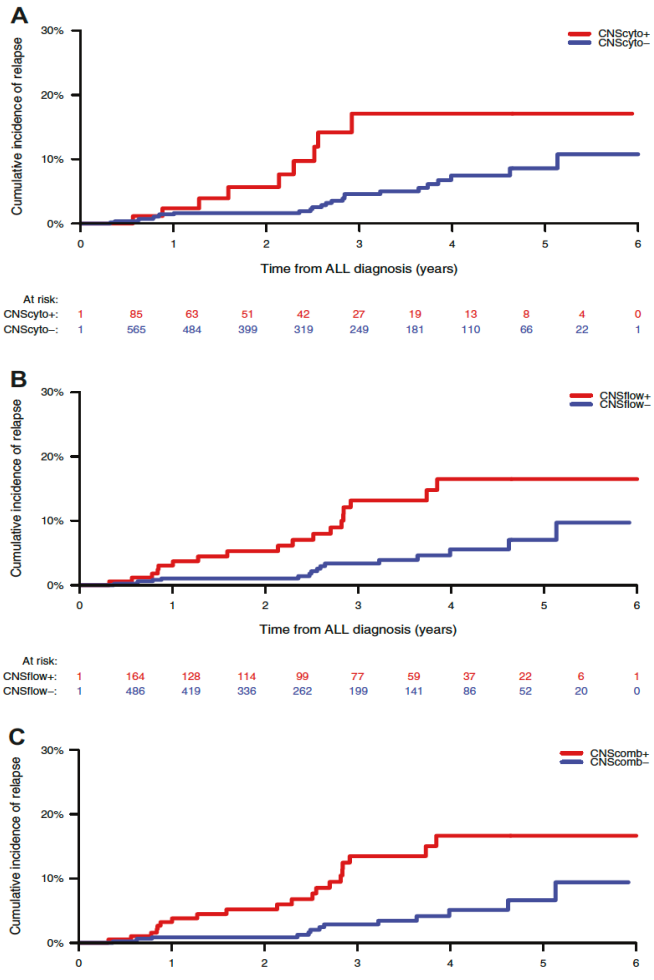
Del Principe et al. Haematologica. 2021;106(1):39-45.

Advances in Flow Cytometry:
from research to the clinic for personalized medicine

2 Marzo 2023 - Roma
Università degli Studi di Roma "Tor Vergata"
Facoltà di Medicina e Chirurgia

MFC detection of leukemic blasts in CSF predicts risk of relapse in childhood ALL: a Nordic Society of Pediatric Hematology and Oncology study

Fig. 2 Cumulative incidence of relapse according to CNS status at diagnosis. CNS status was classified by a cytospin (CNS_{cyto+} = CNS2 or CNS3; CNS_{cyto-} = CNS1; HR = 2.7 (95% CI 1.3–5.8; *P* = 0.010)), b flow cytometry (CNS_{flow+} ≥ 10 blasts detected; CNS_{flow-} < 10 blasts detected; HR = 3.0 (95% CI 1.5–5.9; *P* = 0.0013)), and c combined cytospin/flow cytometry (CNS_{comb+} = CNS_{flow+} and/or CNS_{cyto+}; CNS_{comb-} = CNS_{flow-} and CNS_{cyto-}; HR = 3.4 (95% CI 1.7–6.8; *P* < 0.001)). CNS = central nervous system. ALL = acute lymphoblastic leukemia



Thastrup M Leukemia. 2020 Oct;34(10):2822.



Technical Pitfalls of MFC

- 1) Low cellularity
- 2) Rapid decline of leucocytes count upon lumbar puncture
- 3) Blood contamination



ESCCA/ISCCA recommendations for the analysis of cerebrospinal fluid by MFC in HM

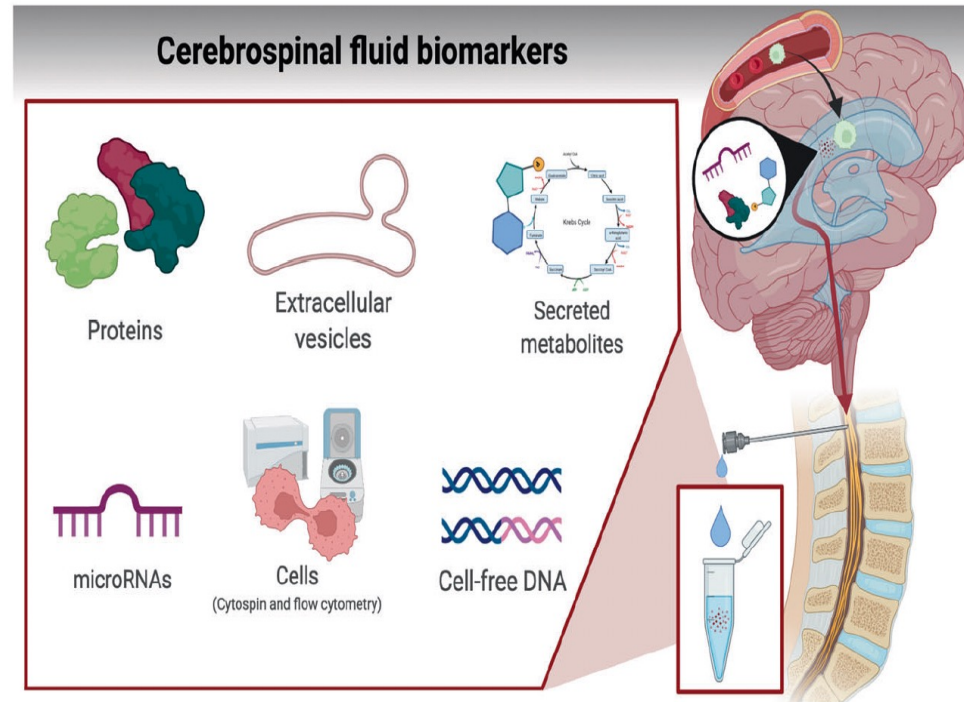
Issue	Recommendation
Scarce Cell Events in CSF	Acquisition of at least 1000 total events recommended
	Even if a few clustered events with suggestive phenotype can be considered diagnostic
Time of Processing and Transport	The CSF sample should be processed within 60 minutes from harvesting or stored in Transfix tubes
Threshold	At least 10 phenotypically abnormal events to consider the sample as positive
Blood contamination	Simultaneous PB sample required to exclude blood contamination
	Presence of blasts in CSF should be always reported even if blood contamination is suspected
Panels	8 or more color panels should be preferred to characterize all events present in the sample
Interpretation of MFC findings	To avoid inaccurate reporting, on routine practice, analysis should be restricted to patient with known HM or, when diagnosis is not known, to samples with more than 5 cells/ μL

Del Principe MI et al. Cytometry B Clin Cytom. 2021 ;100(3):269-281



Central nervous system involvement in childhood ALL : challenges and solutions

BETTER BIOMARKERS



Biomarker(s)	Detection method	Study design
Extracellular vesicles (leukemia-derived exosomes for CSF-barrier transmigration) [83]	Fluorescent microscopy	Experimental
microRNA [89]	qPCR	Cross-sectional observation

Biomarker(s)	Detection method	Study design	Patient cohort
Circulating leukemic blasts in CSF [10]	Flow cytometry (6-color)	Prospective non-intervention study	Newly diagnosed pediatric ALL (n = 255)
DNA from CSF cells [76]	PCR for VDJ rearrangements	Prospective non-intervention study	Newly diagnosed pediatric ALL without TLP (n = 37)
DNA from CSF cells [74]	PCR for VDJ rearrangements	Prospective non-intervention study	Newly diagnosed pediatric ALL (n = 30)
DNA from CSF cells [77]	PCR for VDJ rearrangements	Prospective non-intervention study	Newly diagnosed pediatric ALL without TLP (n = 65)
DNA from CSF cells [75]	PCR for VDJ rearrangements	Prospective non-intervention study	Newly diagnosed pediatric ALL (n = 38)
Soluble biomarkers			
Cerebrospinal fluid proteome during PEG-asparaginase treatment [78]	Quantitative label-free LC-MS/MS (tryptic digest)	Cross-sectional observation	Newly diagnosed B- and T-ALL (n = 4) and lymphoblastic lymphoma (n = 1)
Cerebrospinal fluid proteome alterations comparing before vs after induction therapy [80]	Quantitative label-free LC-MS/MS (tryptic digest)	Cross-sectional observation	Pediatric patients with confirmed CNS B-ALL (n = 6)

ALL acute lymphoblastic leukemia, CNS central nervous system, CSF cerebrospinal fluid, TdT terminal deoxynucleotidyl

Thastrup M et al. Leukemia. 2022.36:2751 – 2768



Convegno Regionale SIES Delegazione Emilia Romagna

Biopsia liquida: CHE TRAFFICO IN PERIFERIA!

Bologna

28 Febbraio – 1 Marzo 2025

Central nervous system involvement in childhood ALL : challenges and solutions

BETTER DRUGS^A

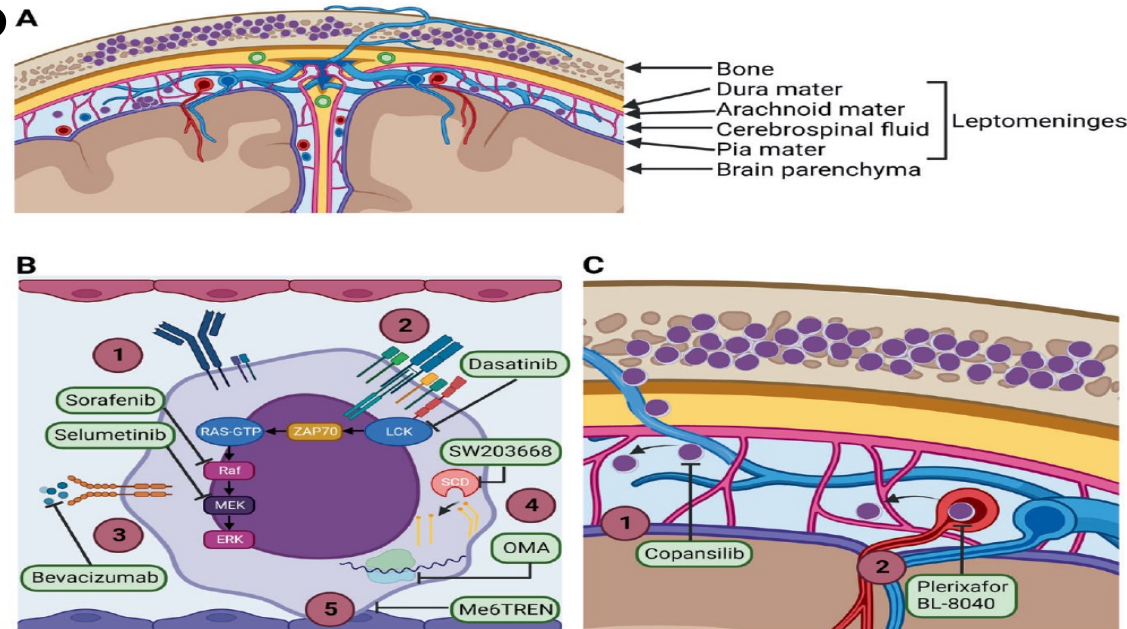


Fig. 2 Mechanisms of action of drugs that target leukemic cells within the CNS. **A** Coronal section of human brain showing the meninges and the meningeal vasculature. The leptomeninges consist of the arachnoid mater, the pia mater and the subarachnoid space. The subarachnoid space is filled with CSF, veins, arteries and arachnoid trabeculae extending from the arachnoid mater to the pia mater. **B** Novel drugs that target survival mechanisms employed by leukemia cells in the leptomeninges [1]. Sorafenib and selumetinib inhibit Ras/Raf/MEK/ERK signaling downstream of B-cell receptor activation [2]. Dasatinib inhibits LCK signaling downstream of T-cell receptor activation [3]. Bevacizumab sequesters VEGF-A and inhibits binding to the VEGFR2 [4]. SW203668 inhibits SCD-mediated enzymatic conversion of saturated fatty acids to mono-unsaturated fatty acids and OMA inhibits ribosome mRNA translation [5]. Me6TREN inhibits adhesion of leukemia cells to meningeal cells. **C** Novel drugs that target invasion mechanisms employed by leukemia cells during dissemination to the leptomeninges [1]. Copansilib inhibits integrin $\alpha 6$ -mediated migration of leukemia cells along emissary vessels [2]. Plerixafor or BL-8040 block CRCX4-mediated migration across meningeal blood vessels. LCK lymphocyte specific cell-kinase, CNS central nervous system, CSF cerebrospinal fluid, SCD stearoyl-CoA desaturase, VEGF vascular endothelial factor, OMA omacetaxine mepesuccinate.

Thastrup M et al. Leukemia. 2022.36:2751 – 2768



CONCLUSIONS: where are we in 2025?

- ✓ Comprehensive determination of pre-treatment (karyotype, genetics) and post treatment (MRD) refines prognosis
 - MRD is a biomarker that identifies patients with different outcome at various time-points within homogeneous biological subgroups
- ✓ MFC and RT-qPCR are the techniques of choice
 - High technical standard requirement
 - Complementary application (according to specific transcript or phenotypic array)
 - High-throughput techniques under validation process
- ✓ Optimizing treatment of the CNS remains a challenge in ALL
 - Some biomarkers, such as CSF-flow cytometry, are now being tested in prospective trials.
 - Novel drugs are also being tested in Phase I/II trials, although wider access for iCNS relapse patients is needed.
 - The future is hopeful for improved management of the CNS over the next decade.





THANK YOU!

