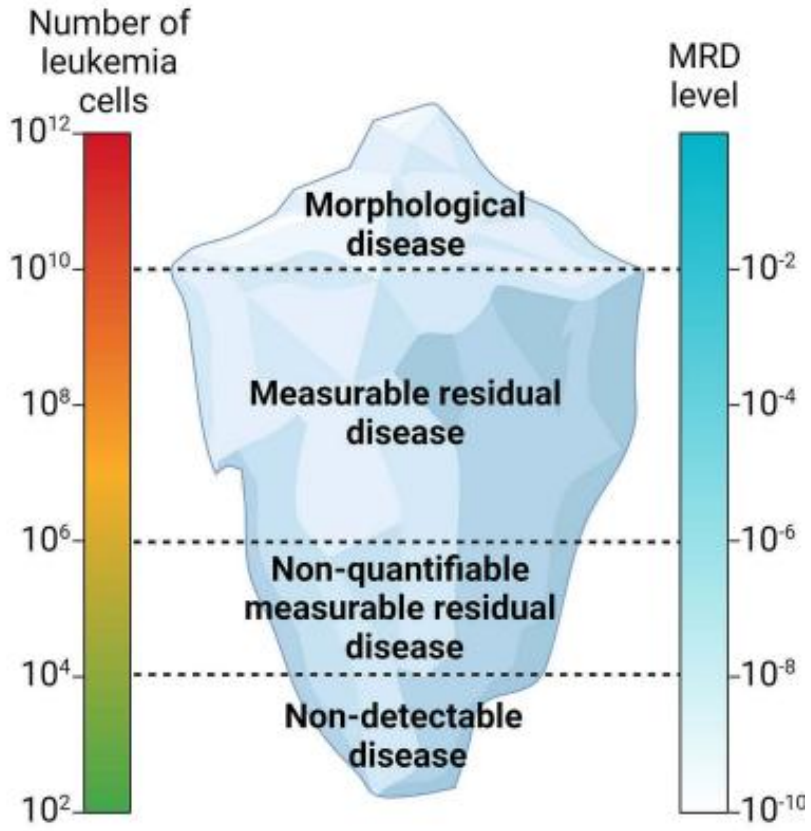
Measurable Residual Disease in Acute Leukemia: Where We Are and Where We Are Going?



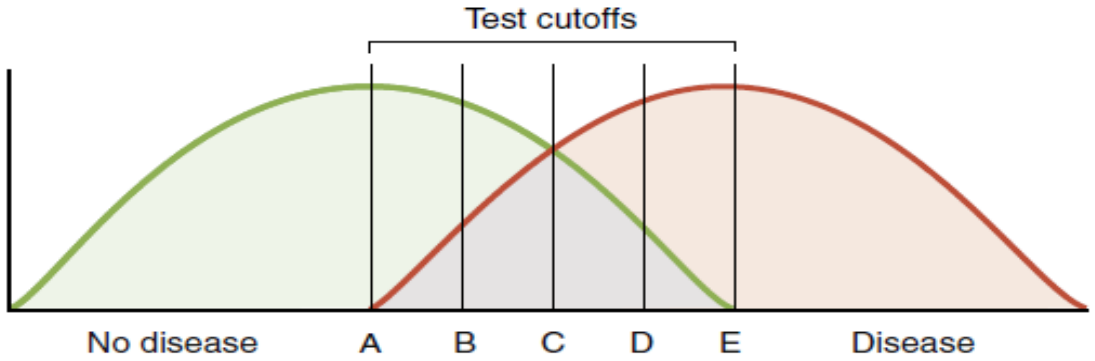


Figure 8.3 Effects of varying the test cutoff on overlapping populations of patients with and without disease.

- **Changing the cutoff from C to A:**
  - All diseased persons will have a positive test, and the sensitivity will be 100%.
  - Increased sensitivity is associated with decreased specificity, and the (FPs) increases.
- **Changing the cutoff from C to E:**
  - All non-diseased persons will have a negative test, and the specificity will be 100%.
  - Accompanied by concomitant decreased sensitivity and additional FN results.

MRD Modalities(AML)

	Method	Target	Sensitivity	Applicable in % of AML	Turn-around time (days)	Limitations/ problems
Established	Multi-parameter flow cytometry (MFC)	Leukemia-associated immunophenotype (LAIP) or different from normal (DfN)	10^{-3} to 10^{-4}	85-90	2	Less sensitive, more subjective analysis
Established	Real-time quantitative PCR (RT-qPCR)	Robust data: *NPM1*, *CBFB::MYH11*, *RUNX1::RUNX1T1* Less validated: *KMT2A::MLLT3*, *DEK::NUP214*, *BCR::ABL1*, *WT1*	10^{-4} to 10^{-5}	40-50*	3-5	Limited applicability
Exploratory	Next-generation sequencing (NGS)†,‡	Potentially any somatic mutation†	10^{-2} to 10^{-4}	~100	5-10	Less sensitive, costly, technically challenging
Exploratory	Digital PCR (dPCR)	Specific targeted mutations	10^{-3} to 10^{-4}	~70	3-5	Specific assay necessary for every mutation, limited sensitivity

*Less frequent in elderly patients with AML.

†The NGS-MRD threshold has not been defined for individual mutations; NGS-MRD positivity is provisionally defined as ≥ 0.1% variant allele frequency, excluding mutations related to clonal hematopoiesis and germline mutations.

‡Common gene mutations consistent with pre-malignant clonal hematopoiesis such as *DNMT3A*, *TET2*, and *AXSL1* excluded; further study is required to determine which mutations are truly indicative of residual AML and not clonal hematopoiesis.

MRD Modalities(ALL)

	Multi-color flow cytometry	qPCR for fusion genes	ASO-qPCR for IG/TR genes	High-throughput NGS
Sensitivity	10^{-4}	10^{-4} to 10^{-5}	10^{-4} to 10^{-5}	10^{-6}
Applicability	>90%	40-50%	90-95%	>90%
Advantages	- Rapid - Relatively inexpensive - DfN method does not require access to diagnostic specimen	- Sensitive - Standard primers used for specific fusions	- Sensitive - Applicable to most patients - Standardized guidelines in Europe	- Very sensitive - Applicable to almost all patients - Clone-unbiased (can track multiple clones and evolution) - Only US FDA-approved assay (ClonoSEQ) - Data for MRD use in peripheral blood
Limitations	- Variable sensitivity - Requires technical expertise - Fresh cells required - Less standardized - Immunophenotypic shifts can lead to false negative results	- Not applicable to all patients	- Time-consuming - Expensive - Relies on pre-treatment sample - Requires extensive experience and labor	- Expensive - Longer turn-around time than MFC - Requires diagnostic pre-treatment sample

2022 https://doi.org/10.3324/haematol.2022.280638

- **Mass cytometry time of flight (CyTOF)** :
  - Labeling antibodies with heavy metal isotopes instead of fluorochrome.
  - Evaluating samples using time-of-flight mass spectrometry .
  - CyTOF allows a larger number of parameters (greater than 40) to be evaluated and eliminates much of the “background noise” which can be a problem in high sensitivity MFC assays.
  - It may allow for earlier assessment of MRD and a personalized optimization of therapy.
  - Need for complex analytical tools, lack of validation in large clinical trials and expense.

Front. Oncol. **2019**, *9*, 655.

HemaSphere **2019**, *3*, S2.

Case Presentation

- An 7-year-old child presented with a 2-week history headache and weight loss.

- **Physical examination:**
  - Cervical lymphadenopathy
  - Hepatosplenomegaly.

- **Laboratory tests:**

  - WBC: 23000/ul.
  - Hb level of 6.5 g/dL
  - Platelet:53000/uL.
  - PB smear showed granulocytic hyperplasia and 25% blasts.

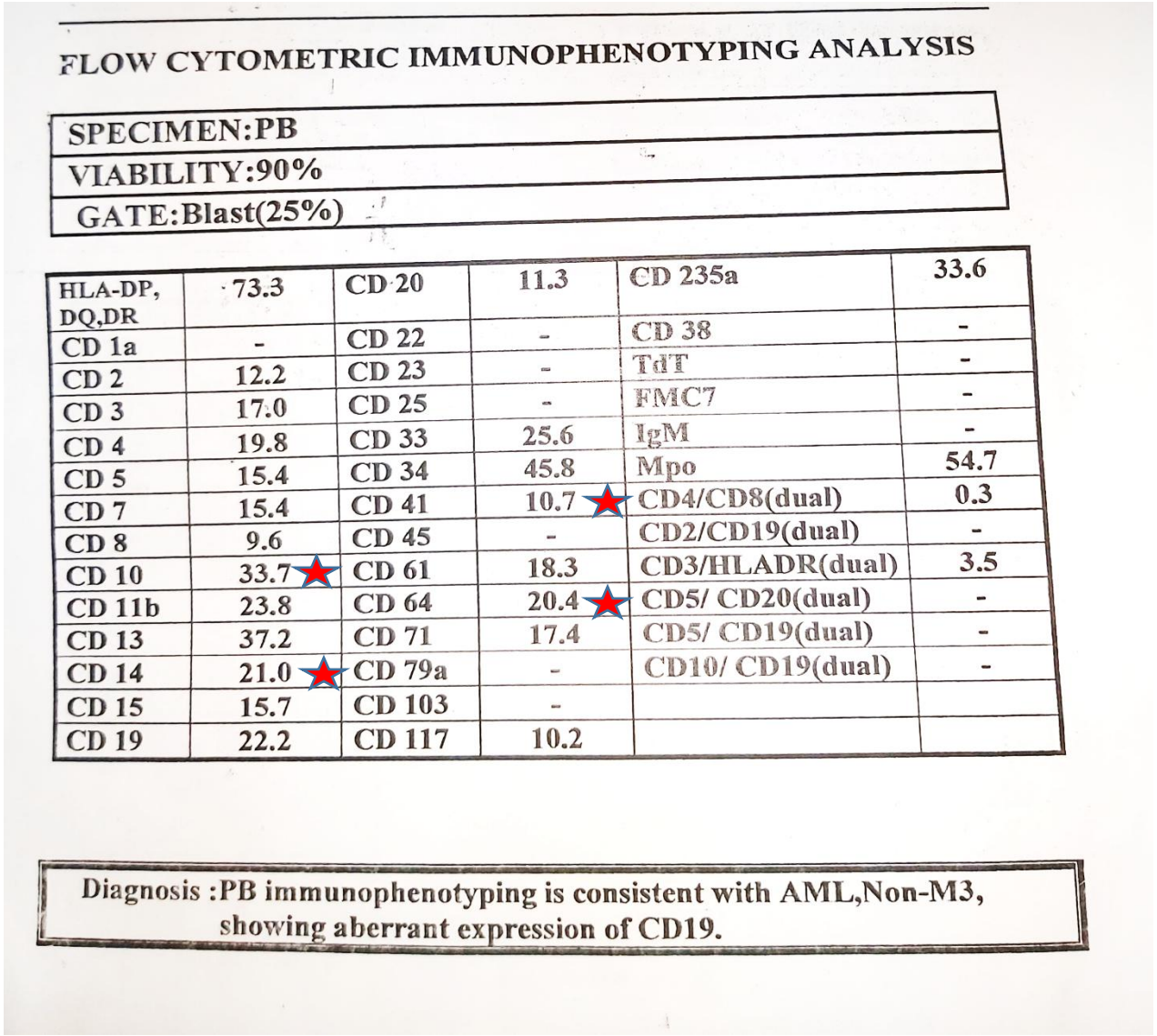
- **RT-PCR :**
  - BCR/ABL rearrangement (p210).

- **Immunophenotyping on peripheral blood:**
  - 25% blasts
  - Diagnosed as AML with aberrant expression of CD19 .

- Cerebrospinal fluid analysis was negative for leukemic involvement.

- She was treated with AML-BFM protocol and achieved remission following AML induction chemotherapy.

Immunophenotyping: (DX)



FLOW CYTOMETRIC IMMUNOPHENOTYPING ANALYSIS

SPECIMEN:PB
VIABILITY:90%
GATE:Blast(25%)

HLA-DP, DQ,DR	73.3	CD 20	11.3	CD 235a	33.6
CD 1a	-	CD 22	-	CD 38	-
CD 2	12.2	CD 23	-	TdT	-
CD 3	17.0	CD 25	-	FMC7	-
CD 4	19.8	CD 33	25.6	IgM	-
CD 5	15.4	CD 34	45.8	Mpo	54.7
CD 7	15.4	CD 41	10.7 ★	CD4/CD8(dual)	0.3
CD 8	9.6	CD 45	-	CD2/CD19(dual)	-
CD 10	33.7 ★	CD 61	18.3	CD3/HLADR(dual)	3.5
CD 11b	23.8	CD 64	20.4 ★	CD5/ CD20(dual)	-
CD 13	37.2	CD 71	17.4	CD5/ CD19(dual)	-
CD 14	21.0 ★	CD 79a	-	CD10/ CD19(dual)	-
CD 15	15.7	CD 103	-		
CD 19	22.2	CD 117	10.2		

Diagnosis :PB immunophenotyping is consistent with AML,Non-M3, showing aberrant expression of CD19.

Harmonization and standardization

Pacific Blue™	OC515™	FITC	PE	PerCP-Cyanine5.5	PE-Cyanine7	APC	APC-C750™
CyCD3	CD45	CyMPO	CyCD79a	CD34	CD19	CD7	SmCD3

- **CD34**
- **CyMPO**
- **CD19**
- **CyCD79a**
- **CD7**
- **SmCD3**
- **CyCD3**

Myeloid Markers: CD117, HLA—DR, CD33, CD13, CD11b, CD15,
Monocyte Marker: CD14, CD36, CD64,CD4, CD11c

B cells Marker: CD22, CD10, CD20 and TdT

T cells Marker: CD1a, CD2, CD4, CD5, CD8, CD10, and TdT

Flow-Cytometric Monitoring of Minimal Residual Disease

Dx: 20/1/1398
Flow MRD: 15/4/1398

Clinical history:

IMMATURE CELL MARKERS	%	GATE
CD117	4.6	Total
CD34	2.4	Total
CD10	11.6	Total
CD123	2.9	Total
MPO	-	Total
TDT	-	Total
LYMPHOCYTE MARKERS		
CD1a	-	Total
CD2	9.6	Total
CD3	7.9	Total
CD5	8.5	Total
CD7	8.6	Total
CD19	14.7	Total
CD20	10.6	Total
CD22	-	Total
HLA DR	7.8	Total
CD4	-	Total
CD8	-	Total
MYELOMONOID MARKERS		
CD13	9.5	Total
CD33	18.4	Total
CD14	7.0	Total
CD64	21.7	Total
CD15	58.2	Total

CYTOCHEMISTRY	Reaction
Myeloperoxidase	
Periodic Acid Schiff	
Non Specific Esterase	
Differential	% of Total
PMN	
Lymph	
Mono	
IMMATURE CELLS	
VIABLE CELLS	

Diagnosis : BM immunophenotyping revealed relative polymorphic hematopoietic cells.There is no evidence of minimal residual disease (MRD) by multicolor flow cytometric immunophenotyping. (Please see attached sheet)

How to report MRD

- The minimum number of cells needed for accurate reporting of MRD
- AML: at least 500 000 to 1 million (0.1%)
- ALL: at least 5 million (0.01%)

- **Elements in an MRD report should contain the following parameters:**
- Absolute numbers of LAIP cells and WBCs
- Threshold level
- Quality of the sample
  - viability,
  - insufficient regeneration
  - and PB contamination
- **LLOD**
- **LLOQ**

Standardization of MFC

- There are still ~25% of patients who relapse despite achieving MRD negativity:

  - **Technical:**
    - Lack of standardization of assays between laboratories:
      - Differing surface markers used
      - Varying levels used to define positivity and negativity
    - Inherent need for a subjective interpretive component

  - **Biological:**
    - Presence of LSCs which are undetectable by routine methods.
    - ELN recommends the inclusion of a tube to determine residual LSCs in MFC MRD analysis.

Blood **2018**, *131*, 1275–1291.

N. Engl. J. Med. **2015**, *373*, 1136–1152.

Technical

ELN 2021 MFC-MRD recommendations based on a Delphi poll

Multiparameter flow cytometry MRD recommendation	LoE	GoR	LoA (%)
Request first-pull BM aspirate for MRD, and process sample within 3 days of storage, undiluted, in ambient conditions.	V	A	94
Implementation of a minimum required set of tubes/ fluorochromes combination is a prerequisite for harmonized LAIP/DfN MRD detection, analysis and reporting.	I	A	94
We recommend harmonized use of the integrated diagnostic LAIP and DfN strategy for MRD detection that incorporates the core MRD markers CD34, CD117, CD45, CD33, CD13, CD56, CD7, and HLA-DR, to assess all samples.	V	B	88
When immunophenotypic abnormalities in specific samples may reflect transient features of regenerating or "stressed" hematopoiesis, the MRD report should comment on this possibility and note that a repeat sample in 2-4 wk, if clinically indicated, may be informative.	V	C	94
The standard for determining MFC MRD negativity is the acquisition of >500 000 $CD45^+$ cells and ≥100 viable cells in the blast compartment assessed for the best aberrancy(ies) available.	V	B	76
For samples stored at ambient temperature >3 d, the MRD report should make specific note of potentially compromised cell viability.	V	B	94
LLOD and LLOQ should be calculated to assess MFC-MRD assay performance.	V	B	93

GoR, grade of recommendation; LoA, level of agreement; LOD, limit of detection; LoE, level of evidence; UMI, unique molecular identifier.

Biological

ELN 2021 future improvement of MRD recommendations based on a Delphi poll

No.	Future improvement of MRD recommendation	LoE	GoR	LoA (%)
C1	LSCs can be immunophenotypically defined as $CD34^{+}/CD38^{-}$ cells[55] combined with an aberrant marker not present on HSCs (eg, CD45RA, CLL-1, or CD123).	IV	A	95
C2	Measurements of LSCs may have prognostic value and should be further validated in prospective clinical trials.	IV	B	86
C3	LSC detection requires optimally 4 million events, most likely best achieved with a 1-tube assay.	V	B	78
C4	High-quality flow cytometry data (standardized instrument settings, preanalytics, and measurements) are necessary for future automated analyses.	IV	A	100

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Qualitative BCR/ABL assay(MRD)

Dx: 20/1/1398
Mol MRD: 15/4/1398

Test	t(9:22) BCR-ABL P210
Result	Positive

Method: Real Time PCR

The translocation t(9;22)(q34;q11.2) results in the generation of the Philadelphia (Ph) chromosome. The BCR-ABL1 fusion gene formed then translates a protein with tyrosine kinase activity. The t(9;22) had been reported in chronic myeloid leukemia (CML), AML and ALL.

Ph-positive ALL is characterised by presence of intragenic deletion of the IKZF1 (Ikaros) gene, which is not found in Ph- positive CML and AML . The incidence of Ph- positive ALL increases with age from 2% in children to 29% in adults (30-39 years), and to 40% in older adults (40+ years). Although the Ph chromosome in ALL is associated with a poor prognosis, treatment with tyrosine kinase inhibitors have improved the event free survival rates of ALL patients compared to patients treated with traditional chemotherapy and stem cell transplantation. However, Ph- positive ALL with IKZF1 deletions has an unfavourable outcome even with imatinib therapy compared to those with IKZF1 wild-type.

It involves band 34 of the long arm of chromosome 9, splicing the proto- oncogene c-abl to band 11 of the long arm of chromosome 22 in the bcr gene. In 50-80% of ALL cases, the breakpoint in 22q11 falls between exons b1 and b2 of the major breakpoint cluster region (M-bcr).

Clinically, children with Ph-positive blasts are older, have higher leukocyte counts, larger percentages of circulating blasts, a higher frequency of FAB L2 morphology, a higher frequency of CNS leukaemia and more prevalent pseudodiploid karyotypes than are found in Ph negative cases. Most Ph+ blasts have a B- lineage immunophenotype, although isolated cases with a T-cell or mixed phenotype have been reported. The consistent lack of success in treating this form of ALL has prompted most investigators to consider BMT during first remission as a therapeutic option. In addition, it was recently noted that children with Ph+ALL and low initial blood cell counts may have a durable response to chemotherapy.

Quantitative BCR/ABL assay(MRD)

Dx: 20/1/1398
Mol MRD: 15/4/1398

Test	t(9:22) BCR-ABL P210 *(quantitative)*
Result	**Positive : 0.01%** Limit of detection: (<0.0001%)

Method: Real Time PCR

The translocation t(9;22)(q34;q11.2) results in the generation of the Philadelphia (Ph) chromosome. The BCR-ABL1 fusion gene formed then translates a protein with tyrosine kinase activity. The t(9;22) had been reported in chronic myeloid leukemia (CML), AML and ALL.

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Standardization of Quantitative Polymerase Chain Reaction(qPCR)

ELN 2021 molecular MRD recommendations based on a Delphi poll

Molecular MRD recommendation	LoE	GoR	LoA (%)
Techniques for molecular MRD assessment should reach an LOD of 10^{-3} or lower. To achieve this LOD, qPCR, dPCR, or error-corrected NGS using UMIs is recommended.	IV	B	100
Either EDTA or heparin can be used on samples as an anticoagulant for molecular MRD analysis.	V	C	76
Only 5 mL of BM aspirate should be used for molecular MRD assessment from the first pull (or the first pull after repositioning, if the initial pull is used for flow-MRD).	V	B	94
The method of cell isolation should be kept consistent, as it may alter the leukemic cell percentage (eg, Ficoll separation to reduce dilution of leukemic cells with normal granulocytes or lysis of whole blood).	V	B	82
Leukemia-specific PCR assays (eg, for *NPM1*, *PML-RARA*, or *CBF* AML) are preferred over fewer specific markers, such as *WT1* or *EVI1* expression.	V	B	78

GoR, grade of recommendation; LoA, level of agreement; LOD, limit of detection; LoE, level of evidence; UMI, unique molecular identifier.

Standardization of Next Generation Sequencing (NGS)

ELN 2021 molecular MRD recommendations based on a Delphi poll

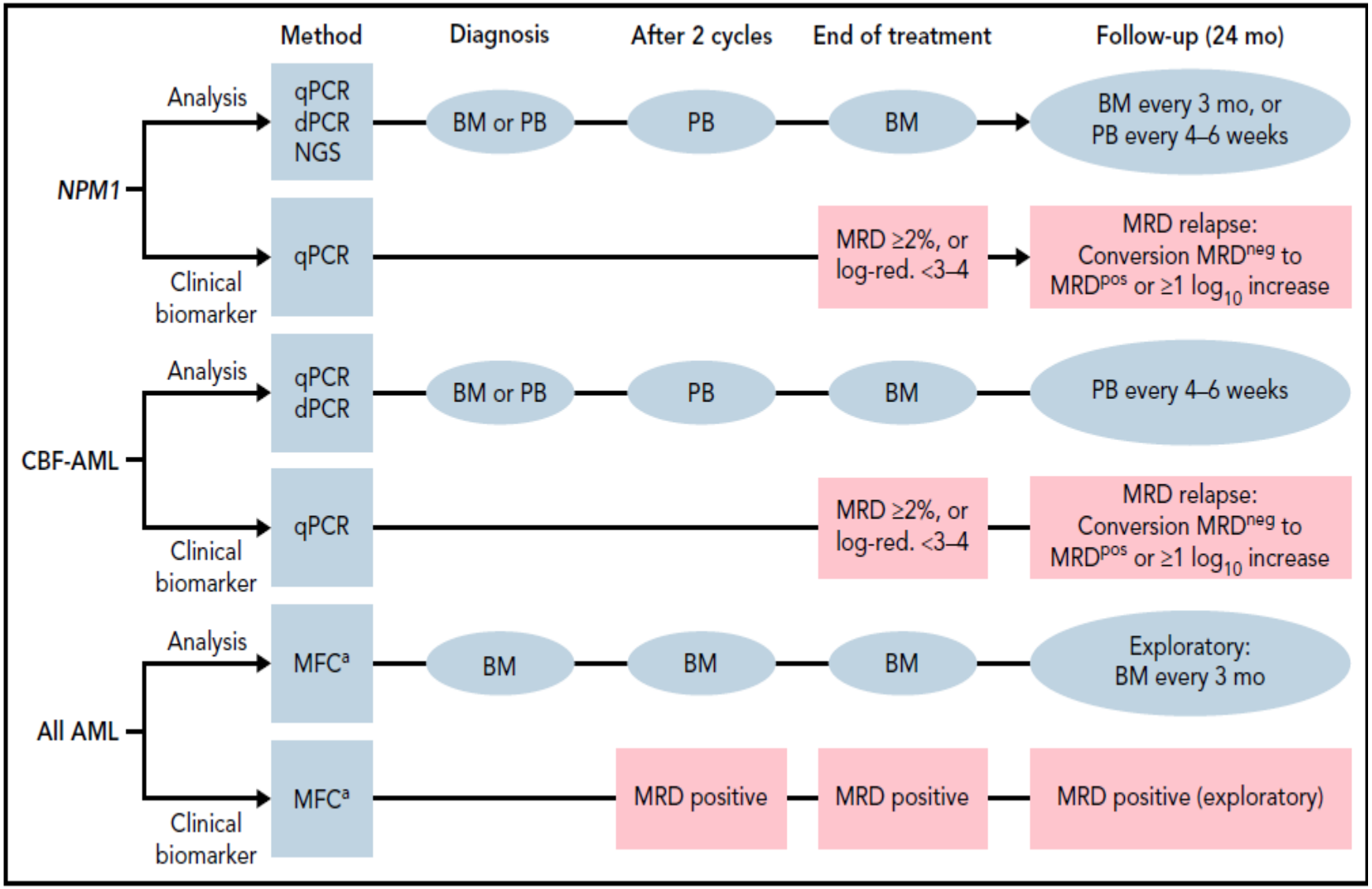
Germline mutations (*ANKRD26*, *CEBPA*, *DDX41*, *ETV6*, *GATA2*, *RUNX1*, and *TP53*) should be excluded as NGS-MRD markers, as they are noninformative for MRD.	V	A	94
Mutations in *DNMT3A*, *TET2*, and *ASXL1* (DTA) can be found in age-related clonal hematopoiesis and should be exduded from MRD analysis.	IV	A	100
Mutations in signaling pathway genes (*FLT3-ITD*, *FLT3-TKD*, *KIT*, and *RAS*, among others) most likely represent residual AML when detected, but are often subclonal and have a low negative predictive value. These mutations are best used in combination with additional MRD markers.	IV	B	94

GoR, grade of recommendation; LoA, level of agreement; LOD, limit of detection; LoE, level of evidence; UMI, unique molecular identifier.

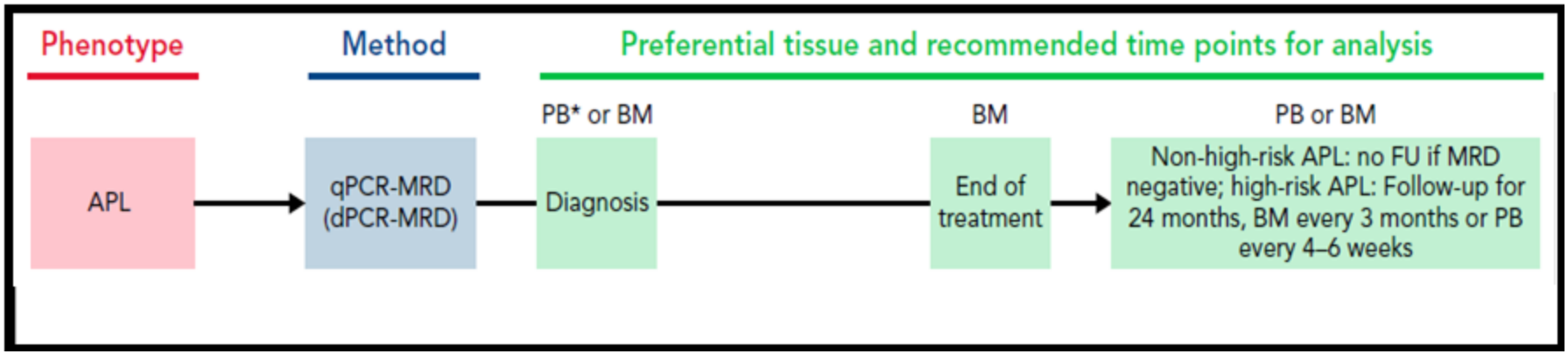
MRD Time Point

Definitions for MRD response categories and MRD relapse

Response category	Abbreviation	Defining criteria
CR with negative MRD	CR_{MRD}^{-}	1. Complete morphologic remission and 2. MRD^{-} in all MRD technologies that were used: a. FC-MRD^{-} in BM (if MFC-MRD was used). b. qPCR-MRD^{-} in BM (or in PB after cycle 2 for *NPM1*- and CBF-MRD) (if qPCR-MRD was used). c. NGS-MRD^{-} in BM (if NGS-MRD was used).
CR with positive MRD	CR_{MRD}^{+}	1. Complete morphologic remission, and 2. MFC-MRD^{+} in PB and/or BM, or 3. NGS-MRD^{+} in PB and/or BM, or 4. qPCR-MRD^{+} in PB and/or BM.
CR with molecular MRD detection at low level	CR-MRD-LL	1. Morphologic CR, and 2. Molecular MRD detectable at low level in PB and/or BM (ie, qPCR for *NPM1* <2% or NGS-MRD <0.1%, but above the detection limit of the assay).
MRD relapse	—	1. Conversion of MRD negativity to MRD positivity independent of the MRD technique, or 2. increase in MRD copy numbers ≥1 log_{10} in patients with CR-MRD-LL who are monitored by qPCR. 3. The result of (1) or (2) should be rapidly confirmed in a second consecutive sample, preferably from the BM.

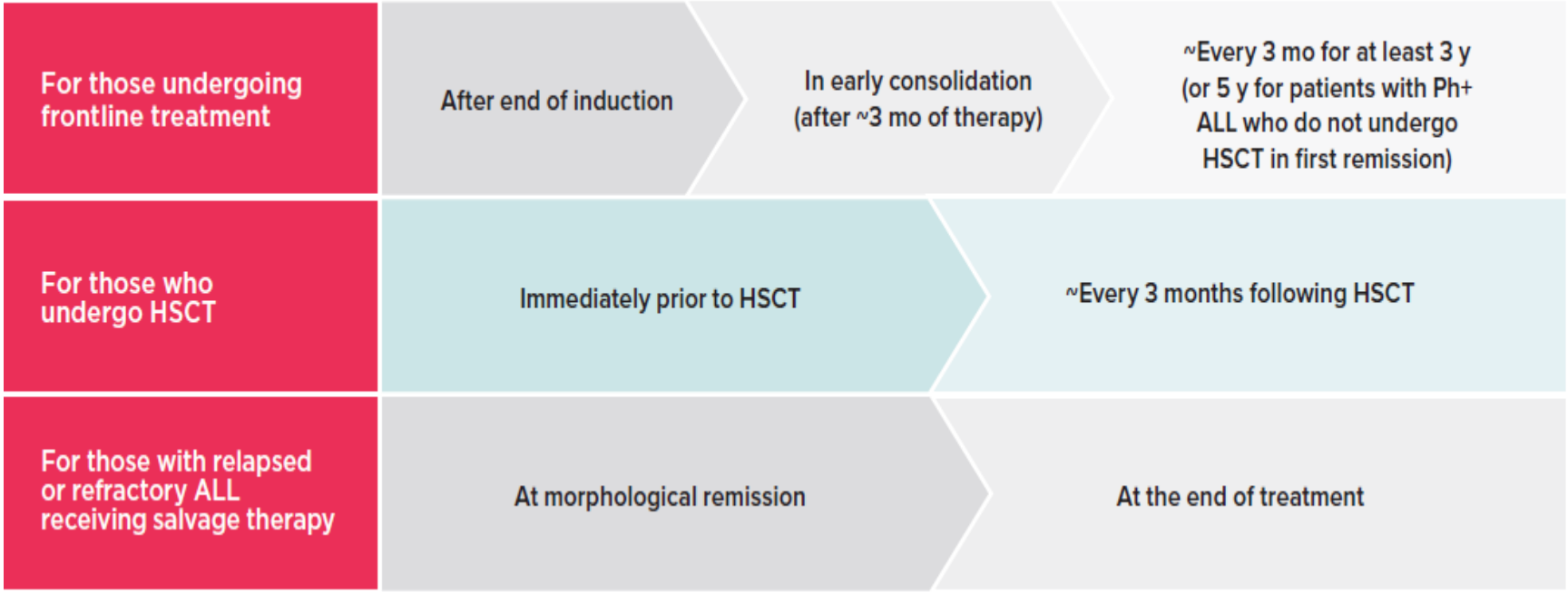


Algorithm of MRD assessment and time points at which MRD is considered a clinically relevant biomarker. Blue squares indicate timepoints of assessment and source of material; pink squares indicate timepoints for treatment modification based on a clinical relevant biomarker:



MRD assessment algorithm for different subtypes of AML. *For NPM1 and CBF AML, PB may be used for MRD assessment at diagnosis if there are ≥20% blasts in the PB. If log reduction is used as a measure of MRD response both PB and BM should be analyzed at diagnosis to have both tissues as baseline comparators.

When to assess MRD in adult ALL (in BM samples)



A consensus of North American experts. *Am J Hematol.* 2019;94(2):257-265.

- She received Imatinib (400mg) prior to allogeneic HSCT therapy

- Six months later (December, 2019) , she underwent allo-HCT from her fully HLA-matched 19 year-old brother after BuCy conditioning.

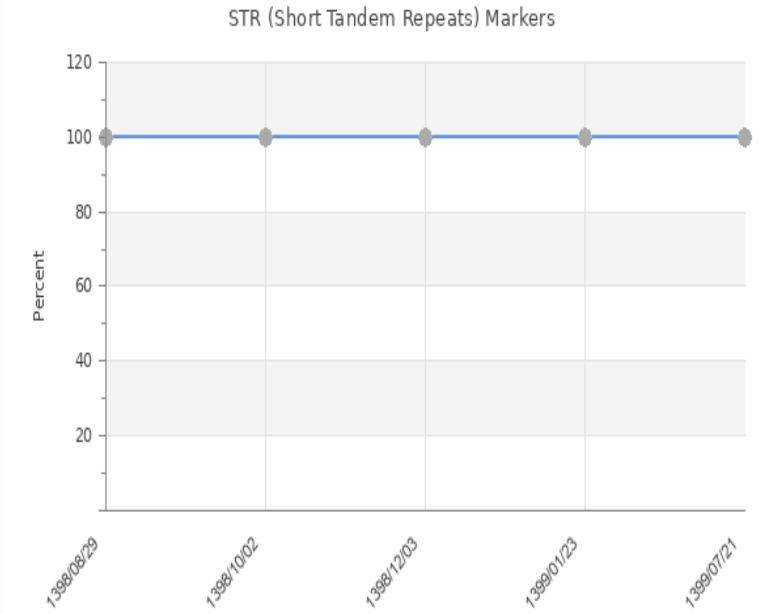
- At the time of HSCT normalized copy number of **BCR-ABL was undetectable**

- Engraftment was confirmed by chimeric exam and patient remained in CR after transplantation with controlled chronic graft versus host disease (cGvHD), that responded to low-dose steroids, and immunosuppressive medications were discontinued 10 months after transplant.

STR technique showed complete donor chimera

Result:	100%

Whole blood showed about 100% donor chimerism.
The specimen contains approximately 100% donor DNA and approximately 0% recipient DNA. The analytical sensitivity of this assay is approximately 5% in a posttransplant specimen (donor and recipient DNA mixed chimerism).



	1398/08/29	1398/10/02	1398/12/03	1399/01/23	1399/07/21
STR (%)	100%	100%	100%	100%	100%

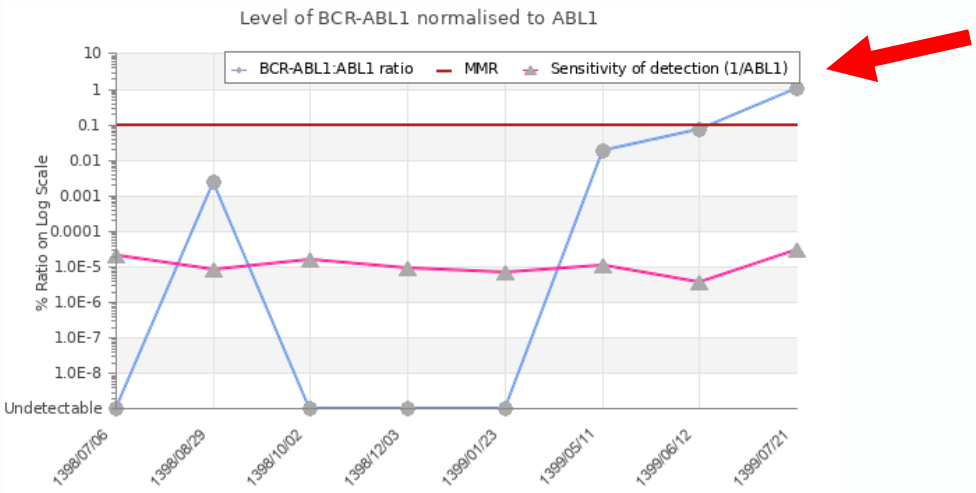
Methods: 1-DNA Extraction, 2-STR-PCR, 3-Fragment Analysis

A significant increase in BCR-ABL level with full chimerism

BCR-ABL1 monitoring report (transcript type=e13a2/e14a2)

BCR-ABL1:ABL1 % ratio	MR level
1.060309	CHR

11 months after HSCT



Date	Copies of BCR-ABL1	Copies of ABL1	BCR-ABL1:ABL1 ratio	BCR-ABL1:ABL1 ratio (%)	Sensitivity of detection (1/ABL1)	Molecular Response (MR)
1398/07/06	Undetectable	46765	0	0	0.000021	MR^5
1398/08/29	3	119204	0.000025	0.002517	0.000008	$MR^{4.5}$
1398/10/02	Undetectable	63269	0	0	0.000016	MR^5
1398/12/03	Undetectable	105290	0	0	0.000009	MR^5
1399/01/23	Undetectable	143664	0	0	0.000007	MR^5
1399/05/11	17	87221	0.000195	0.019491	0.000011	MR^3
1399/06/12	200	279424	0.000716	0.071576	0.000004	MR^3

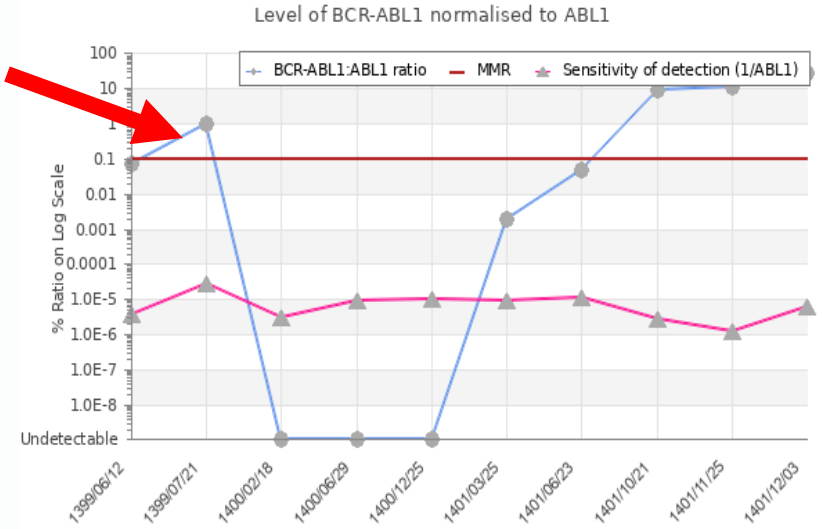
Significant increase in the amount of BCR/ABL was detected, and in the subsequent monitoring, this increase continued by more than 1 logarithm and reached 1%. BM aspirate by flow < 1% blast and cytogenetic study showed all 10 metaphases had a normal male karyotype

- **To confirm imatinib resistance** :
  - BCR/ABL kinase domain mutation analysis was performed by carrying out reverse-transcriptase PCR and direct sequencing.
  - Heterozygote missense mutation at the 253th nucleotide, with a substitution of tyrosine to histidine mutation at **[Y253H]**.
  - Imatinib was immediately changed to dasatinib
- Dasatinib was immediately started, and the patient's follow-up BCR/ABL evaluation revealed a **dramatic decrease** .

A significant increase in BCR-ABL level after Dasatinib treatment

BCR-ABL1:ABL1 % ratio	MR level
28.575723	

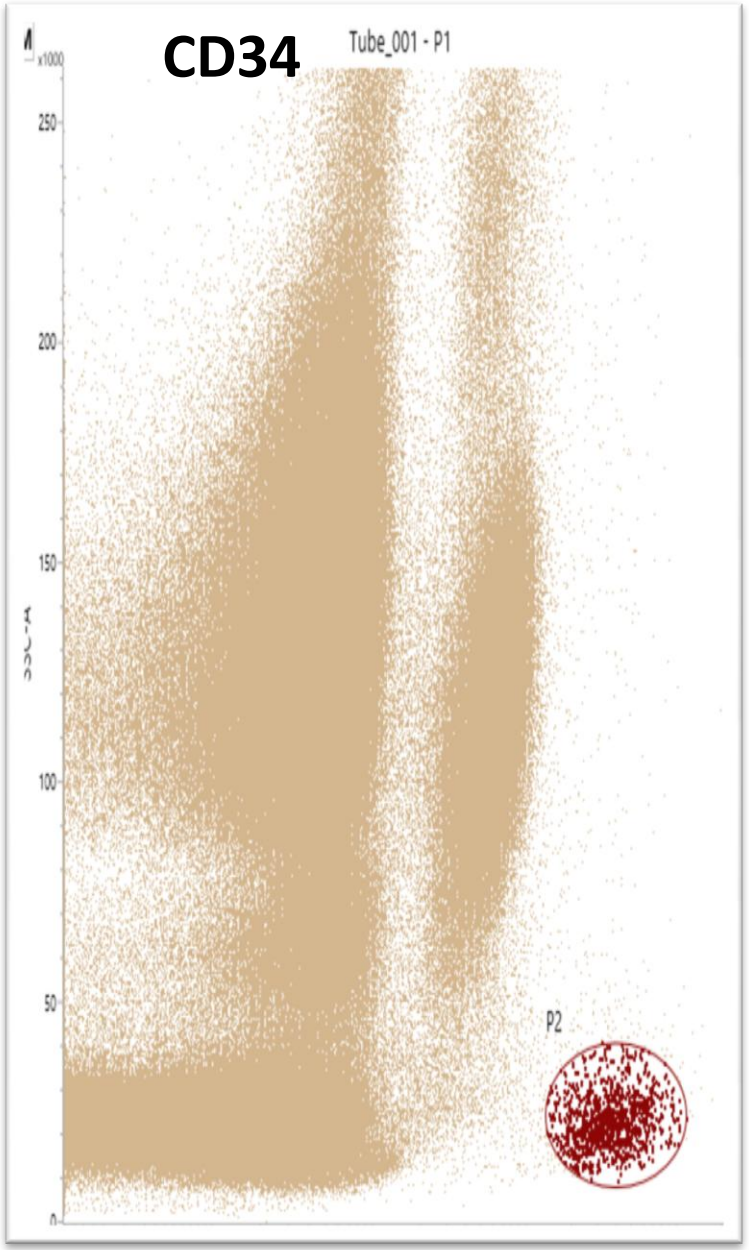
Starting Dasatinib

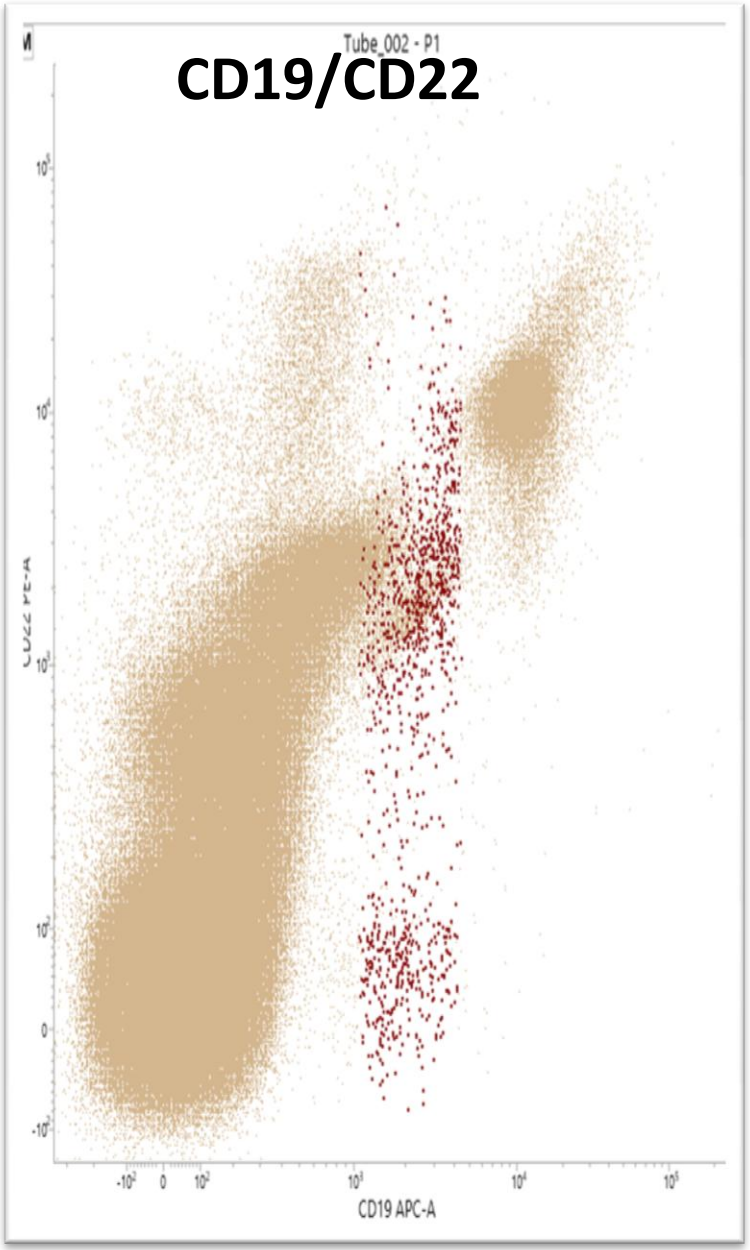


Date	Copies of BCR-ABL1	Copies of ABL1	BCR-ABL1:ABL1 ratio	BCR-ABL1:ABL1 ratio (%)	Sensitivity of detection (1/ABL1)	Molecular Response (MR)
1399/06/12	200	279424	0.000716	0.071576	0.000004	MR^3
1399/07/21	362	34141	0.010603	1.060309	0.000029	CHR
1400/02/18	Undetectable	324574	0	0	0.000003	MR^5
1400/06/29	Undetectable	103386	0	0	0.00001	MR^5
1400/12/25	Undetectable	93315	0	0	0.000011	MR^5
1401/03/25	2	106068	0.000019	0.001886	0.000009	$MR^{4.5}$
1401/06/23	43	90782	0.000474	0.047366	0.000011	MR^3
1401/10/21	33626	374222	0.089856	8.985575	0.000003	CHR
1401/11/25	91340	782303	0.116758	11.675783	0.000001	
1401/12/03	44673	156332	0.285757	28.575723	0.000006	

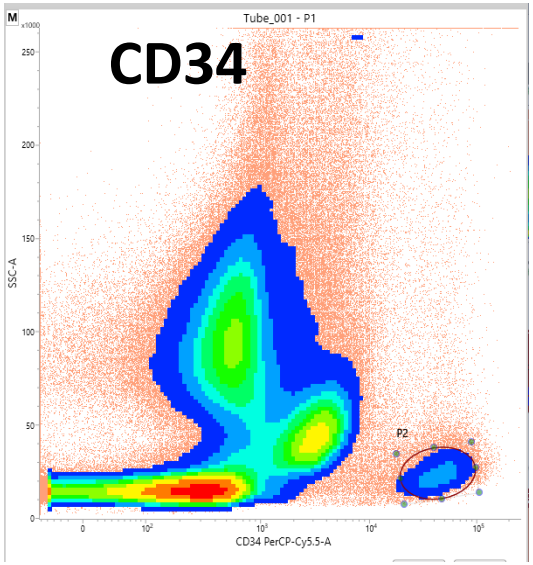
Eighteen months after Dasatinib, a significant change in BCR-ABL level was detected.

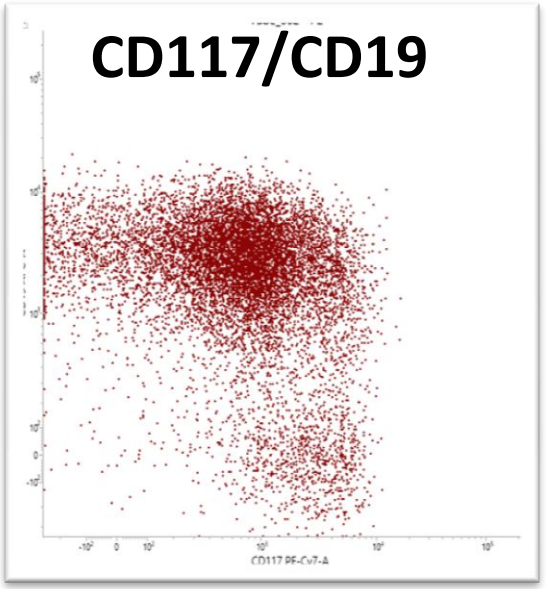
Flow MRD :3.12.1401

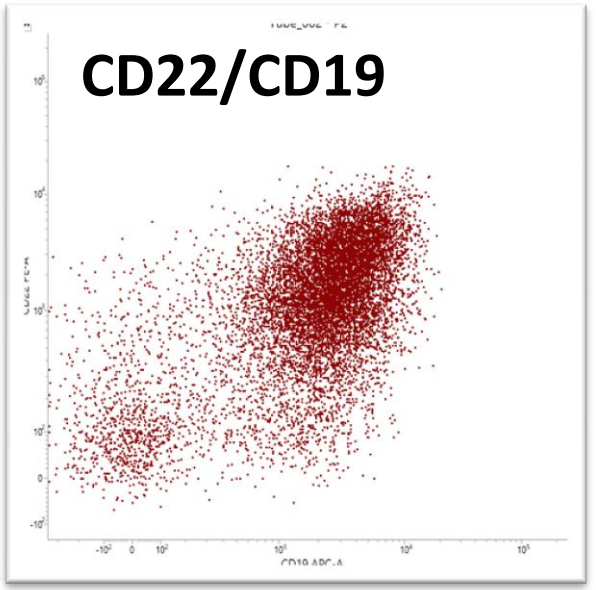


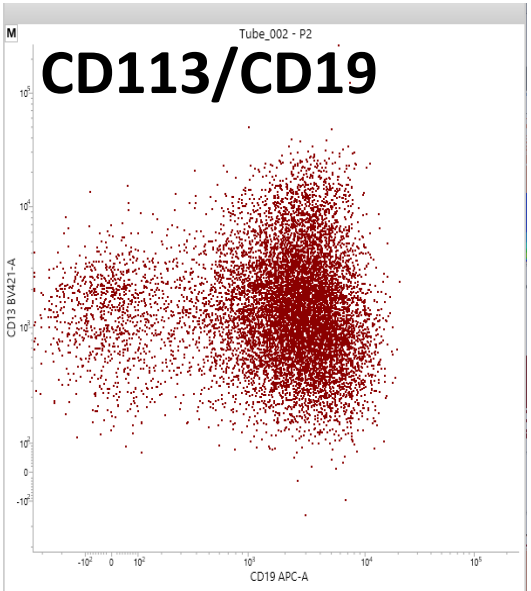


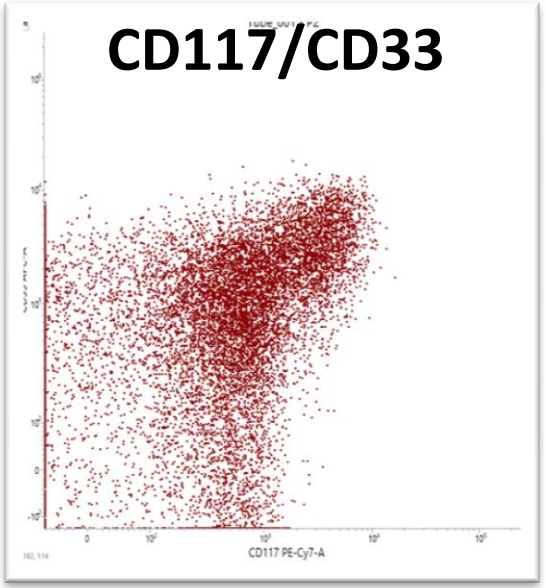
Flow MRD :3.12.1401

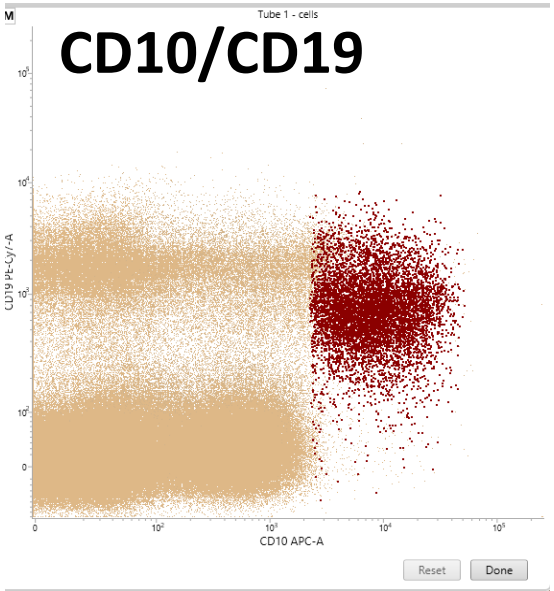












Minimal residual disease (MRD) Test for B-Cell Precursor Acute Lymphoblastic Leukemia by Next Generation Flow cytometry (NGF)

Clinical Data: Known case of AML

Flow Differential (%)

Granulocytes	40	Myeloblasts	0.5	Monocytic Components	-
Monocytes	5	Monoblasts	-	Erythroid cells	6.5
Lymphocytes	46	Blast cells	2	Hematogones	

Immunophenotype:

The Minimal residual disease (MRD) for B-Cell Precursor Acute Lymphoblastic Leukemia (BCP-ALL) was done by two eight-color EuroFlow panels with sensitivity of $\leq 10^{-5}$ by Next Generation Flow cytometry discriminating between normal and malignant BCP cells in 99% of studied patients.

Tube 1	CD20	CD45	CD81	CD66c+CD123	CD34	CD19	CD10	CD38
Expression	Neg	Pos (Mod)	Pos (Mod)	Neg	Pos	Pos	Pos (bright)	Pos (Mod)

Sample Quality: Acceptable
Cellularity: Low
Hemodiluted: No
Blast morphology: 1%
LLOD:0.001
LLOQ:0.002

Statistics

	CD45+ cells acquired	$CD19^{+}$ cells	MRD
Events	**788,000**	**78,000**	**12,480**
% Total	**100**	**8**	**1.58**

Conclusion:

Minimal/Measurable Residual Disease, MRD: 1.58% (POSITIVE)

Comment:

According to ELN recommendations for MRD testing; To define "MRD-negative" and "MRD-positive" patient groups, a cut off of 0.01% is recommended.

- **At her last follow-up(Feb 2023):**
  - **MRD by 8 color MFC:**
    - PB : 0.1%
    - BM showed: 2% (MPAL)
  - **Chimerism Analysis by STR:**
    - BM : 99%
  - **Chimerism Analysis by FISH**:
    - BM: 99.6
  - **MRD evaluation by BCR/ABL quantitation:**
    - BM:28%

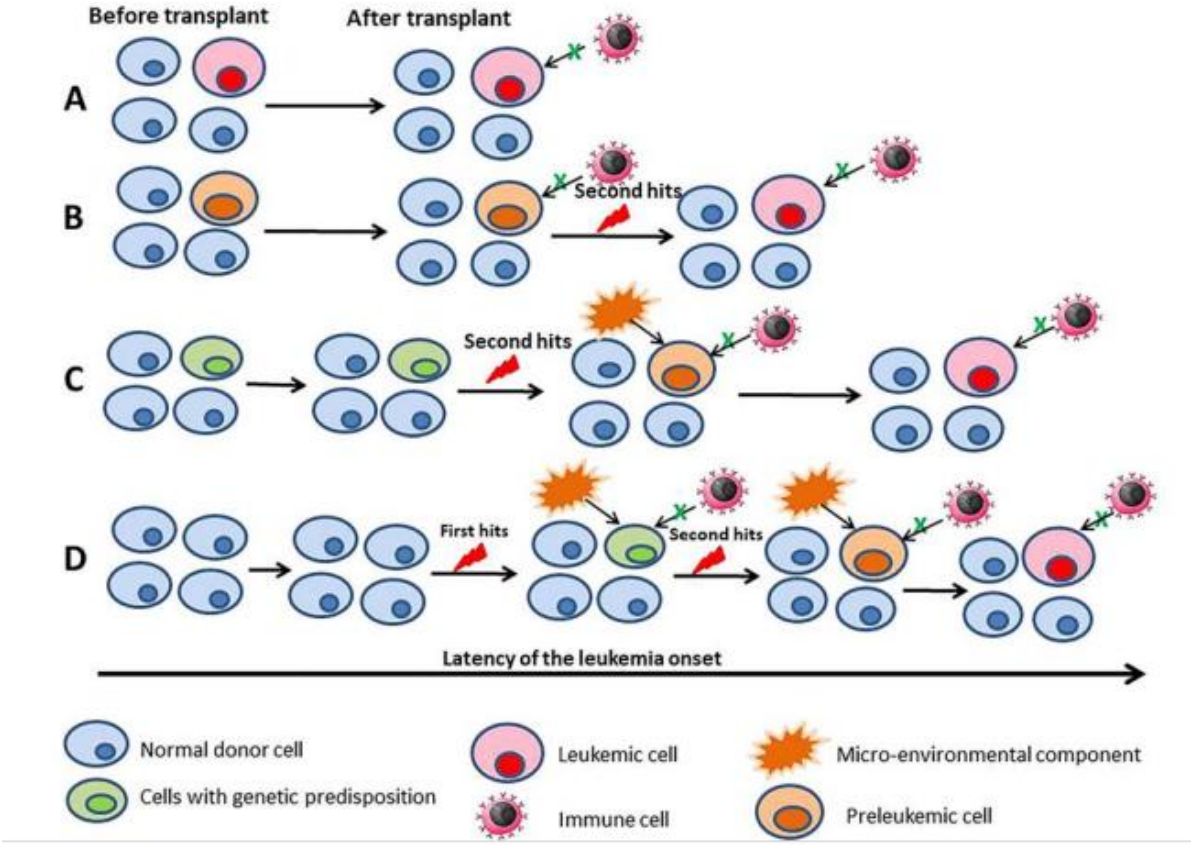
What's your interpretation?

Donor cell leukemia (DCL)

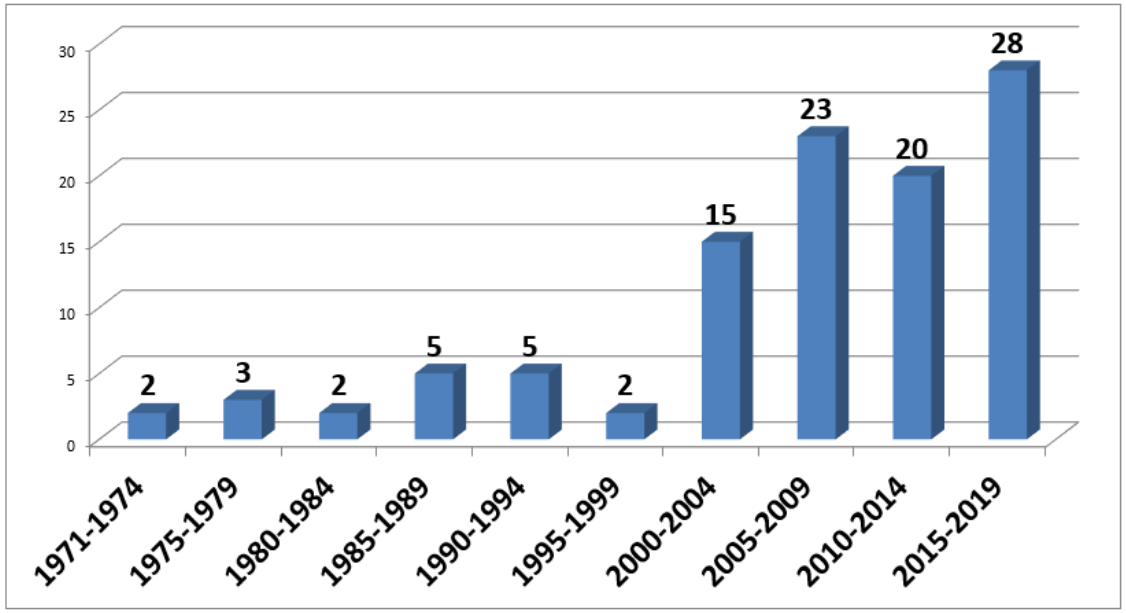
- Donor cell leukemia (DCL) is a very rare event. Although Boyd et al. suggested that DCL accounted for approximately 5% of relapses on the basis of cytogenetic studies of 54 relapses in sex-mismatched bone marrow transplants
- n an EBMT survey, the occurrence rate is reported as 14 cases in 10,489 transplants (0.13%)between 1982 and 2003

- Several hypotheses have been offered to date to explain how DCL might arise:
  - occult leukemia in the donor
  - transfer of oncogenic material from host to donor cells
  - impaired immune surveillance
  - incorrect identification of origin of leukemic cells,
  - leukemic transformation of engrafted bone marrow cells

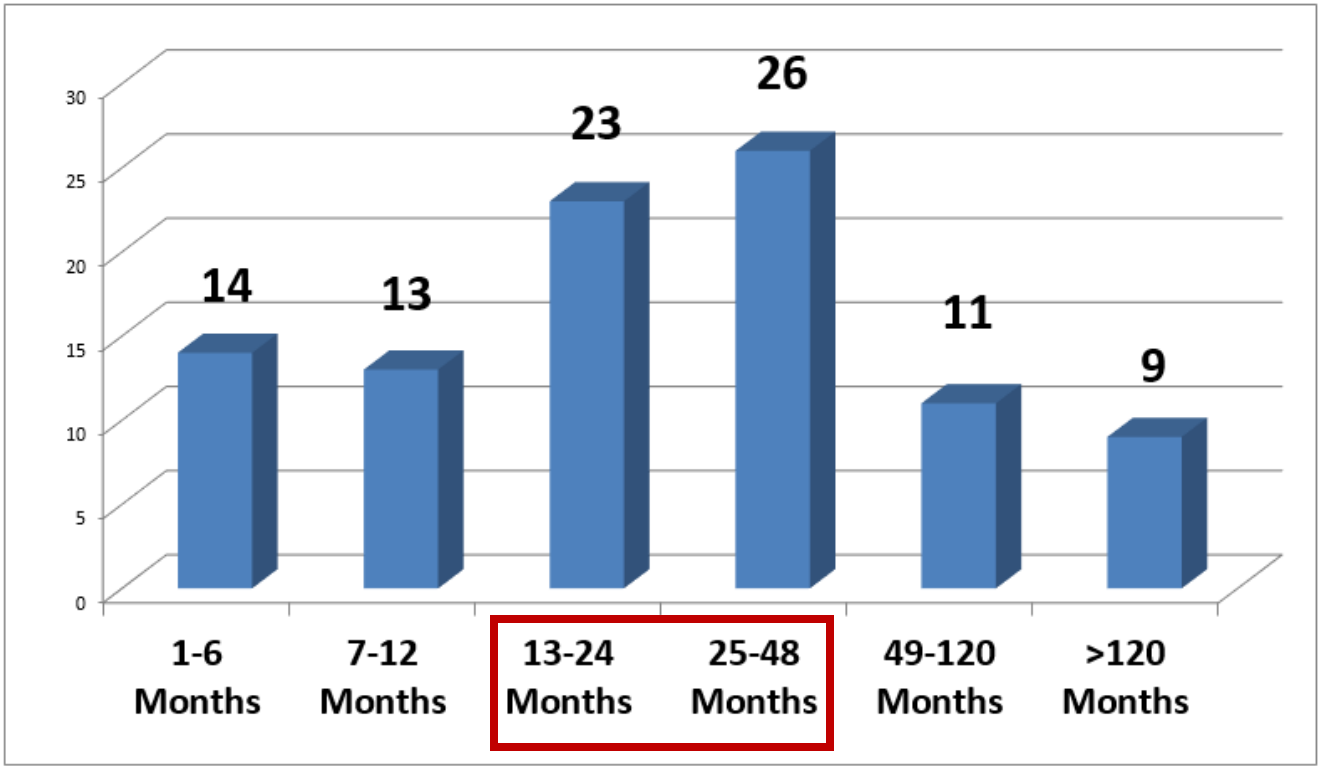
Possible modes of leukemia development in donor cell leukemia



Cases reported with the time of occurrence of DCL after HSCT



The latent period of occurrence of DCL after HSCT



Detection methods for the DCL diagnosis and proofs of donor origin

- Gold standard for chimerism analysis especially for the verification of DCL(recommended by EBMT):
  - STR
  - FISH(sex mismatch only)

Multiple donor-derived leukemias in a recipient of allogeneic hematopoietic cell transplantation for myeloid malignancy

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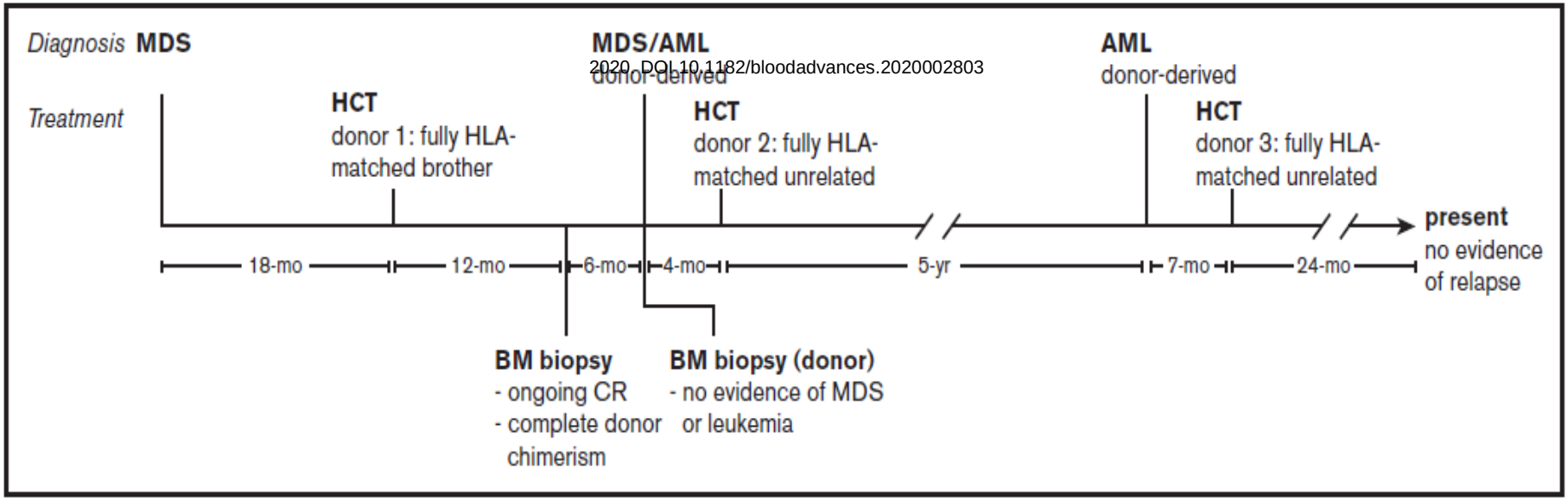

2020. DOI 10.1182/bloodadvances.2020002803

46,XX,del(7)(q11.2q32), del(20)(q11.2q13.1)

46,XY,t(8;21)(q22;q22)[2], 45,sl,-Y [7], 46,sl,del(9)(q13q22)[2], 46,XY[10]

46,XX

2020. DOI 10.1182/bloodadvances.2020002803

Journal of Hematology & Thromboembolic Diseases

Farhan et al., J Hematol Thromb Dis 2013, 2:1
DOI: 10.4172/2329-8790.1000124

Case Report Open Access

The Inevitable: Donor Derived Chronic Myeloid Leukemia Following Matched Related Stem Cell Transplant for Acute Lymphoblastic Leukemia

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Abstract

Donor Derived Cell Leukemia (DCL) is a rare complication of Hematopoietic Stem Cell Transplantation (HSCT). DCL represents a unique form of leukemogenesis in which donor cells become transformed or proliferate following engraftment in a foreign host environment. We report a 58-year old patient with B-cell acute Lymphocytic leukemia who underwent an allergenic HSCT from his Human Leukocyte Antigens (HLA)-matched sibling. Three months after HSCT, chromosomal analysis revealed a female karyotype with a (9;22) translocation. This was confirmed with Fluorescence in Situ Hybridization and polymerase chain reaction studies in the recipient and the donor confirming the transmission of occult leukemia.

MRD Lab Validation

Facilities Conducting MRD Testing

The following is a list of facilities that are CLIA-certified and accept external MRD samples. CLIA certification was validated using the CDC website* and acceptance of external samples was confirmed by reviewing facility websites and/or contacting facilities directly. Amgen neither recommends nor endorses, and may or may not have financial relationships with, any facility that appears on this list. This list is not intended to be a comprehensive list nor as a referral to any provider listed. If you would like to suggest a facility to be added to this list, please contact Amgen MedInfo at 800-77-AMGEN.

LOCATION	FACILITY NAME	MRD TEST	WEBSITE	PHONE NUMBER
National	Adaptive Biotechnologies	NGS	https://www.clonoseq.com	(888) 552-8988
Salt Lake City, UT	ARUP Laboratories	Flow Cytometry	https://www.aruplab.com	(800) 242-2787
Seattle, WA	CellNetix	Flow Cytometry, PCR [Ph(+) only]	https://cellnetix.com	(844) 344-4209
Los Angeles, CA	Children's Hospital Los Angeles (Pathology and Laboratory Medicine)	Flow Cytometry	https://www.chla.org/pathology-and-laboratory-medicine	(877) 543-9522
Cincinnati, OH	Cincinnati Children's Hospital (Immunopathology Laboratory)	Flow Cytometry	https://www.cincinnatichildrens.org/service/c/cancer-blood/hcp/clinical-laboratories/immunopathology-lab	(513) 803-2567
Durham, NC	Duke University Health System Clinical Laboratories	Flow Cytometry	https://clinlabs.duke.edu/molecular-diagnostics-laboratory	(919) 684-2698
Seattle, WA	Fred Hutchinson Cancer Research Center (Molecular Oncology Laboratory)	PCR [Ph(+) only]	https://research.fredhutch.org/molecular-oncology/en.html	(206) 667-2592
Seattle, WA	Hematologics, Inc.	Flow Cytometry	https://www.hematologics.com	(206) 223-2700
Baltimore, MD	Johns Hopkins Medicine (Pathology)	Flow Cytometry, PCR [Ph(+) only], NGS	https://pathology.jhu.edu/patient-care/testing	(410) 955-1921
Boston, MA	Massachusetts General Hospital (Pathology)	Flow Cytometry	https://mghlabtest.partners.org OR https://mghlabtest.partners.org/clinicians/directory-of-labs/	(617) 724-5227
Rochester, MN	Mayo Clinic Laboratories	Flow Cytometry	https://www.mayocliniclabs.com	(800) 533-1710
Houston, TX	MD Anderson Cancer Center (Molecular Diagnostics Laboratory)	Flow Cytometry, PCR	https://www.mdanderson.org/research/research-resources/core-facilities/molecular-diagnostics-lab.html	(713) 794-4780
National	NeoGenomics	Flow Cytometry, PCR [Ph(+) only]	https://neogenomics.com	(866) 776-5907
Columbus, OH	The Ohio State University Wexner Medical Center James Molecular Laboratory	Flow Cytometry, PCR	https://pathology.osu.edu/divisions/Clinical/molpath/ordering.html	(614) 293-0665
National	Quest Diagnostics	PCR [Ph(+) only]	https://www.questdiagnostics.com	(866) 697-8378
Grand Rapids, MI	Spectrum Health Advanced Technology Laboratories	Flow Cytometry	https://www.spectrumhealth.org/for-health-professionals/advanced-technology-laboratories	(866) 989-7999
Chapel Hill, NC	UNC Medical Center (McLendon Clinical Laboratories)	Flow Cytometry	https://www.uncmedicalcenter.org/mclendon-clinical-laboratories/directory	(984) 974-8320
Kansas City, KS	University of Kansas Health System (Pathology and Laboratory Medicine)	Flow Cytometry	https://www.kansashealthsystem.com/care/specialties/pathology	(913) 588-1227
Ann Arbor, MI	University of Michigan (Department of Pathology)	Flow Cytometry	https://www.pathology.med.umich.edu/handbook/#/details/5337	(800) 862-7284
Dallas, TX	UT Southwestern Medical Center (Department of Pathology)	Flow Cytometry	https://www.utsouthwestern.edu/education/medical-school/departments/pathology/	(214) 648-4088
Seattle, WA	University of Washington (Hematopathology)	Flow Cytometry, PCR [Ph(+) only], NGS	https://dlmp.uw.edu/patient-care/hematopathology	(206) 606-7060
New Haven, CT	Yale School of Medicine (Laboratory Medicine)	Flow Cytometry, PCR [Ph(+) only]	https://medicine.yale.edu/labmed/sections/flowcytometry/	(203) 688-2450

This information is current as of March 2022. Amgen does not guarantee the accuracy of this information, and it is up to the individual physician to conduct their own research.
CDC, Centers for Disease Control and Prevention; CLIA, Clinical Laboratory Improvement Amendments; MRD, measurable or minimal residual disease; NGS, next-generation sequencing; PCR, polymerase chain reaction; Ph(+), Philadelphia chromosome–positive.
*https://www.cdc.gov/clia/LabSearch.html

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THANKS!

Any questions?

Regular Article

blood

LYMPHOID NEOPLASIA

Monitoring of childhood ALL using *BCR-ABL1* genomic breakpoints identifies a subgroup with CML-like biology

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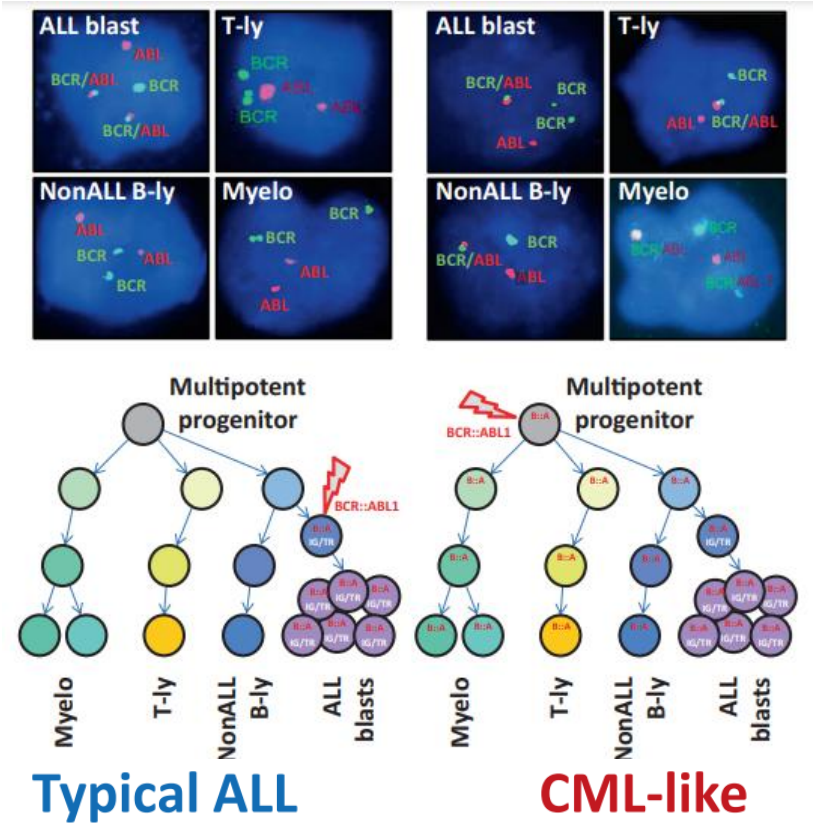
[1]Childhood Leukemia Investigation Prague (CLIP) and [2]Department of Pediatric Hematology and Oncology, Second Faculty of Medicine, Charles University, Prague, Czech Republic; [3]University Hospital Motol, Prague, Czech Republic; [4]Children's Cancer Institute, Lowy Cancer Research Centre, University of New South Wales, Sydney, NSW, Australia; [5]Department of Pediatrics, University Medical Centre Schleswig-Holstein, Campus Kiel, Kiel, Germany; [6]Gennet, Center for Fetal Medicine and Reproductive Genetics, Prague, Czech Republic; [7]Institute of Hematology and Blood Transfusion, Prague, Czech Republic; [8]Blood and Marrow Transplant Services, Children's Hospital at Westmead, Sydney, NSW, Australia; and [9]School of Women's and Children's Health, University of New South Wales, Sydney, NSW, Australia

Key Points

- Combination of Ig/TCR and *BCR-ABL1* genomic approach for MRD monitoring in childhood ALL reveals patients with CML-like disease.
- Monitoring ALL using *BCR-ABL1* genomic breakpoint is feasible and enables the most

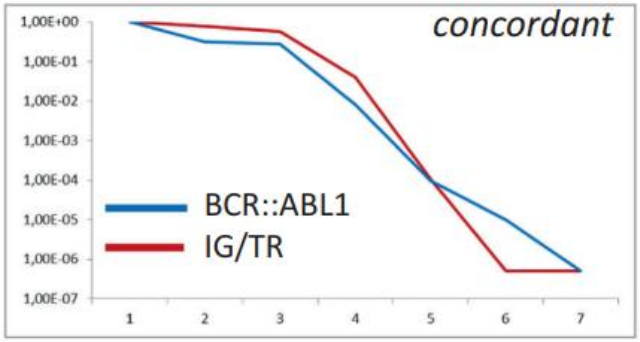
We used the genomic breakpoint between *BCR* and *ABL1* genes for the DNA-based monitoring of minimal residual disease (MRD) in 48 patients with childhood acute lymphoblastic leukemia (ALL). Comparing the results with standard MRD monitoring based on immunoglobulin/T-cell receptor (Ig/TCR) gene rearrangements and with quantification of *IKZF1* deletion, we observed very good correlation for the methods in a majority of patients; however, >20% of children (25% [8/32] with minor and 12.5% [1/8] with major-*BCR-ABL1* variants in the consecutive cohorts) had significantly (>1 log) higher levels of *BCR-ABL1* fusion than Ig/TCR rearrangements and/or *IKZF1* deletion. We performed cell sorting of the diagnostic material and assessed the frequency of *BCR-ABL1*-positive cells in various hematopoietic subpopulations; 12% to 83% of non–ALL B lymphocytes, T cells, and/or myeloid cells harbored the *BCR-ABL1* fusion in patients with discrepant MRD results. The multilineage involvement of the *BCR-ABL1*-positive

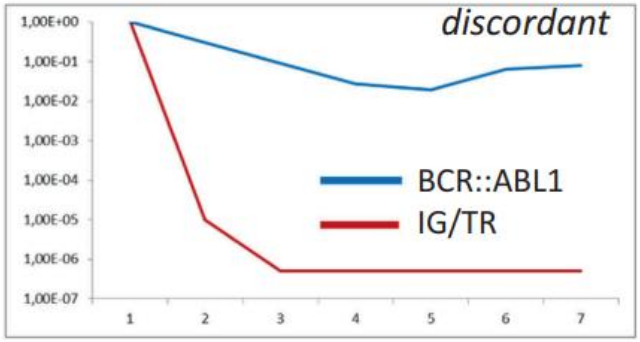
Schematic illustration of key differences between “Typical ALL” and “CML-like” disease



CML-like disease should be based on the proof of BCR::ABL1 fusion in myeloid or stem cell compartment

Schematic illustration of key differences between “Typical ALL” and “CML-like” disease





Typical ALL **CML-like**