Payvand
Clinical Specialty Laboratory

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Webinar: CML Update

Diagnosis & Molecular Monitoring in Pediatric Chronic Myeloid Leukemia, CML

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30.10.1400

Objectives

1. Review diagnostic work up for CML
2. Criteria for response to TKI therapy with emphasis on Molecular monitoring
3. Review the concept of International scale & Conversion factor
4. How to request & How to report the result of Quantitative *BCR-ABL* rt-PCR
5. Review a few reports of CML patients

Diagnostic Work up

- Physical examination with particular reference to spleen & liver size
- CBC with microscopic differential
- BM aspirate for cytologic examination & cytogenetics; core biopsy if dry tap
- Chromosome Banding Analysis (CBA)
- Fluorescence in-situ hybridization (FISH) only in case of Ph-negativity
- Qualitative RT-PCR for the detection of *BCR-ABL1* transcripts & identification of the ***transcript type***
- Electrocardiogram
- Standard biochemical profile with hepatitis B-serology

Leukemia (2020) 34:966–98; doi.org/10.1038/s41375-020-0776-2

Diagnostic Work up, cont.

- **Physical examination**
- **A standard biochemical profile** including hepatitis B/C serology, cholesterol, lipase, and hemoglobin A1c values, *the use of TKIs may be associated with **reactivation** of hepatitis viruses,*
- **CBC with microscopic differential, % blast**
- Compared to adult CML, the **mean leukocyte count** in pediatric CML at diagnosis is more than **3-4 fold higher** ($60x10^9$ cells/L versus $240x\ 10^9$ cells/L), a **broad "buffy coat"** ("**leukocrit**")
- The **median WBC** at baseline in adult CML ranges from 80 to 150×10^9/l; however, in childhood CML, the median WBC count was 250×10^9/l in a study of 200 patients with a median age of 11.6 years

Leukemia (2020) 34:966–98; doi.org/10.1038/s41375-020-0776-2
https://doi.org/10.1038/s41375-020-0776-2; 2020 British Society for Haematology and John Wiley & Sons Ltd

Cancers **2021**, 13, 798.
https://doi.org/10.3390/cancers13040798

CML in Pediatrics: CBC

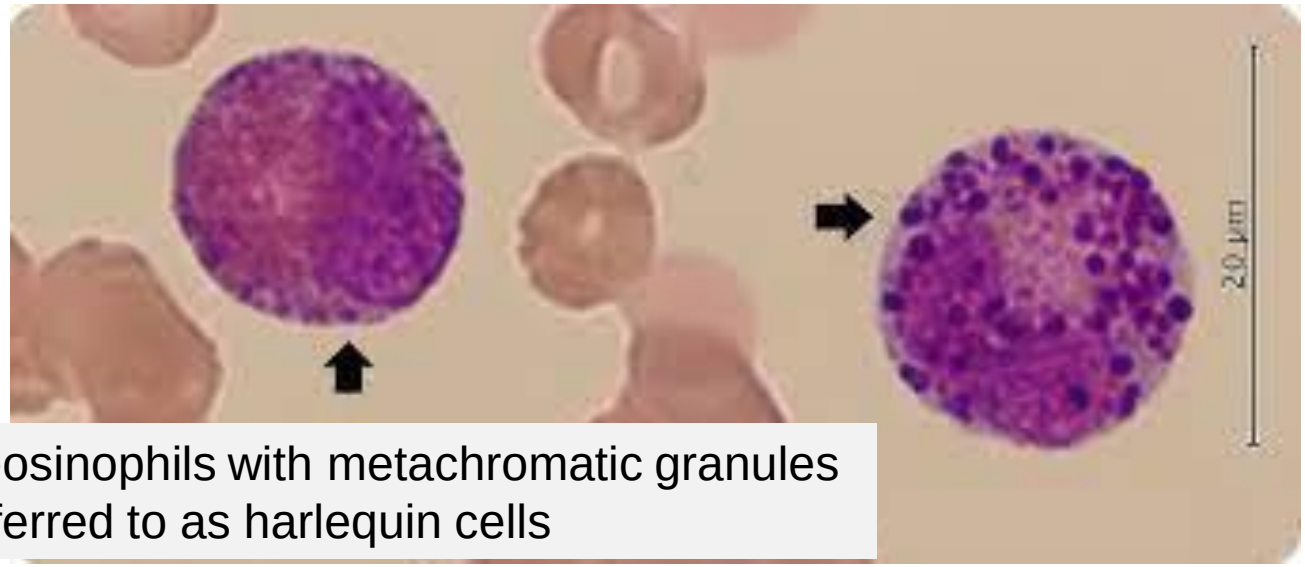
- **Mild normocytic, normochromic anemia** (**median Hb 10.4 g/dL**) is present at diagnosis in **60%** pediatric patients in CML-CP
- **Neutrophil function** in CML is **normal** or **only mildly impaired.**
- These mature granulocytes have ***decreased apoptosis***, resulting in accumulation of long-lived cells with low or absent enzymatic activity, such as alkaline phosphatase.
- **Absolute lymphocytosis** is also often seen.
- **Basophils** in all patients are usually elevated (in the range of **5-10%**) in the PB & **eosinophils** may be mildly increased as well (90% patients).
- The **platelet count** is normal in CML-CP & even **elevated above 500 x 10^9/L in half** of the pediatric patients, thrombocytopenia is seen in **<5.5%** of patients

Cancers **2021**, 13, 798.
https://doi.org/10.3390/cancers13040798

Indian J Med Res 149, May 2019, pp 600-609
DOI: 10.4103/ijmr.IJMR_331_19

CML in Pediatrics: CBC

- Eosinophils with atypical basophilic staining granules known as a **"Harlequin" cell** are also most commonly seen in CML.



Dysplastic eosinophils with metachromatic granules are often referred to as harlequin cells

AYA had clinical features suggestive of ***aggressive disease*** with **larger spleen size**, **higher WBC count**, **higher % of PB blasts**, & **lower Hb** compared to other age groups.

Cancers **2021**, 13, 798.
https://doi.org/10.3390/cancers13040798

CML in Pediatrics: BMA

- **BM aspirate** for **Cytomorphologic exam.,** % blasts, % basophils (to confirm the ***phase of the disease***) & **Cytogenetics** (full karyotype analysis) ***at diagnosis, 3 month and 1 year (at 18 mo. if no CCyR at 12 month)***
- **Core biopsy** if dry tap
- ***Hypercellular marrow*** ***with Myeloid Hyperplasia & Left Shift***
- ***Blasts,*** ***which are mainly myeloid but notice to lymphoid blasts is very important***
- ***Basophilia, eosinophilia, …***
- ***Megakaryocytic proliferation is present in >50%*** of the cases with ***micromegakaryocytes (hypolobated nuclei)***
- ***Grades of fibrosis*** are varying and manifest myelofibrosis has been described as an adverse morphological factor in adult CML which may be detected in **15% (26%** in a large multicenter study**) of pediatric CML,** 15-65% depending on the method of diagnosis in adult CML

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CML in Pediatrics, cont.

- In adults, most BP are myeloid lineage with **20–30%** being ***lymphoblastic***.
- However, ***in pediatrics BP are predominantly lymphoblastic***; an international registry of CML in children & adolescents (n=479) reported 17 children presenting with BP & **12/17 (70%)** had **lymphoblastic BP**.
- **Sheets of blasts** may be seen in **focal areas of the** BM, which can be considered evidence of a BP even if the rest of the marrow shows chronic phase.

Pediatr Blood Cancer. 2019 September ;
66(9): e27827. doi:10.1002/pbc.27827.

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CML in Pediatrics, cont.

- Additionally, the presence of ***lymphoblasts even at lower number*** may herald blast transformation & requires further evaluation.
- As the onset of lymphoid BP may be quite sudden, the detection of any bona fide lymphoblasts in the blood or marrow should raise concern for a possible impending lymphoid BP, and prompt additional laboratory and genetic studies to exclude this possibility).

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Diagnostic Work up
Cytogenetic

- **Chromosome Banding Analysis (CBA),** Giemsa-stained metaphases for detection of ***Additional Cytogenetic Abnormalities*** (**ACA**) in Ph^+ cells, particularly '***major route' abnormalities***: +8, a 2^{nd} Ph-ch. (+Ph), i(17q), +19, −7/7q-, 11q23, or 3q26.2 aberrations, & ***complex aberrant karyotypes***.
- Currently, the panel recommends classifying ACA & treating patients with **high-risk ACA as high-risk patients**.
- High risk ACA predict a **poorer response to TKIs** and a **higher risk of progression to accelerated phase (AP) or blast crisis (BC).**
- **FISH** only in case of Ph-negativity

There is no need for CSF sampling in CML CP

European LeukemiaNet 2020 recommendations for treating chronic myeloid leukemia
https://doi.org/10.1038/s41375-020-0776-2; 2020 British Society for Haematology and John Wiley & Sons Ltd

Pediatr Blood Cancer. 2019 September ; 66(9): e27827. doi:10.1002/pbc.27827.

J. Clin. Med. **2020**, 9, 3671

Genetically Based Risk Assessment

Chromosomal abnormalities	Somatic mutations		
		Frequency of mutation in BC (%)	
High-risk ACA **Wang et al 2016[47]** **Gong et al 2017[78]** **Hehlmann et al 2020[48]**	**Mutated genes, selection**	**Grossmann 2011[79] n = 39**	**Branford 2018[80] n = 46**
+8	RUNX1	33.3	28
+Ph	ASXL1	20.5	23
i(17q)	IKZF1	17.9	33
+19	WT1	15.4	NA
+21	TET2	7.7	NA
+17	IDH1/2	7.7	8
-7/7q-	CBFB/MYH11	NA	6
3q26.2	TP53	2.6	3
11q23	ABL1 kinase domain	33.3	58
Complex aberrant			

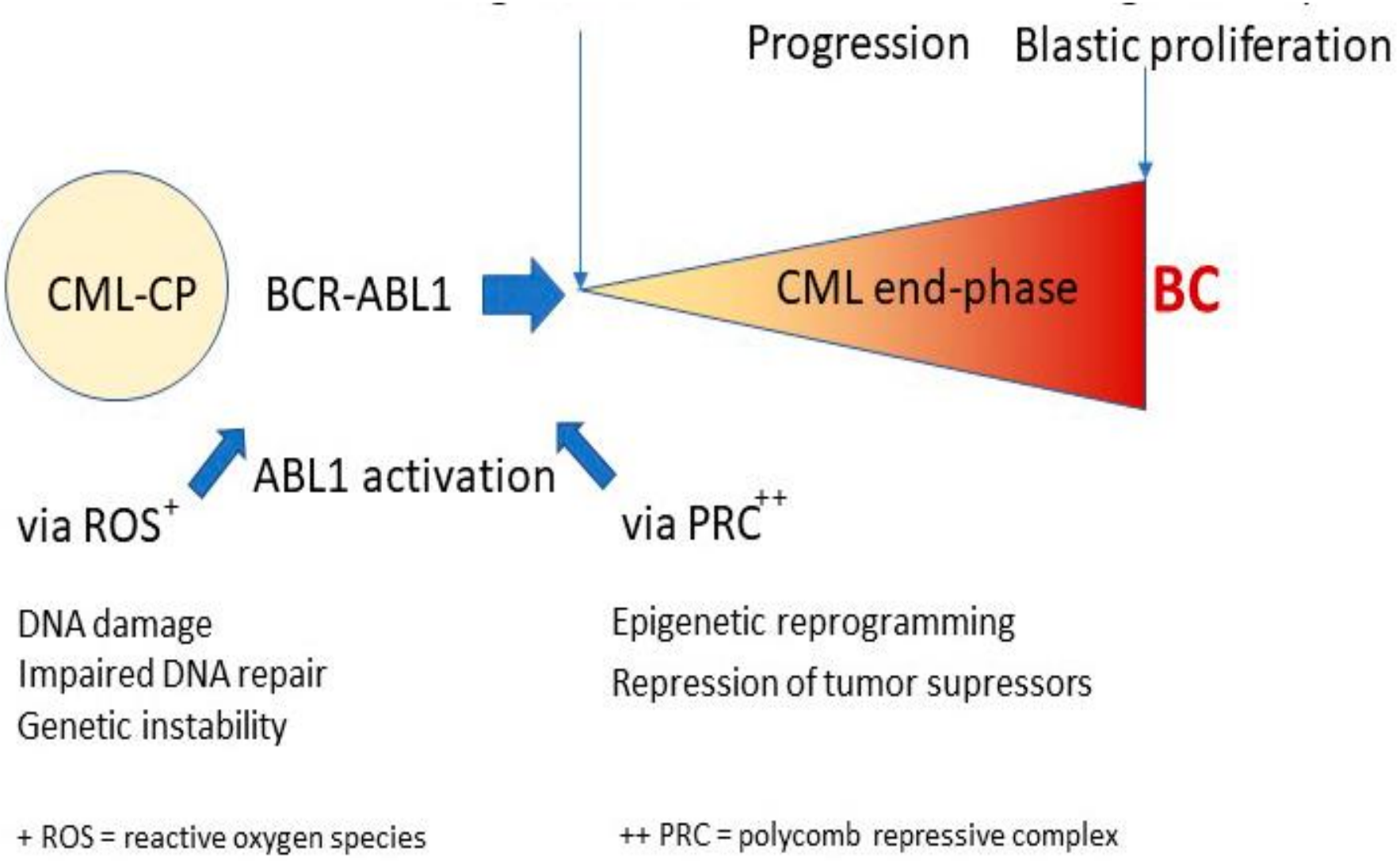
ACA = additional chromosomal abnormalities, BC = blast crisis

Pediatr Blood Cancer. 2019 September ; 66(9): e27827. doi:10.1002/pbc.27827.

Genetically Based Risk Assessment

- Children, adolescents & young adults (**AYA**) tend to have a ***more aggressive clinical presentation than adults***
- Recent data indicate that some **genetic differences** exist in pediatric CML compared to adult disease; for example, **60% of pediatric patients** have ***ASXL1 mutation*** compared to only **15% of adults**.
-

Genetically Based Risk Assessment



ACA = additional chromosomal abnormalities, BC = blast crisis

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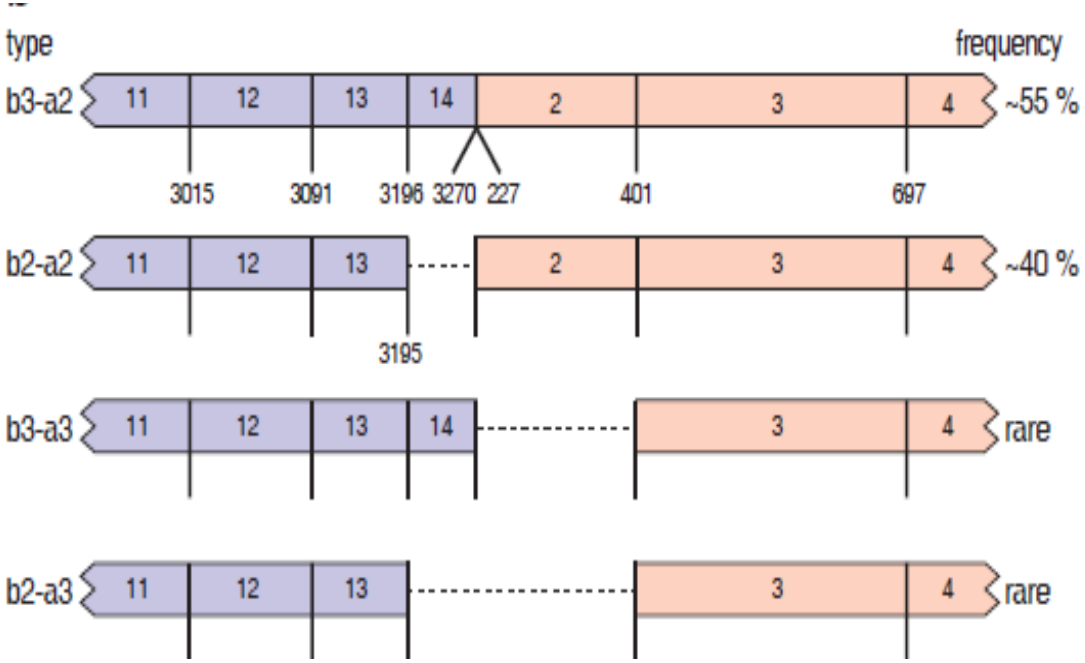
Diagnostic Work up
RT-PCR

- **Qualitative rt-PCR** for the **detection of *BCR-ABL1* transcripts** & **identification of the transcript type**
- A **quantitative PCR** is **not mandatory at diagnosis**.
- If a molecular assay demonstrates *BCR-ABL1*, but the Ph chromosome cannot be identified by cytogenetics, **a FISH test is required**.



Diagnostic Work up
RT-PCR

- **e13a2 (b2a2)** & **e14a2 (b3a2)** transcripts (p210) were identified in **38%** & **62%** of patients, respectively (>45,000 pts)
- e13a2 was more frequent in **males** & the proportion decreased with age in both sexes.
- The BCR-ABL1 transcript e1a2 (P190), typically seen in Ph+ ALL, is found in approximately 1% of CML patients and has also been associated with poor prognosis.
- Most of these patients (P190) do not achieve a molecular response with TKI therapy & are candidates for stem cell transplantation

Diagnostic Work up: Atypical *BCR-ABL1* transcripts

- Approximately **1–2%** (About 2–4%) of patients reveals atypical forms, (e1a2, e19a2, e13a3, and e14a3 were the most frequently identified transcripts).
- Atypical forms may result **false negative PCR** using routine primer/probe sets in qualitative or quantitative rt-PCR protocols.

BCR-ABL1 transcripts were amplified & sequenced to characterize the underlying fusion.

For molecular monitoring of these patients:

The **Individual Molecular Response (IMR)** level based on a log reduction from pretreatment levels.

Leukemia (2020) 34:966–984;doi.org/10.1038/s41375-020-0776-2
Journal of Cancer Research and Clinical Oncology;doi.org/10.1007/s00432-021-03569-8

Disease monitoring based on NCCN Recommendations

	Recommendations
History and physical examination with documentation of spleen size by palpation (indicate cm below costal margin)	Every visit- Weekly until clinically stable Biweekly till complete hematological response Then monthly till 3 months from diagnosis and then every 3 months
CBC with differential	Every visit
BM Karyotyping	Every 3 months until complete cytogenetic response Failure to achieve response milestone Any sign of loss of response (defined as hematologic or cytogenetic relapse)
PB Q-RT-PCR	At diagnosis Every 3 month until CCyR and then every 3 month for 2 years and then every 3 to 6 months. With 1 log increase in with MMR repeat in 1–3 months.
BCR-ABL kinase domain mutation analysis	• In chronic phase if • inadequate initial response • Any sign of loss of response or • 1-log increase in transcript or loss of MMR • Disease progression to AP or BP

Pediatr Blood Cancer. 2019 September ; 66(9): e27827. doi:10.1002/pbc.27827.

Response criteria based on modification from NCCN & ELN

Type of response	Anticipated duration to response
• **Complete hematologic response**	No signs & symptoms of disease with disappearance of palpable spleen, Complete normalization of PB count with WBC count within age appropriate normal values Absence of immature cells such as myelo., Promyelo. or blasts in PB. Platelet count: 150 – 450X 10^9/L
• **Cytogenetic response (a minimum of 20 metaphases)**	
- Major (Complete + partial)	0 - 35% Ph positive metaphase
1. Complete (CCyR)	no or <1% Ph positive metaphases
2. Partial (PCyR)	1 - 35% Ph positive metaphases
- Minor	>36−65% Ph positive metaphases
- Minimal	66-95% Ph positive metaphases
- No response	>95% Ph positive metaphases

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Response criteria based on modification from NCCN & ELN

Type of response	Anticipated duration to response
Molecular response	
Complete molecular response	No detectable BCR-ABL1 mRNA by Q-RT-PCR (IS) using an assay with a sensitivity of **at least 4.5 logs** below the standardized baseline
Major molecular response (MMR)	BCR-ABL1 transcripts **0.1%** by Q-RT-PCR (IS) or more than a 3-log reduction in BCR-ABL1 mRNA from the standardized baseline, if Q-PCR according to IS is not available

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Response criteria based on modification from NCCN & ELN

Type of response	Anticipated duration to response
• **Relapse**	- ***Any sign of loss of response*** (defined as hematologic or cytogenetic relapse) - ***1 log increase in BCR-ABL1 transcript*** levels with loss of MMR should ***prompt BM evaluation*** for ***loss of CCyR & mutational analysis*** but is not defined as relapse

Acceptable time line for therapy response for first line TKI

Time in months	Optimal Response	Warning signs		Failure to respond
		Hematological and cytogenetic	BCR-ABL1 (IS)	
Diagnosis	NA	Blast crisis or AP; del(9q-); additional cytogenetic abnormalities in Ph+ cells	Baseline level	NA
3	CHR, BCR-ABL1 <10% and/or Ph+ <35%	Ph+ 36–95%	>10%	No CHR; stable disease or disease progression Ph^+ >95%
6	BCR-ABL1 <1% CCyR, (Ph+ 0%)	Ph+ 1–35%	1%–10%	Ph+ > 35% BCR-ABL1 >10%
12	CCyR BCR-ABL1 0.1%	-	> 0.1% –1%	Ph+ > 1% BCR-ABL1 > 1%
18	BCR-ABL1 0.1%	-	>0.1% –1%	
Then and at anytime	-	Additional cytogenetic abnormality in Ph- cells	Any rise in BCR-ABL1 transcript level	Loss of CHR; loss of CCyR; presence of new mutation; loss of MMR; additional cytogenetic abnormality in Ph+ cells

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Benefits of Molecular Monitoring of CML

- Molecular monitoring is a ***minimally invasive method*** to optimize treatment & outcome in CML.
- **The assay allows:**

(1) Early (**3-6 months** after initiation of therapy) identification of cases at a ***high risk of progression***;

(2) Detection of cases ***lacking response to TKI*** therapy;

(3) Detection of cases with undetectable disease that could be eligible for ***TKI discontinuation trials*** (***TFR: Treatment Free Remission***).

Milestones for treating CML expressed as *BCR-ABL1^{IS}*

	Optimal	Warning	Failure
Baseline	NA	High-risk ACA, high-risk ELTS score	NA
3 months	≤10%	>10%	>10% if confirmed within 1–3 months
6 months	≤1%	>1–10%	>10%
12 months	≤0.1%	>0.1–1%	>1%
Any time	≤0.1%	>0.1–1%, loss of ≤0.1% (MMR)[a]	>1%, resistance mutations, high-risk ACA

For patients aiming at TFR, the optimal response (at any time) is BCR-ABL1 ≤0.01% (MR^4).

A change of treatment may be considered if MMR is not reached by 36–48 months.

NA not applicable, *ACA* additional chromosome abnormalities in Ph+ cells, *ELTS* EUTOS long term survival score.

[a]Loss of MMR (BCR-ABL1 >0.1%) indicates failure after TFR

European LeukemiaNet 2020 recommendations for treating chronic myeloid leukemia
https://doi.org/10.1038/s41375-020-0776-2

Importance of the International Scale?

- **How much the results obtained in different labs, are *really comparable*?**

  - Different RNA preparation methods,

  - Real-time PCR machines & technologies,

  - Enzymes, fluorophores, quenchers, & standard curve materials.

- As such, *reported BCR-ABL1 measurements vary greatly between laboratories*.

So Harmonization or Comparability is Essential

IS & Conversion Factor

Reference RNA Calibrator, Corresponding to **10%, 1%, 0.1%, 0.01%**

BCR-ABL / Control gene (*ABL*)

BCR-ABL (local value) × conversion factor

Laboratory	MMR^{Eq}	$0.1\%/MMR^{Eq}$ (%) = Conversion Factor	Formula for conversion of a given result to the international scale ($BCR\text{-}ABL^{L} \times CF = BCR\text{-}ABL^{IS}$)
Adelaide	0.08%	0.1/0.08 = 1.25	$BCR\text{-}ABL^{L} \times 1.25$
Mannheim	0.12%	0.1/0.12 = 0.83	$BCR\text{-}ABL^{L} \times 0.83$
London	0.045%	0.1/0.045 = 2.22	$BCR\text{-}ABL^{L} \times 2.22$

BCR-ABL1 transcript levels according to the International Scale

BCR-ABL^{IS}, %	Log reduction from standardised baseline	MR category	Minimum number of *ABL1* transcripts
100	0	–	–
≤0·1	3	MR^{3} (MMR)	>10 000
≤0·01	4	MR^{4}	10 000–31 999
≤0·0032	4·5	MR$^{4·5}$	32 000–99 999
≤0·001	5	MR^{5}	≥100 000

Deep Molecular Response

CML, chronic myeloid leukaemia; MMR, major molecular response; MR, molecular response.

British Journal of Haematology, 2018, 182,777–788

How to Request: Clinician to Laboratory

- ***Good communication*** among members of the multidisciplinary team is essential for ***more effective disease management.***

- **Ideally, the following clinical information should be submitted to the laboratory:**
  - **TKI therapy,** Current TKI therapy & dose, Start date, Mutation status
  - **For patients who are being transferred between hospitals, *BCR-ABL1* transcript type esp. atypical forms**
  - **Any recent known treatment interruptions (e.g. pregnancy, TFR, intolerance)**
  - **Possible issues with *treatment adherence*.**

How to report: laboratory to clinician *minimum required information*

- Test (specific test/method)
- Result [***BCR-ABL1***^{IS},%]
- **Interpretation:**

  - Response status (**MR level**) & level of sensitivity

  - ELN 2020 guideline status (**Optimal**, **Warning**, **Failure**)

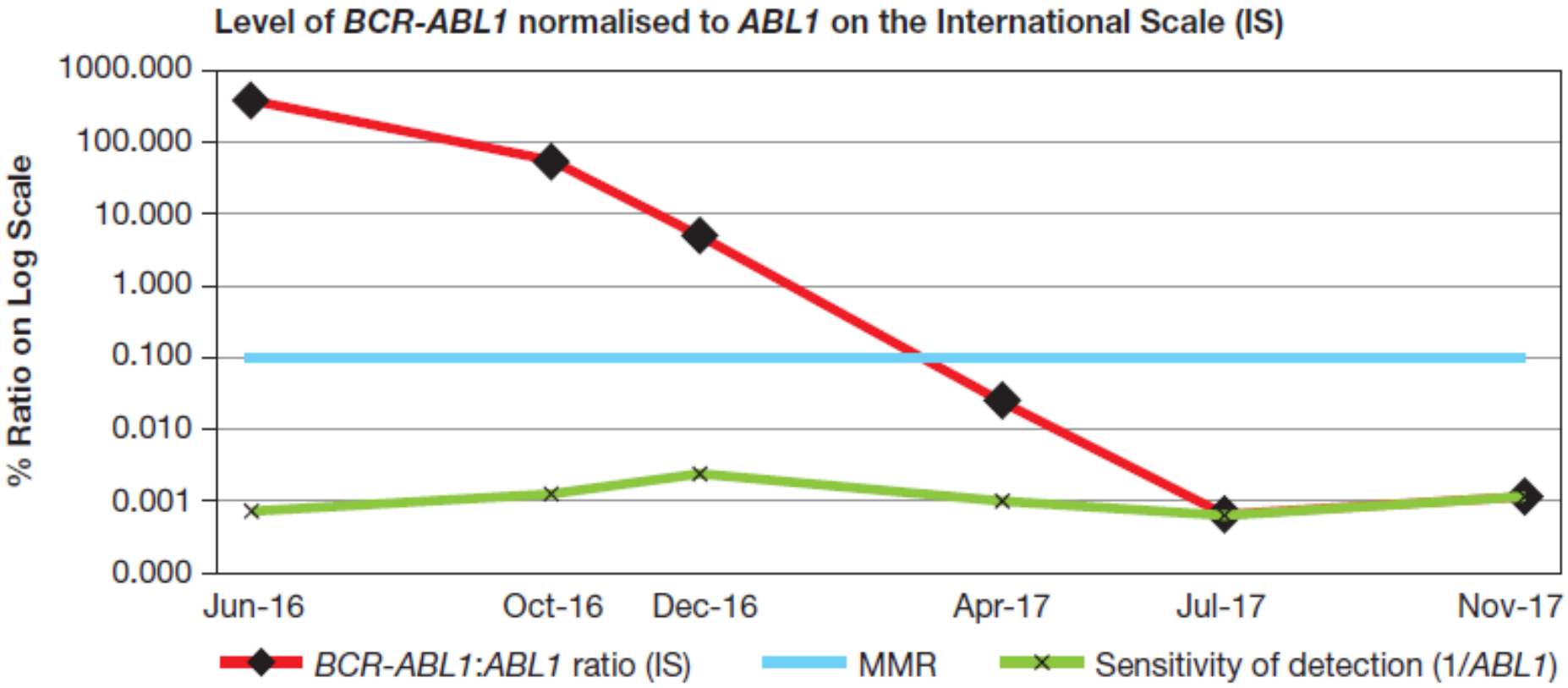
  - **Trends over time** (graph) with interpretation whether current result is significantly different with the previous

  - Suggestion for monitoring frequency/date of next test

Clinical Summary: CML on imatinib for 18 months. For *BCR-ABL1* monitoring by RT-qPCR.

BCR-ABL1 **quantitative PCR (RT-qPCR) monitoring report (transcript type = e13a2/e14a2)**

Treatment response	*BCR-ABL1:ABL1* % ratio on IS	MR level	Date next sample due
Optimal	Undetectable	$MR^{4,5}$	May 2018



•Patient test result & Interpretation:

***BCR-ABL*^{IS}%: 0.00017%**

Patient response based on ELN guideline:

***MR 5.0**

***Optimal**

Treatment commence: Imatib (Cipla): 2 years

then Imatinib: 4 months

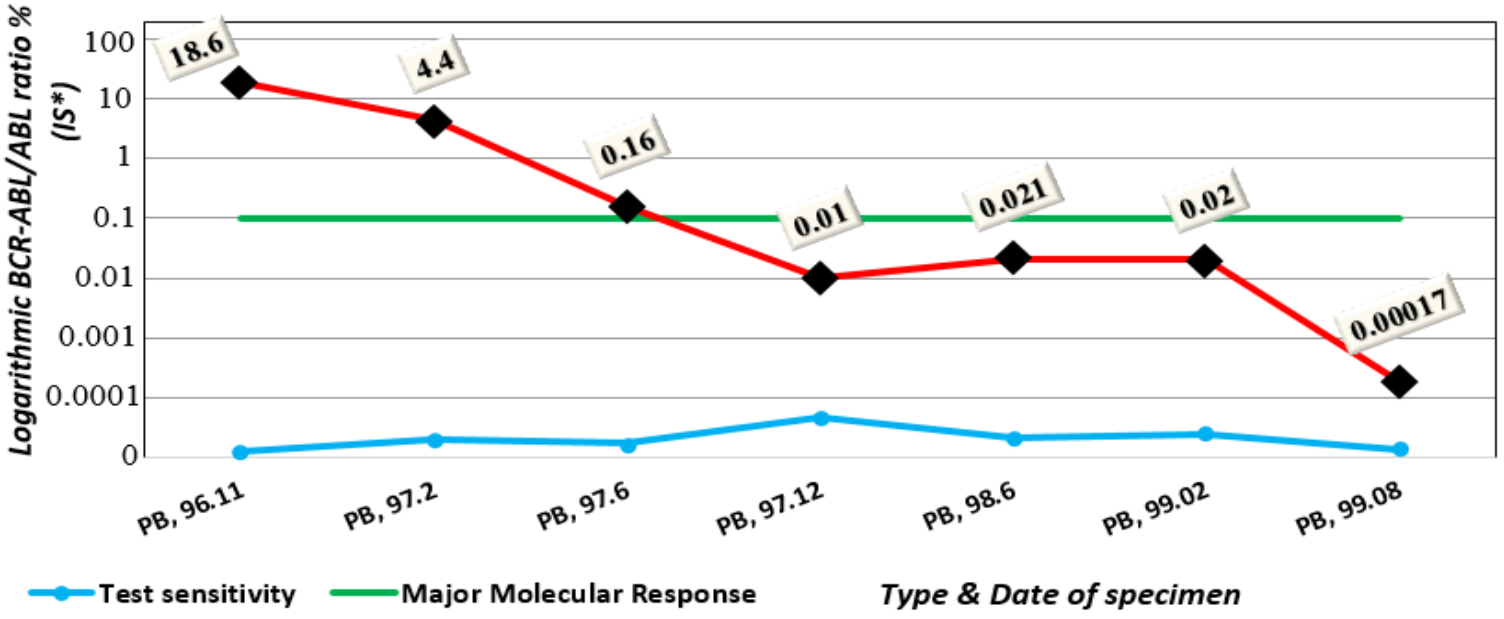
***BCR-ABL* Copies (Total): 0.65**
***ABL* Copies (Total): 302931**
Test Sensitivity (1/ABL1): 0.000003
Transcript variant at diagnosis: Not-Available

***BCR-ABL*^{IS}%**
0.00017%

Log reduction

- >10 %
- MR 1.0
- MR 2.0
- MR 3.0
- MR 4.0
- MR 4.5
- **MR 5.0**

• Patient *BCR-ABL*^{IS}% History:



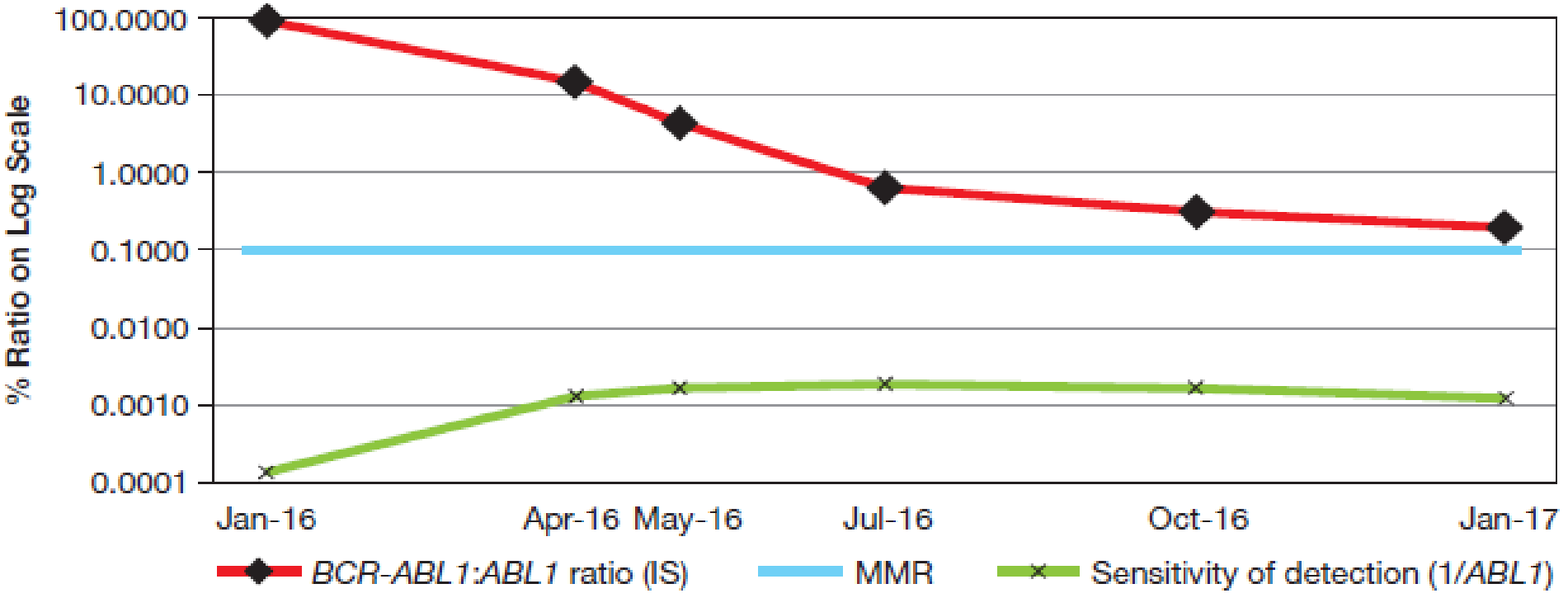
Clinical Summary: CML on imatinib for 18 months. For *BCR-ABL1* monitoring by RT-qPCR.

***BCR-ABL1* quantitative PCR (RT-qPCR) monitoring report (transcript type = e13a2/e14a2)**

Treatment response	*BCR-ABL1:ABL1* % ratio on IS	MR level	Date next sample due
Warning	0.1910	MR^2	April 2017

Please note that this result could signify a resistance to treatment that may be due to an acquired mutation in the *ABL1* kinase domain (AKD) of the *BCR-ABL1* fusion gene and therefore AKD mutation testing is recommended.
Please notify the laboratory if AKD testing is required on the current sample.

Level of *BCR-ABL1* normalised to *ABL1* on the International Scale (IS)

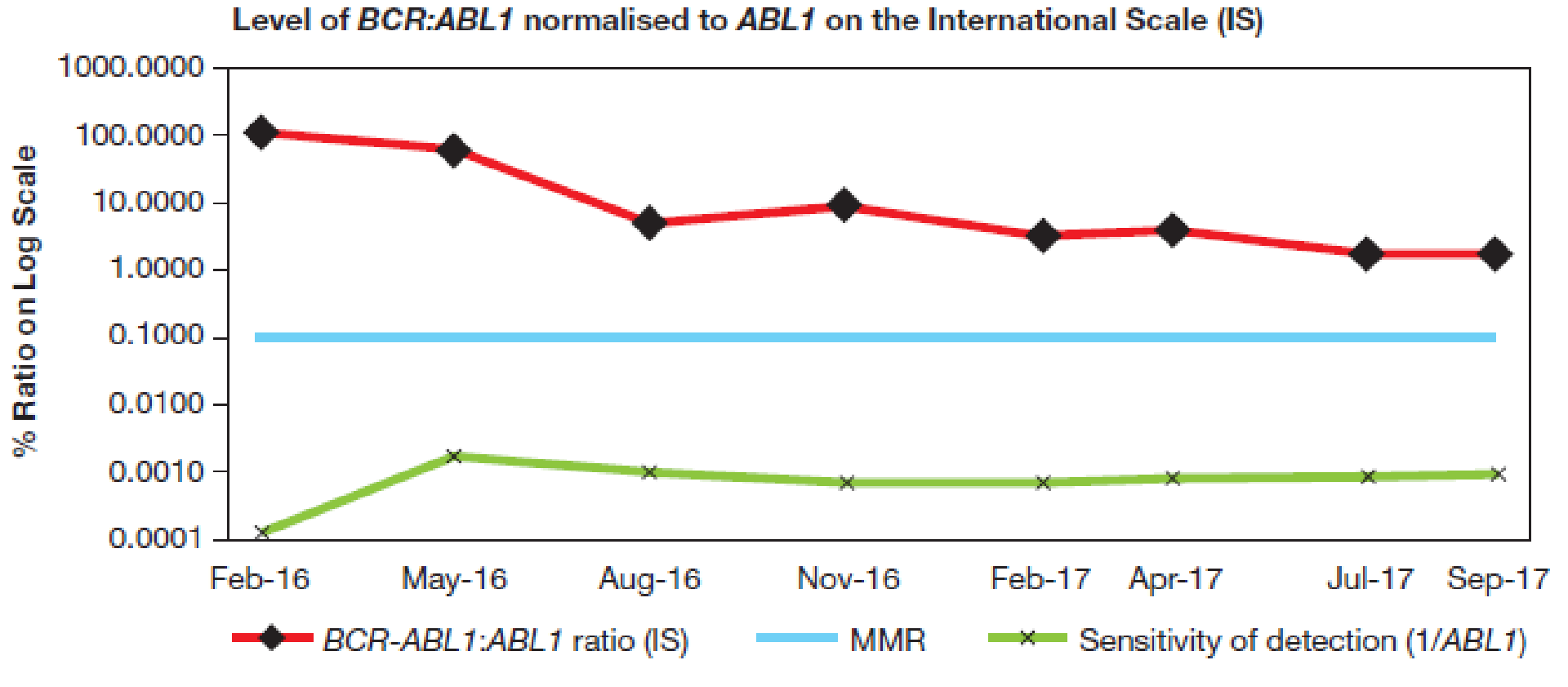


Clinical Summary: CML on imatinib for 18 months. For *BCR-ABL1* monitoring by RT-qPCR.

***BCR-ABL1* quantitative PCR (RT-qPCR) monitoring report (transcript type = e13a2/e14a2)**

Treatment response	*BCR-ABL1:ABL1* % ratio on IS	MR level	Date next sample due
Failure	1.6905	MR[1]	As clinically required

Please note that this result could signify a resistance to treatment that may be due to an acquired mutation in the *ABL1* kinase domain (AKD) of the *BCR-ABL1* fusion gene and therefore AKD mutation testing should be considered. Please notify the laboratory if AKD testing is required on the current sample.



Branford et al showed that **61%** of patients with ***a > 2-fold increase in BCR-ABL*** had detectable mutations, compared with <1% of patients with stable or decreasing BCR-ABL.

- Primary resistance to TKI in patients with uncomplicated CML-CP is **rare**. Hence routine testing for mutations is not required at diagnosis of CML-CP or for those with an optimal response to TKI.
- **Recommendation—**Mutational analyses be performed if a failure to achieve therapy milestones (suboptimal response) is observed, loss of prior response or progression to AP or BP.

Pediatr Blood Cancer. 2019 September ; 66(9): e27827. doi:10.1002/pbc.27827.

•Patient test result & Interpretation:

BCR-ABL^{IS}%: **4.05%**

Patient response based on ELN guideline:

***MR 1.0**

***Failure**

Treatment commence: Second line therapy:

Imatinib: 2 years then Kimonil: 2 years

BCR-ABL **Copies (Total): 9931**

ABL **Copies (Total): 198909**

Test Sensitivity (1/ABL1): 0.000005

Transcript variant at diagnosis: Positive for BCR/ABL1

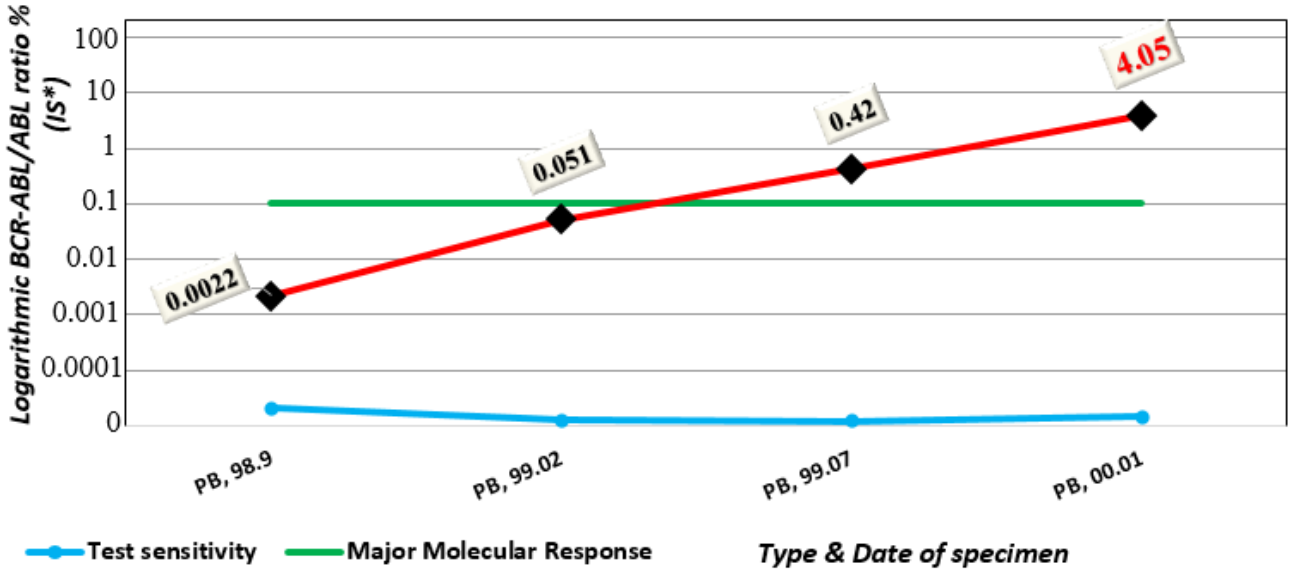
P210 fusion b3a2.

BCR-ABL^{IS}%

4.05%

Log reduction

- >10 %
- **MR 1.0**
- MR 2.0
- MR 3.0
- MR 4.0
- MR 4.5
- MR 5.0



ABL Kinase Domain Mutation

Test Name: Detection of ABL Kinase Domain Mutations in CML

Specimen Type: EDTA anticoagulated peripheral blood

Method: RNA was isolated and reverse transcribed into cDNA. The BCR-ABL1 kinase domain was amplified in a two steps nested PCR reaction that does not amplify non-translocated ABL1. Then PCR product analyzed by Sanger sequencing.

Result: One heterozygous likely pathogenic variant defined as c.1075T>G (p.F359V) in *ABL1* gene (catalytic domain) detected.

Interpretation: Approximately 85% of all **Imatinib resistant mutations** are associated with amino acid substitutions at just seven residues (P-loop: M244V, G250E, Y253F/H and E255K/V; contact site T315I; and catalytic domain:M351T and F359V/C/I/L). Four agents are currently approved for second-line treatment of patients with CML who demonstrate resistance (or intolerance) to Imatinib, Nilotinib, Dasatinib, Bosutinib and Pontatinib. **Based on the NCCN guideline (version 3.2020); Nilotinib is absolutely contraindicated in the patients harboring F359V/C/I mutation. In this cases, clinical trials recommend that Dasatinib may be more effective than the other agents.**

Recommended TKI in case of *BCR-ABL1* resistance mutations

T315I	Ponatinib
F317L/V/I/C, T315A	Nilotinib, bosutinib[a], or ponatinib
V299L	Nilotinib or ponatinib
Y253H, E255V/K, F359V/I/C	Dasatinib, bosutinib[a], or ponatinib

[a]There are limited data available regarding mutations associated with clinical resistance to bosutinib in vivo. Some in vitro data suggest that the E255K and, to a lesser extent, the E255V mutation, might be poorly sensitive to bosutinib.

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https://doi.org/10.1038/s41375-020-0776-2

Recommended TKI in case of *BCR-ABL1* resistance mutations

TABLE 3. *ABL* Mutations and Treatment Options

Mutation	Treatment Options
T315I	Ponatinib, HSCT, omacetaxine, or clinical trial Or Asciminib
V299L	Nilotinib, ponatinib, HSCT, or omacetaxine
T315A	Nilotinib, imatinib, bosutinib, ponatinib, HSCT, or omacetaxine
F317L/V/I/C	Nilotinib, bosutinib, ponatinib, HSCT, or omacetaxine
Y253H, E255K/V, F359V/C/I	Dasatinib, bosutinib, ponatinib, HSCT, or omacetaxine
Any other mutation	Dasatinib, nilotinib, bosutinib, ponatinib, high-dose imatinib, HSCT, or omacetaxine

HSCT, hematopoietic stem cell transplant.

European LeukemiaNet 2020 recommendations for treating chronic myeloid leukemia
https://doi.org/10.1038/s41375-020-0776-2

Conditions raise concerns for progression of disease

1. Resistance to two TKIs,
2. Detection of a *BCR-ABL1* kinase domain (KD)- mutation,
3. Emergence of additional chromosome abnormalities in Ph+ cells (ACA)

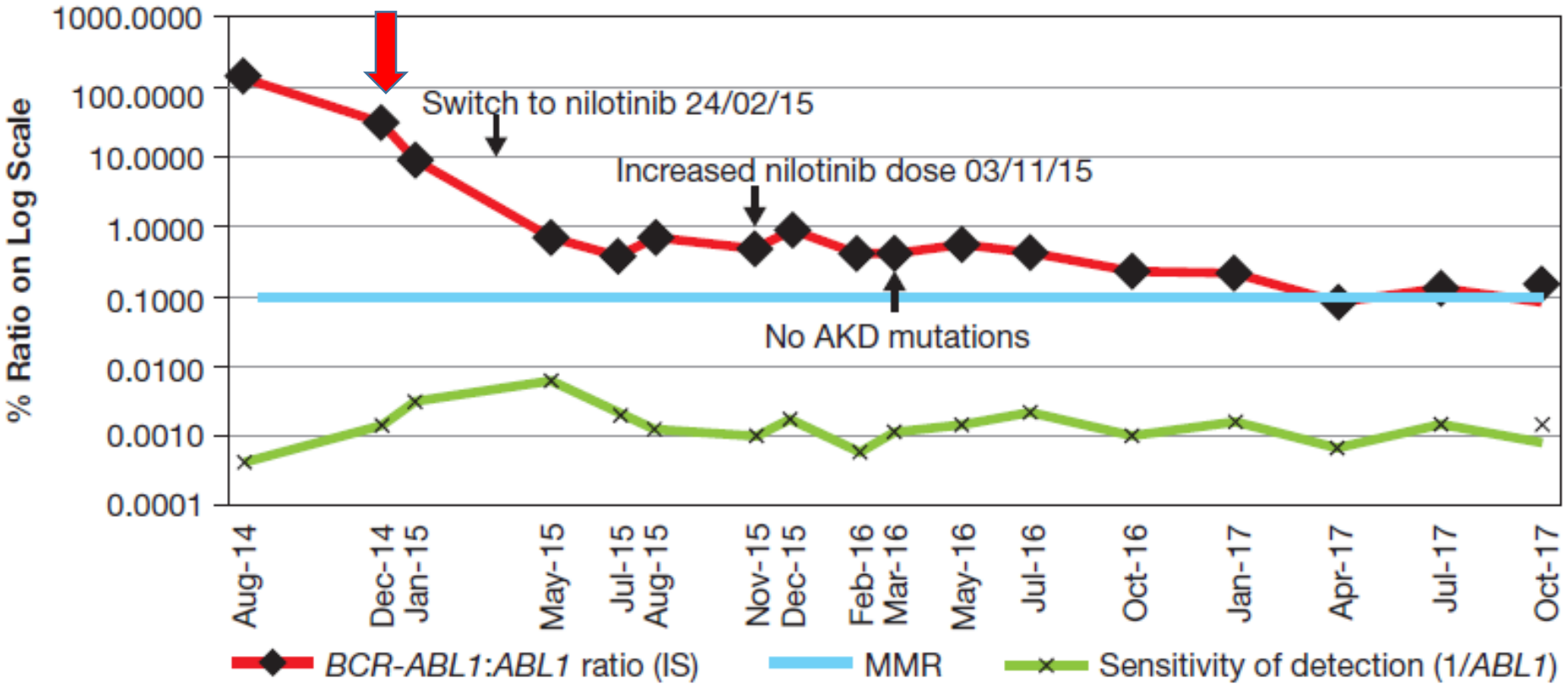
European LeukemiaNet 2020 recommendations for treating chronic myeloid leukemia
https://doi.org/10.1038/s41375-020-0776-2

Clinical Summary: CML on second-line nilotinib for 2 years and 8 months. For *BCR-ABL1* monitoring by RT-qPCR.

***BCR-ABL1* quantitative PCR (RT-qPCR) monitoring report (transcript type = e13a2/e14a2)**

Treatment response	*BCR-ABL1:ABL1* % ratio on IS	MR level	Date next sample due
Optimal	0.080	MR^3	January 2018

Level of *BCR-ABL1* normalised to *ABL1* on the International Scale (IS)



CML: Molecular monitoring of 7 Y/O girl

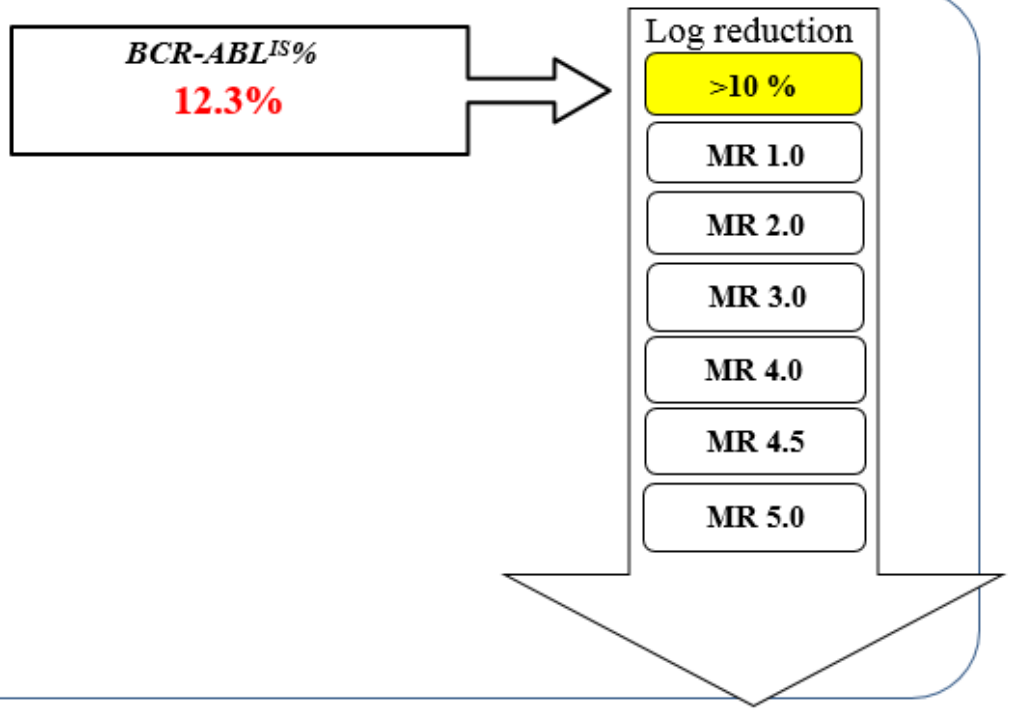
•Patient test result & Interpretation:

***BCR-ABL*^{IS}%: 12.3%**

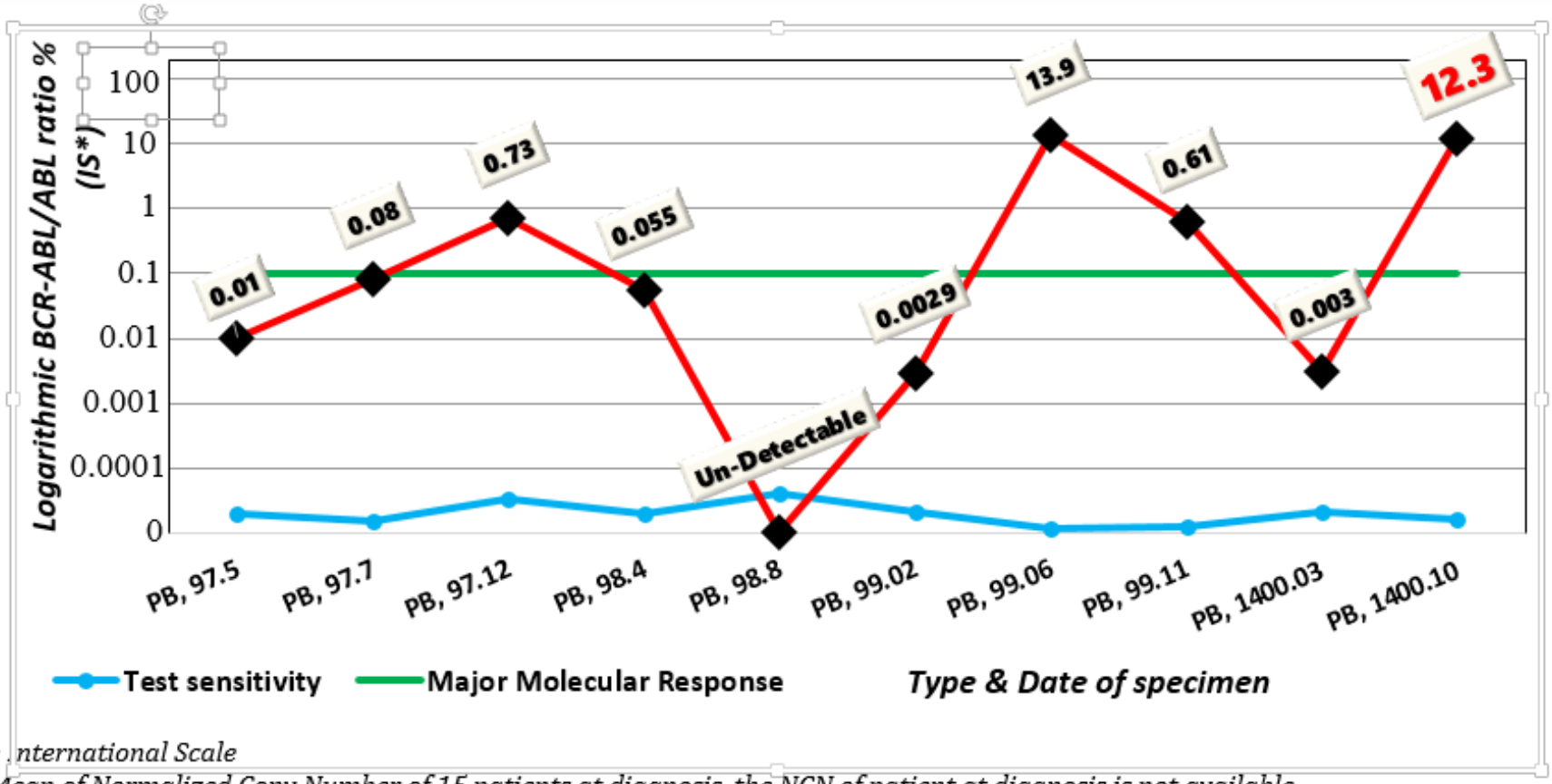
Patient response based on ELN guideline:

Treatment commence: **Gleevec: 6 years and 5 months then treatment discontinued since 3 months ago**

***BCR-ABL* Copies (Total): 25291**
***ABL* Copies (Total): 166653**
Test Sensitivity (1/ABL1): 0.000006
Transcript variant at diagnosis: **Positive for BCR-ABL1^{P210} fusion b3a2.**



CML: Molecular monitoring of 7 Y/O girl



*IS: International Scale

** Mean of Normalized Copy Number of 15 patients at diagnosis, the NCN of patient at diagnosis is not available

How to Report: Laboratory to Clinician

- The laboratory should promptly notify the clinician &/or multidisciplinary team when there is a ***marked change*** **in *BCR-ABL1* level** and/or ***when a change in monitoring frequency is required***.
- **'Marked' Change** needs to be defined locally on the basis of the level of disease & the measured variation of the essay used, but **in general a 1-log increase or loss of MMR** would be considered as a marked change

  – **Changes to monitoring frequency** should be finalized after the laboratory has consulted with the treating hematologist/oncologist; this is usually determined by the clinician rather than the laboratory

BCR-ABL1 Fluctuation

- ***It is important to realize that:***

  - It is not unusual for PCR results to *fluctuate up and down over time*, ***in part because of laboratory technical reasons.***

- The precision of this assay at **low *BCR/ABL1* levels** is **more variable**, such that inter-run variation can be as high as **+ or - 0.5 log**.
- ***Only level changes above 0.5 log should be considered clinically significant.*** For example, if a result is given as 0.1% *BCR/ABL1:ABL1,* then any result between 0.05% and 0.5% should be considered essentially equivalent. If the results are being used to make major therapeutic decisions, ***significant changes during monitoring should be verified with a subsequent specimen***.

How to Report: Patient-Directed Communication

- Increasingly, patients have access to Lab. results, & complex or poorly worded reports can lead to unnecessary alarm/confusion.
- If reports are being sent to the patient,

Current test result:

Date: _____

Your *BCR-ABL1* level, as measured on the International Scale, is_____%.

Your *BCR-ABL1* level has_____[increased / decreased / remained stable] since your last test on_____[date].

Please contact your doctor if you have any questions or concerns about this test result.

Previous test results:

On _____[date], your *BCR-ABL1* level was _____%.

On _____[date], your *BCR-ABL1* level was _____%.

Is there a role for stopping TKI in pediatric patients with a good response? 2019

- So far there are no data to show the feasibility of stopping TKI in the pediatric CML population. The limited available data are mainly based on case reports of non-compliant pediatric patients.
- ***Current adult guidelines for stopping TKI cannot be applied for children and adolescents*** without proper prospective clinical trials.
- **Recommendation**—TKI therapy should only be stopped in children and adolescents in the context of a clinical trial.

Pediatr Blood Cancer. 2019 September ; 66(9): e27827. doi:10.1002/pbc.27827.

Is there a role for stopping TKI in pediatric patients with a good response? 2021

- Recommendations regarding discontinuation apply only for adults because childhood CML is a very rare disease and represents a separate entity.
- The aim of our retrospective study was to assess within the ***International Registry of Childhood CML***, the rate of children remaining in molecular response after discontinuation of imatinib in a context of DMR defined as BCR-ABL1/ABL1 < 0.01% (MR4) for at least two years.
- Eighteen patients less than 18 years old at diagnosis of CML exhibiting a sustained DMR followed by imatinib discontinuation were identified.
- ***After discontinuation, the molecular free remission rate was 61%, 56% and 56% at 6, 12 and 36 months, respectively.***
- **Our findings represent the basis of recommendation regarding discontinuation for physicians involved in the pediatric CML field.**

Cancers **2021**, 13, 4102.
https://doi.org/10.3390/cancers13164102

TFR & Molecular Monitoring

Following discontinuation, monitoring should be:

1. Monthly for 6 months
2. Six-weekly from 7 to 12 months
3. Two-monthly from 13 to 36 months
4. Three-monthly for year ≥3

- We show that late relapses after TKI discontinuation in CML do occur in ~10% of patients in TFR at 36 months.
- Molecular status at 36 months, not being in MR4, is highly predictive of subsequent loss of MMR.

Leukemia, 2021
https://doi.org/10.1038/s41375-021-01173-w

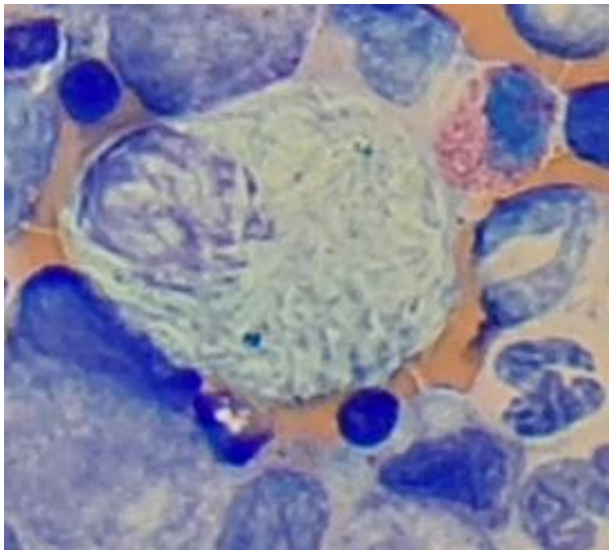
Enabling access to molecular monitoring for CML patients is cost effective in China

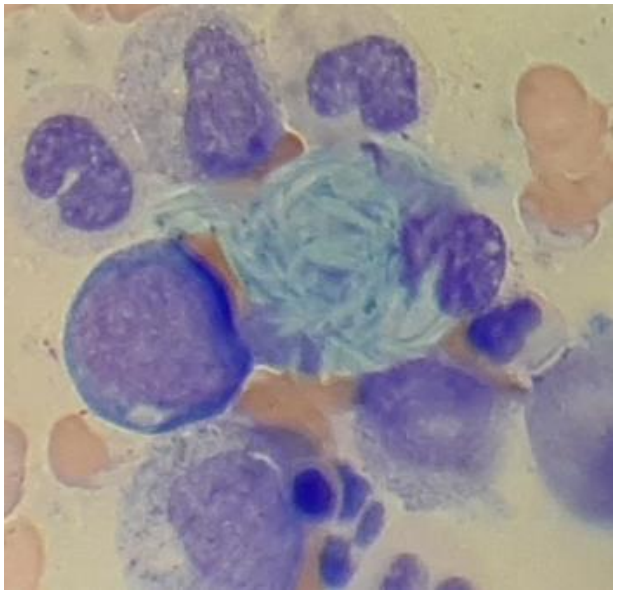
- **Molecular monitoring was dominant to no molecular monitoring**, with increased Lys, total life years (1.52) and QALYs, quality-adjusted life years (1.90) and costs savings (¥93,840) over a lifetime compared to no monitoring in discounted analyses.
- The opportunity of patients that receive molecular monitoring to discontinue treatment during treatment-free remission,
- Overall, this analysis demonstrates that adherence to guideline recommendations of regular molecular monitoring of patients with CML-CP treated with TKIs provides significant clinical benefit that leads to substantial cost savings compared to no molecular monitoring from the perspective of a Chinese payer. In a time where healthcare systems have limited resources to allocate to optimal patient care, investment in molecular monitoring is an ideal choice for improving patient benefits at a reduced cost.

PLOS ONE | https://doi.org/10.1371/journal.pone.0259076 October 25, 2021

Thank you, any question?

Pseudo Gaucher Cells in BMA of CML





Pseudo Gaucher cells and see-blue histiocytes can be found in homogenously distributed in the BMA smears in approximately one third of pediatric.