Multi-institutional Hematopathology Interesting Case Conference

Dr. Oluwole Odujoko
Fellow, Hematopathology,
MSKCC

Memorial Sloan Kettering
Cancer Center

Past history and chief complaints

61-year-old female with prior history of HR+HER2- pT2pN3 stage IIIC invasive ductal carcinoma in 2017

She was treated with right lumpectomy and axillary lymph node dissection in 2017 followed by adjuvant chemotherapy and radiation therapy.

She was placed on Letrozole afterwards

In April, 2022, patient was noticed to have pancytopenia on routine bloodwork.

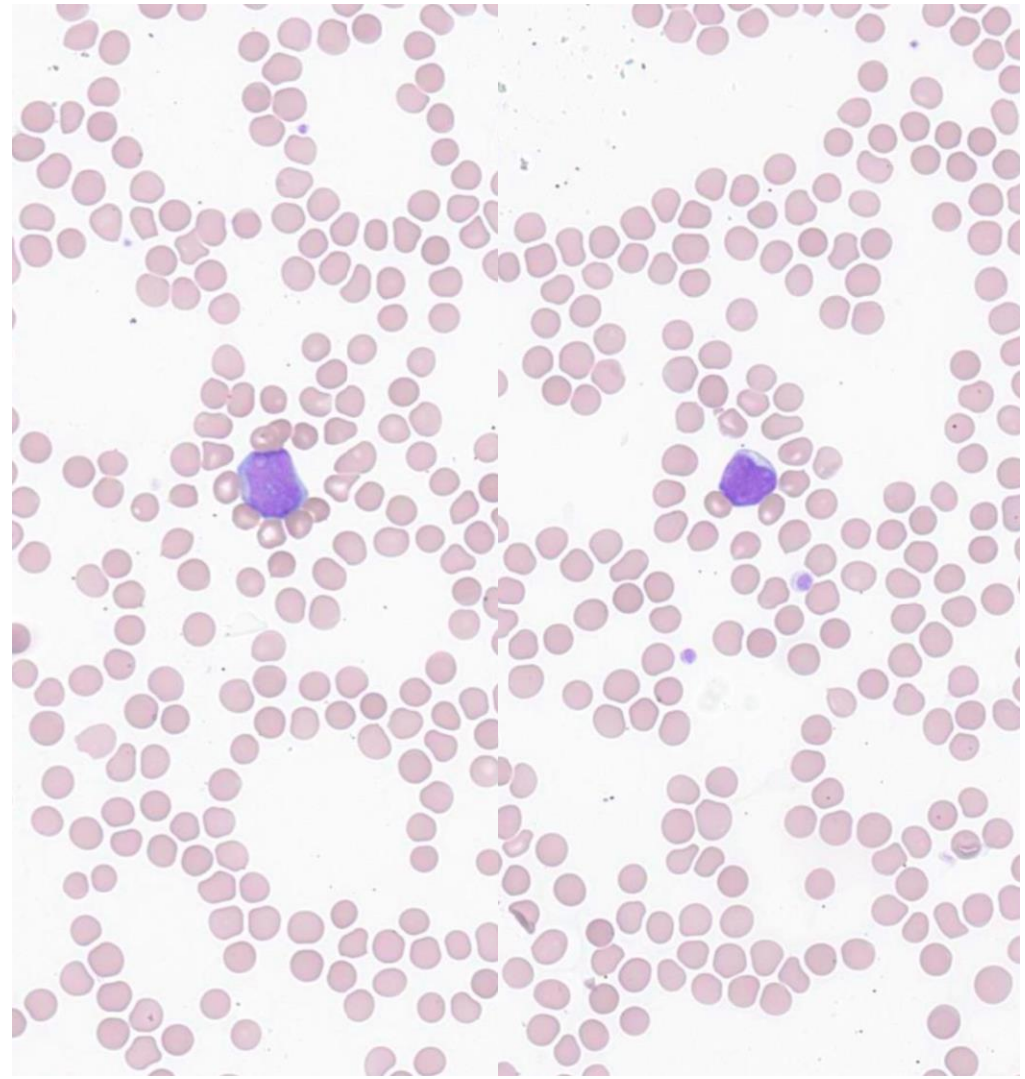
In-house CBC showed WBC of 2.6 and 7% circulating blasts

PERIPHERAL BLOOD

CBC:

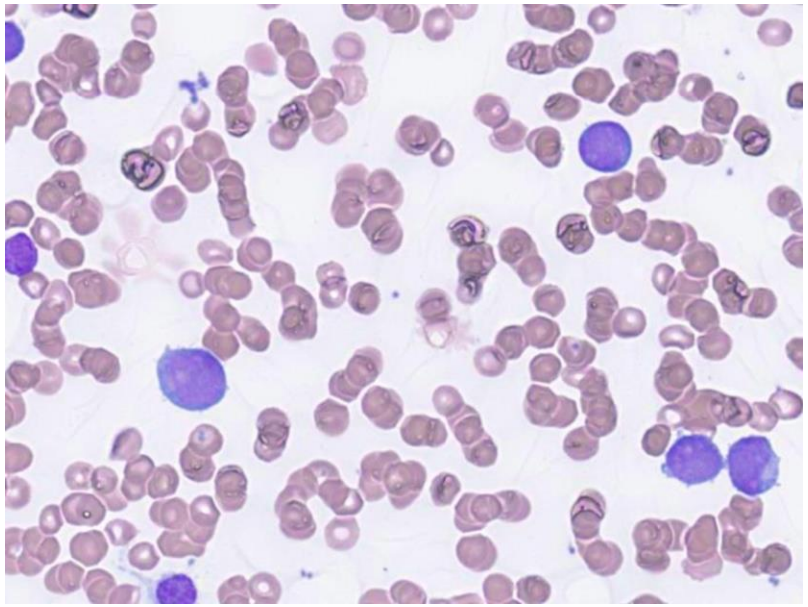
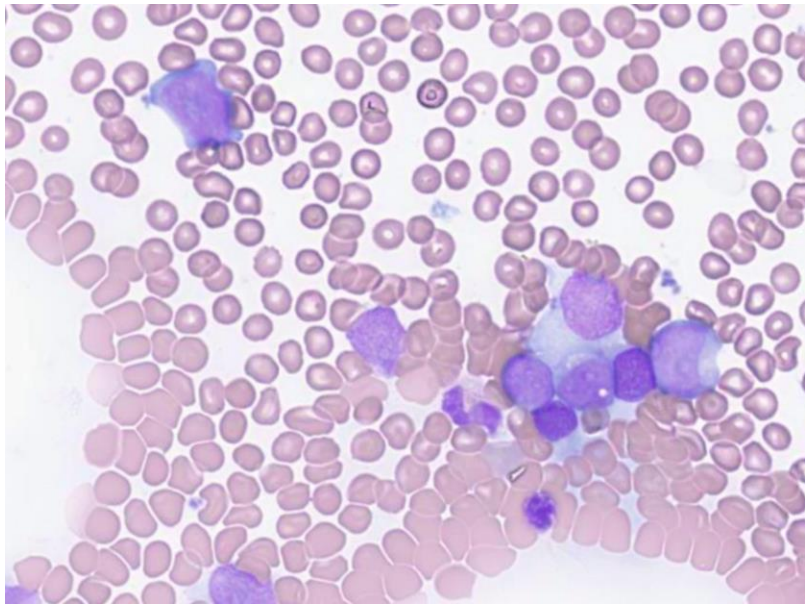
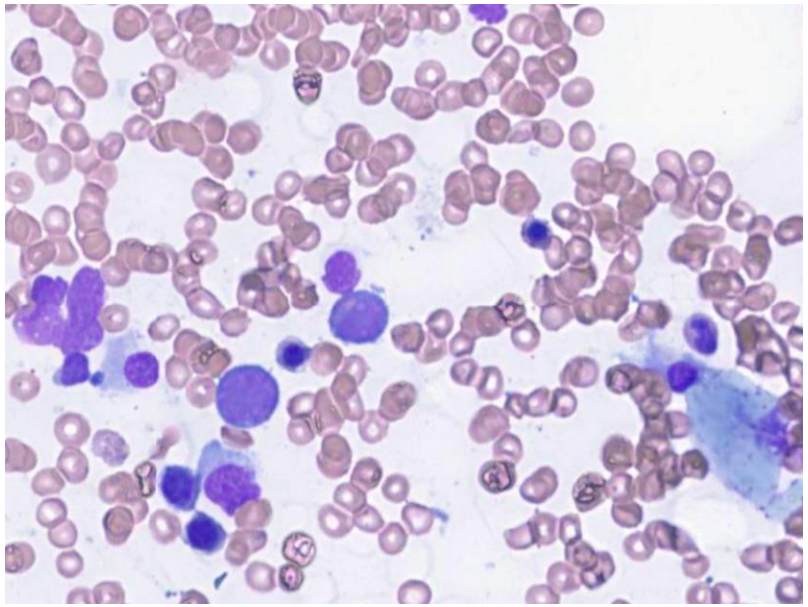
WBC 2.5 L [4.0-11.0 K/mcL]
RBC 3.40 L [3.80-5.00 M/mcL]
HGB 10.9 L [11.2-15.4 g/dL]
HCT 32.4 L [34.3-46.0 %]
MCV 95 [80-98 fL]
MCH 32.1 [27.0-33.0 pg]
MCHC 33.6 [31.0-36.5 g/dL]
RDW 14.0 [12.2-15.1 %]
Platelets 126 L [160-400 K/mcL]

Neutrophil 29.0 L [32.5-74.8 %]
Mono 5.0 [0.0-12.3 %]
Eos 1.0 [0.0-4.9 %]
Baso 0.0 [0.0-1.5 %]
Blast 12.0 H [0.0-0.0 %]



Bone marrow aspirate

4/20/2022

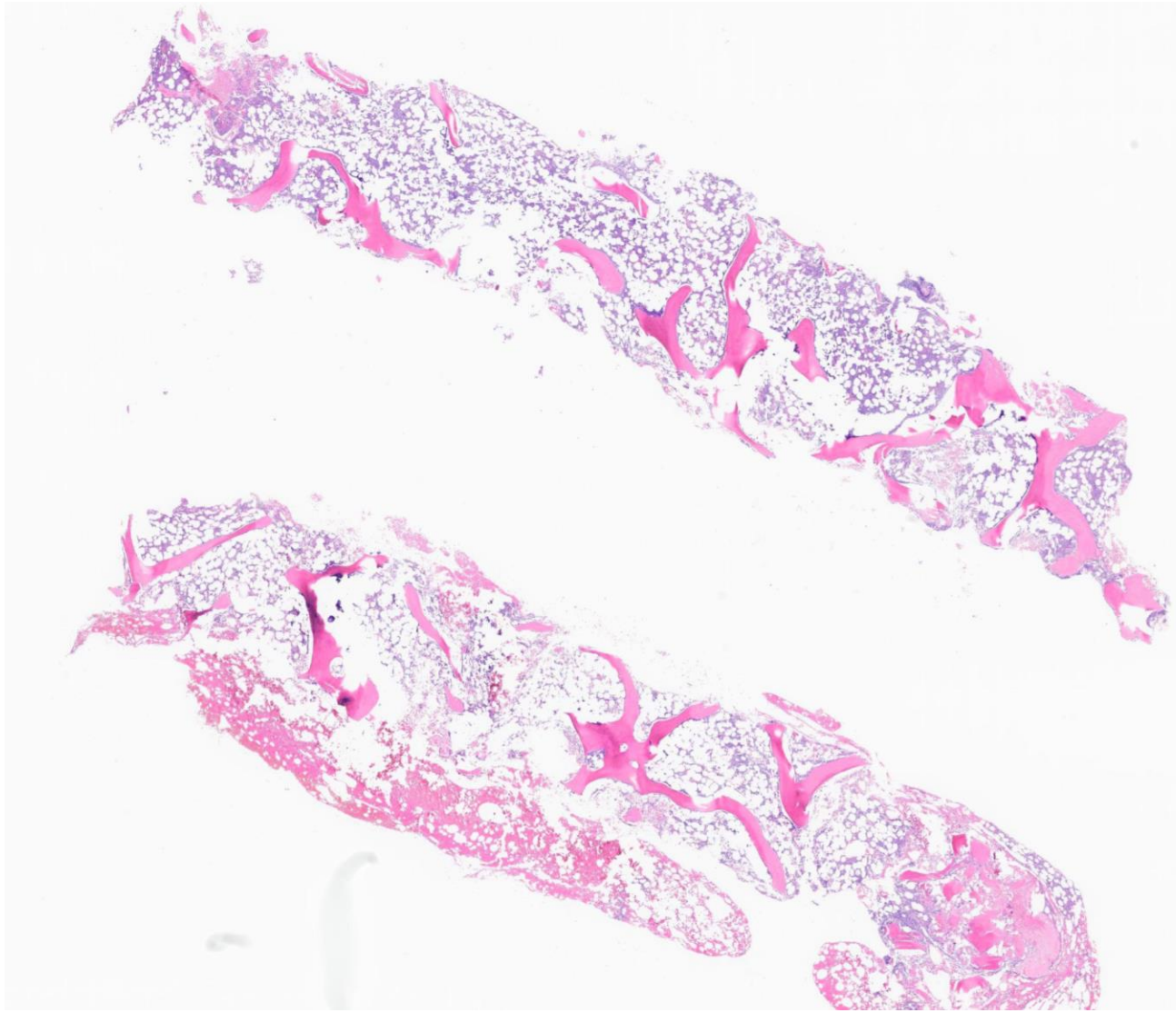




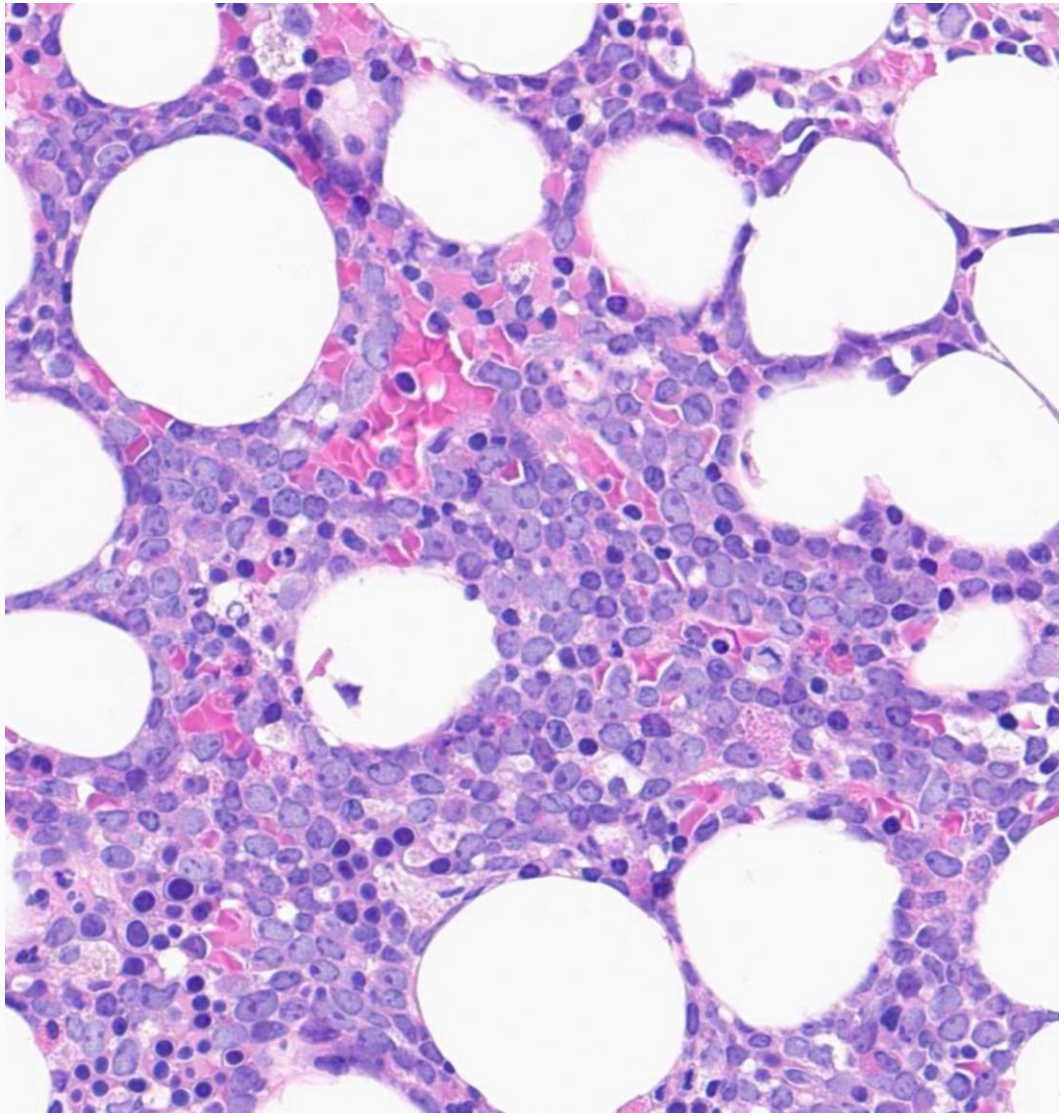
Blasts 65%
Myelocytes 2%
Metamyelocytes 1%
Neutrophils/Bands 7%
Monocytes 1%

Eosinophils 1%
Erythroid Precursors 9%
Lymphocytes 14%
Number of Cells Counted 500
M:E Ratio 1.3

Bone marrow biopsy

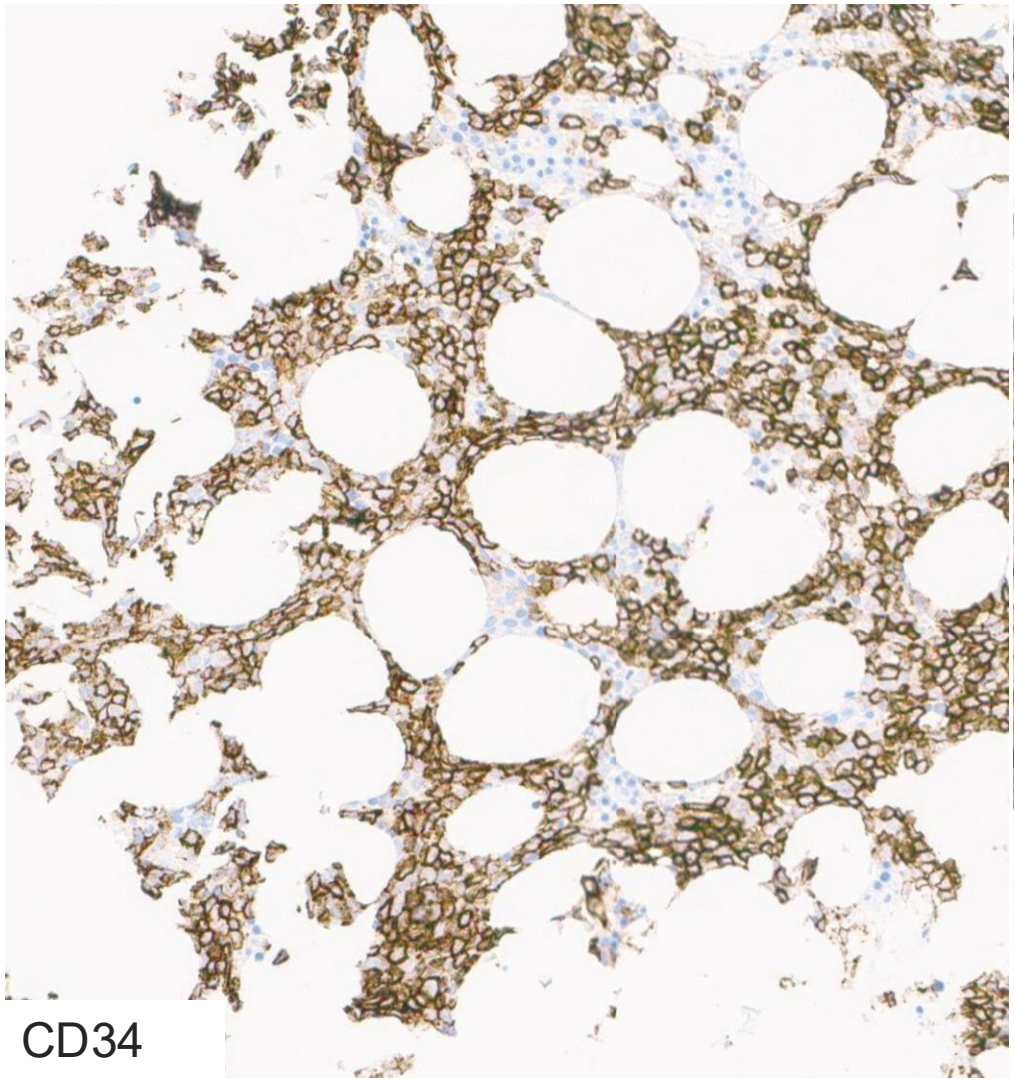
4/20/2022



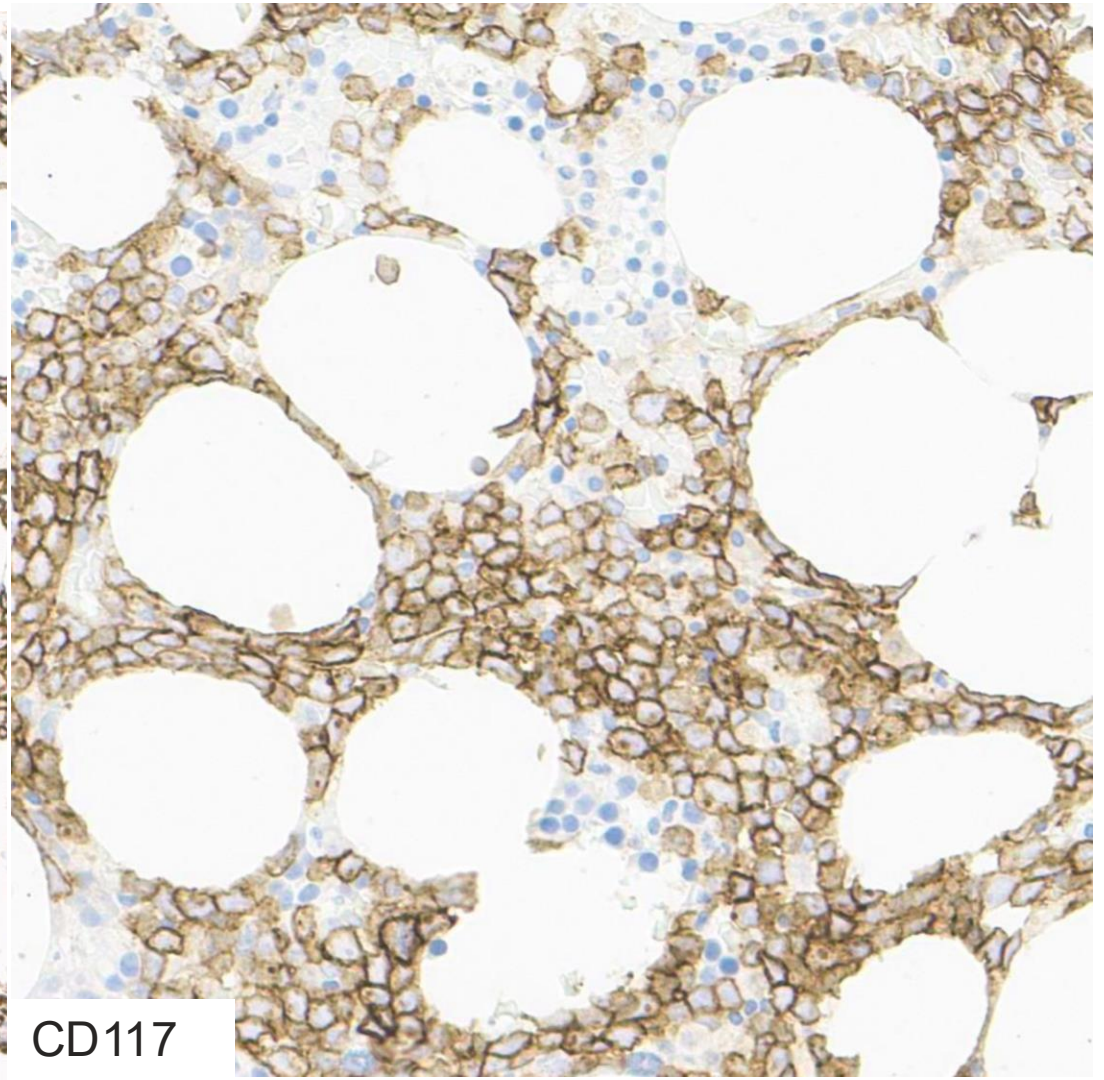


Immunohistochemistry

4/20/2022

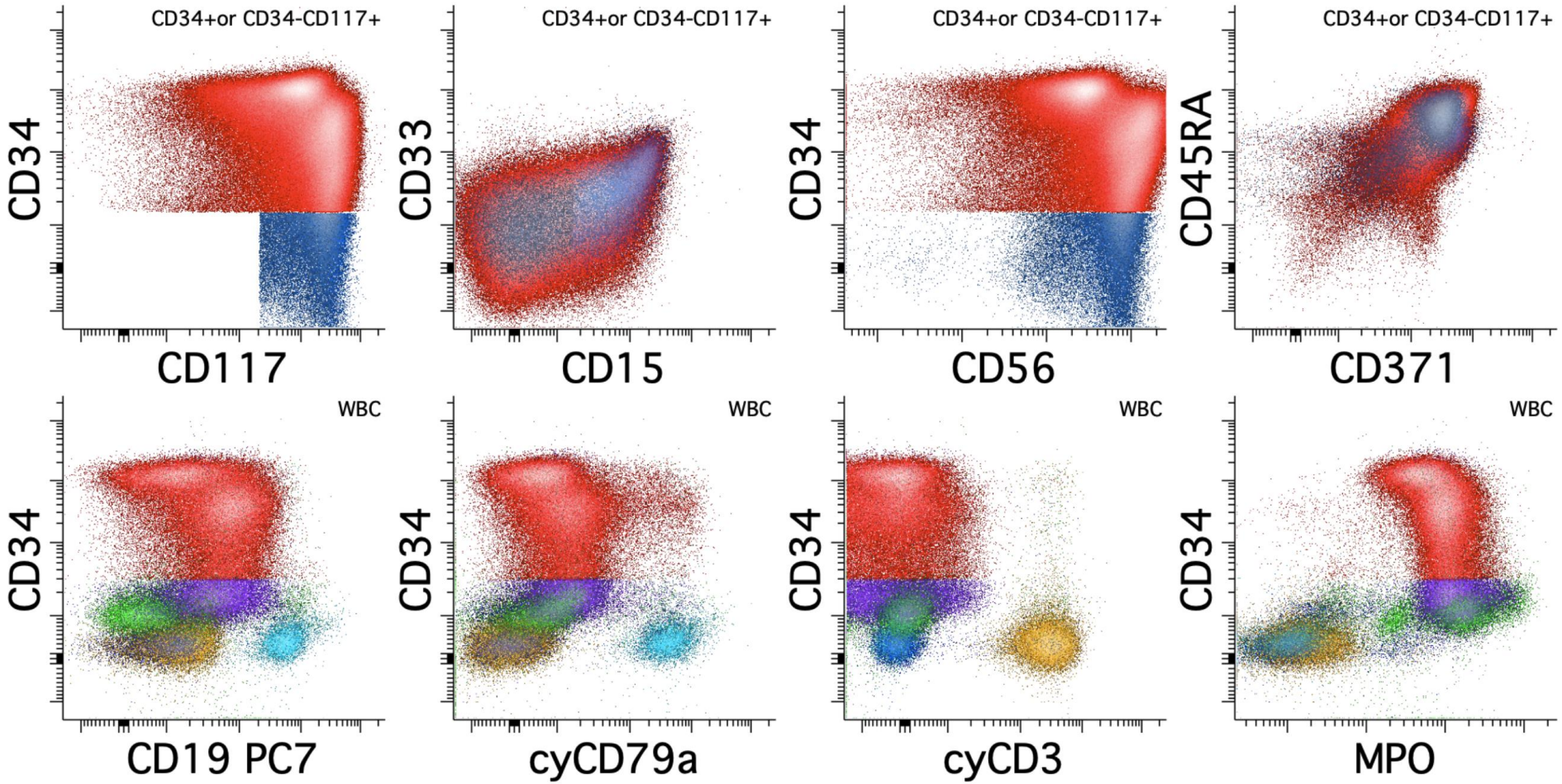


CD34



CD117

Flow cytometry



?Differential diagnosis

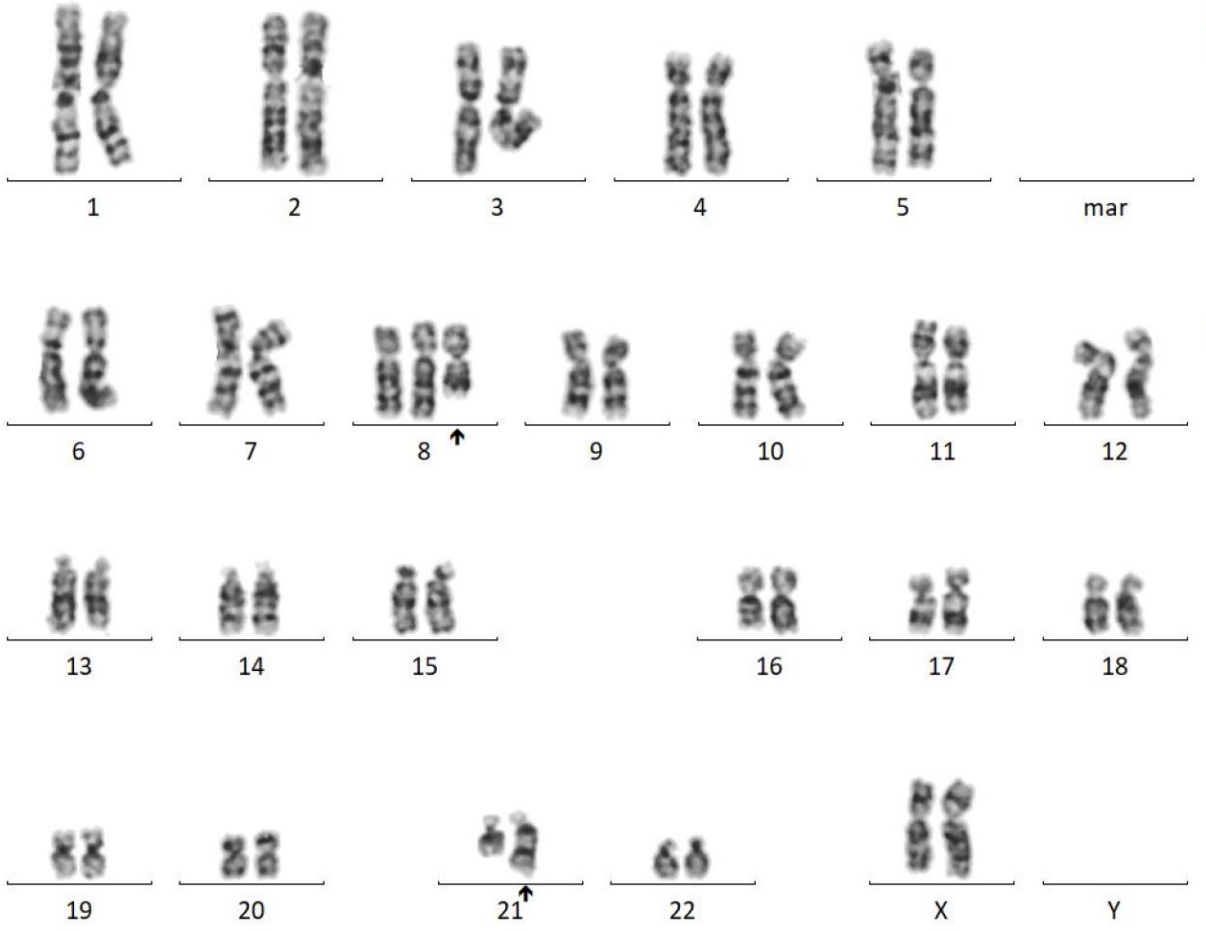
Differential diagnosis for acute leukemias with mixed phenotype

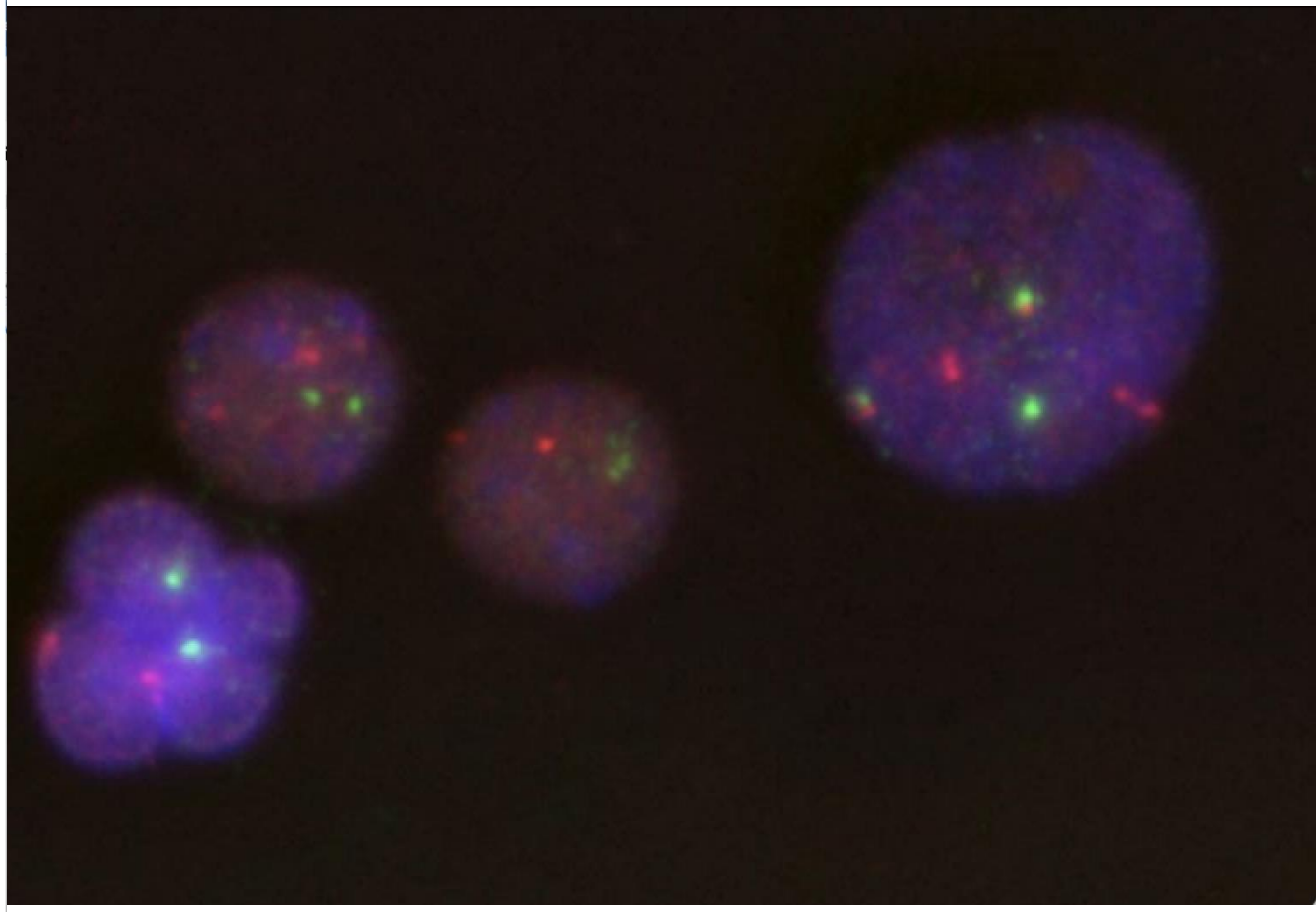
De novo mixed phenotype acute leukemia (MPAL)

Acute myeloid leukemia with mixed phenotype (AML-MP)

- Leukemias with specific genetic abnormalities
- Myeloid/lymphoid neoplasms with eosinophilia and tyrosine kinase gene fusions (ex PDGFRA, etc)
- Chronic myeloid leukemia in blast crisis (blast phase of MPNs)
- AML with myelodysplasia related changes
- Therapy-related AML

Cytogenetics





Diagnosis

DIAGNOSIS:

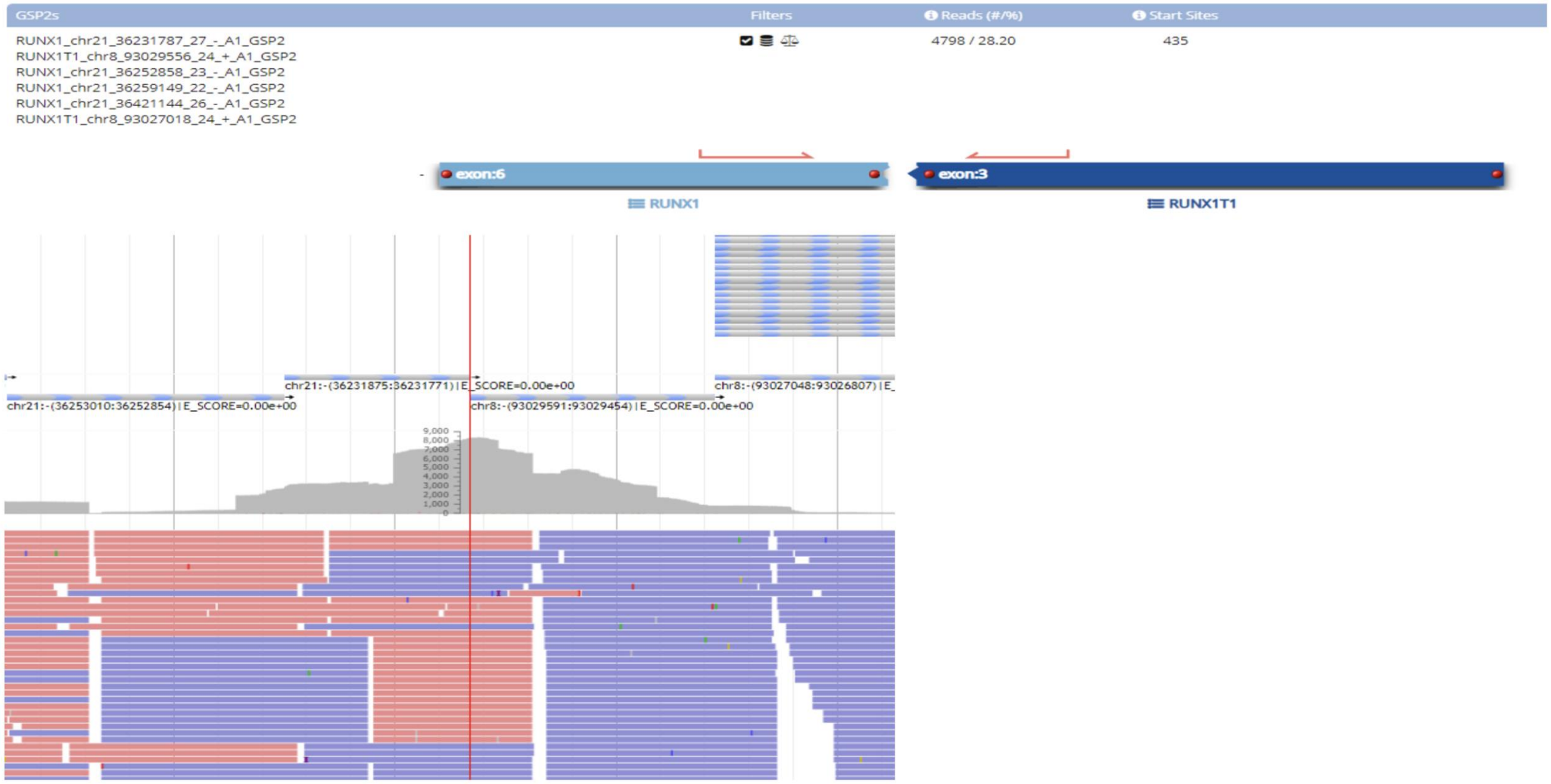
1-3. Bone marrow, right posterior iliac crest, biopsy, aspirate and peripheral blood smears:

- Therapy related myeloid neoplasm presenting as Therapy related acute myeloid leukemia with t(8;21)(q22;q22); RUNX1-RUNX1T1.

According to current WHO,

Acute myeloid leukemia with t(8;21), post cytotoxic therapy

Molecular (qRT-PCR)



The rearrangement is an in-frame fusion between genes RUNX1 Exon6 (NM_001754) and RUNX1T1 Exon3 (NM_001198679).

Mutational profile

1. **KIT (NM_000222) exon17 p.D816G (c.2447A>G)**
2. KDM6A (NM_021140) exon23 p.V1113Sfs*8 (c.3334_3335dupGT)
3. **KIT (NM_000222) exon11 p.V559G (c.1676T>G)**
4. RAD21 (NM_006265) exon5 p.D128_V129insLTFF*SDID

NEGATIVE for FLT3 Internal Tandem Duplication (ITD)

NEGATIVE for FLT3 TKD mutation

Treatment course

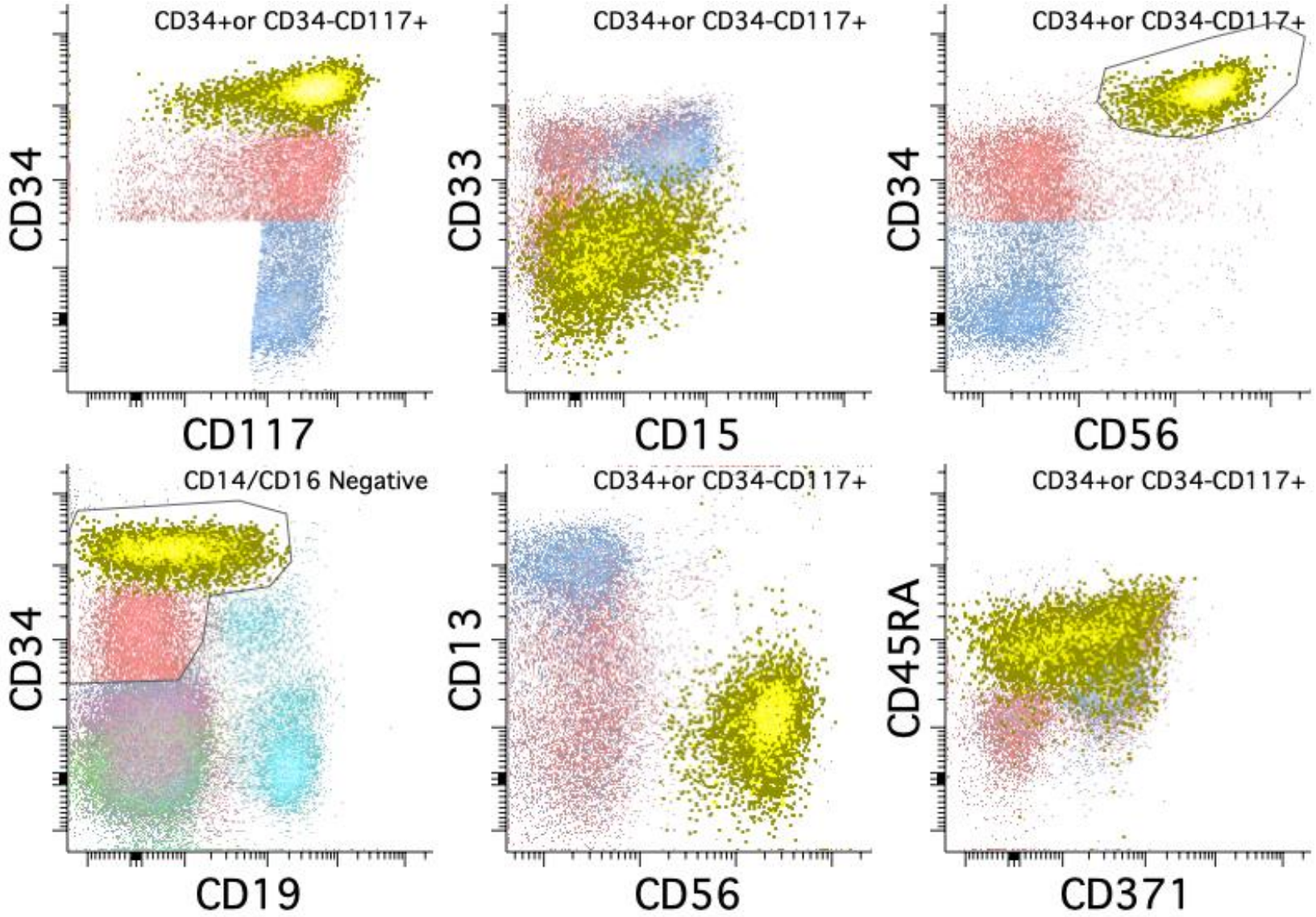
5/2022	Standard induction (7+3)
06/2022	Consolidation (HiDAC)
07/2022.	Bone marrow biopsy CR, MRD negative by flow cytometry Molecular detected RUNX1::RUNX1T1
09/02/2022.	Bone marrow biopsy No abnormality, flow cytometry negative Molecular did not detect RUNX1::RUNX1T1
09/14/2022	Transplanted with MUD

Treatment course

10/14/2022	Molecular detected RUNX1::RUNX1T1
12/19/2022	BMBx negative for disease, flow negative Molecular did not detect RUNX1::RUNX1TI
03/20/2023	Flow cytometry

3/20/2023

Bone marrow flow cytometry



Abnormal myeloid blast population

1.8% of total WBCs

3/20/2023

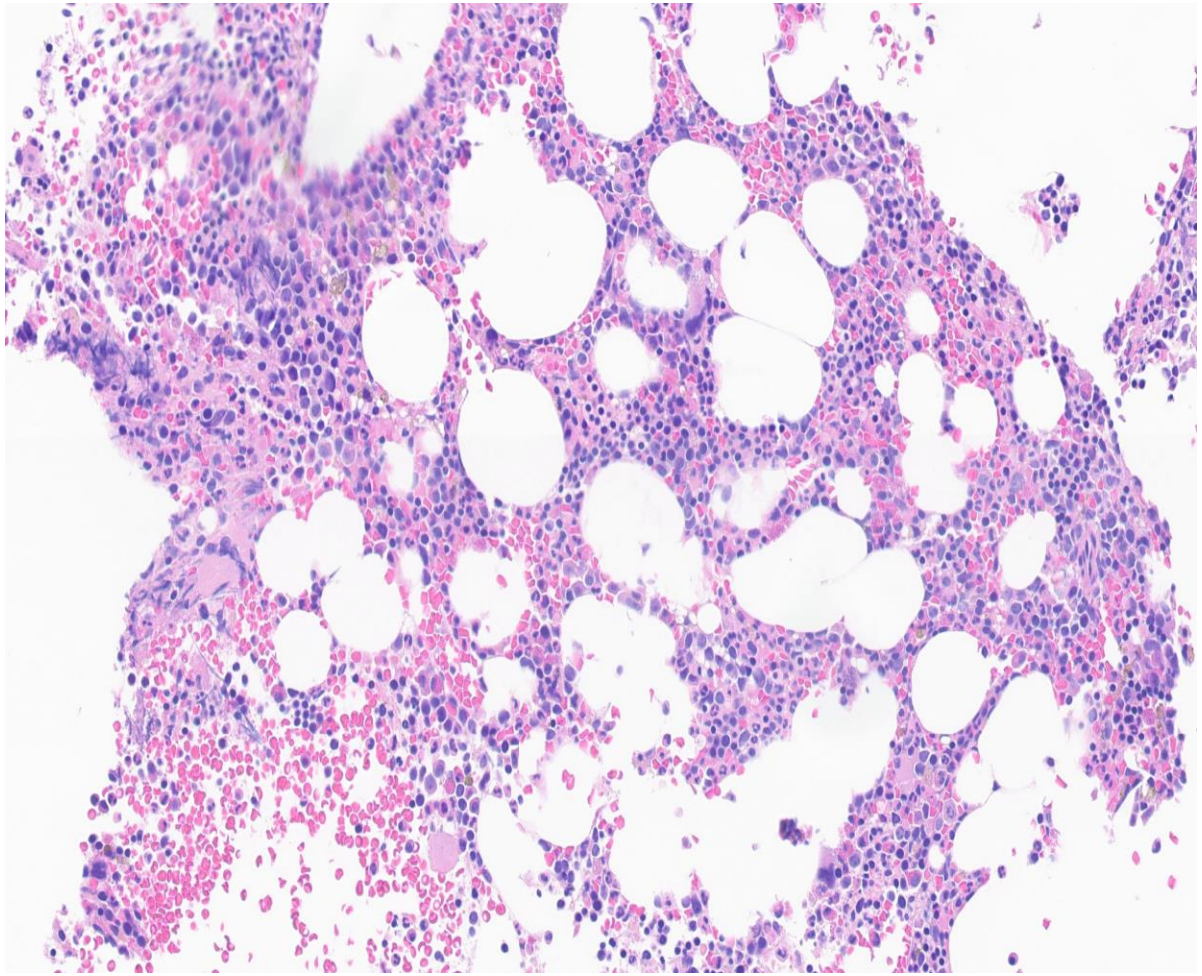
FISH – for chimerism, RUNX1-RUNXT1 fusion

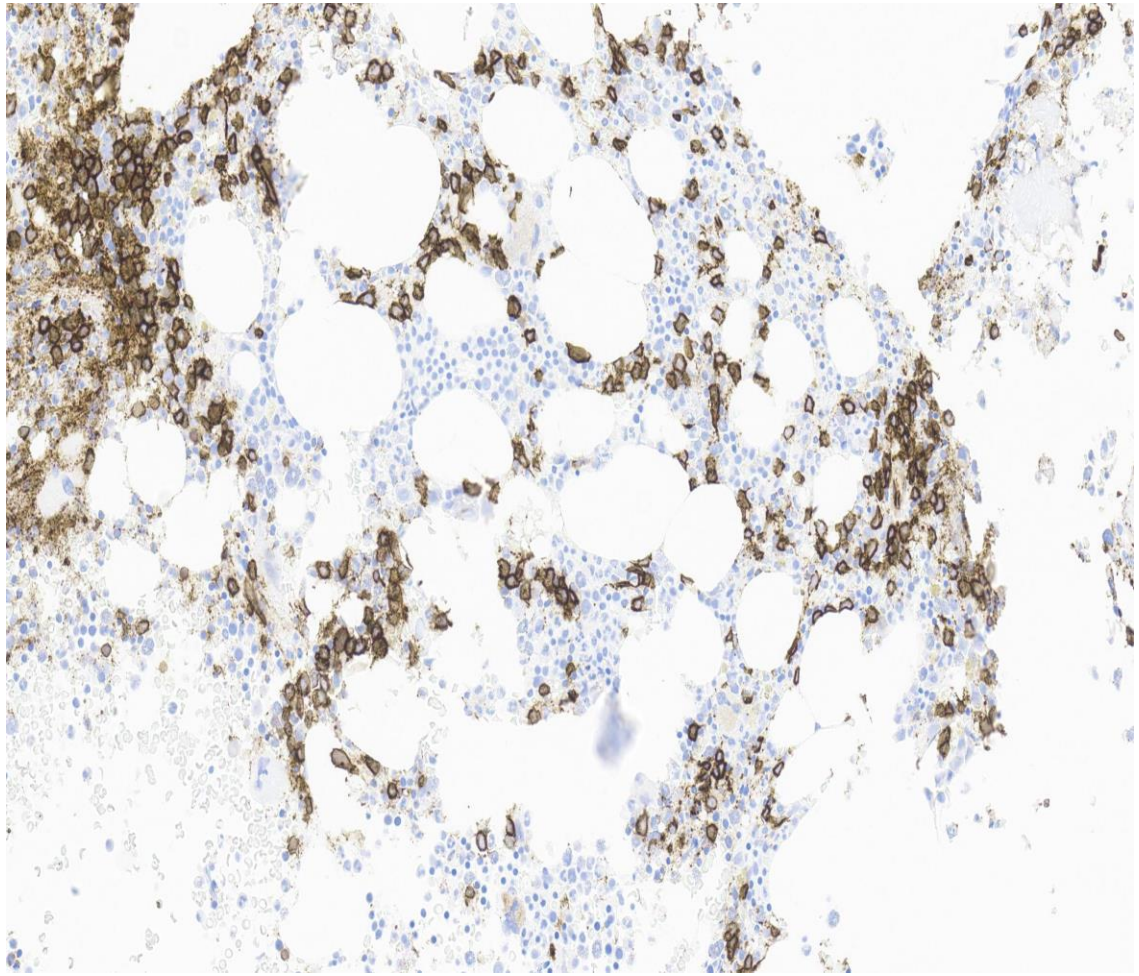
ENGRAFTMENT STATUS:
Chimeric, mostly donor 2.4% host cells 97.6% donor cells

RUNX1-RUNX1T1 fusion/t(8;21) detected in 2.3% of cells, with a signal pattern of two fusions, two signals of RUNX1T1 (8q21.3) and one signal of RUNX1 (21q22).

4/06/2023

Pt later has morphologic relapse (10% blasts by CD34)





Treatment after relapse

Patient was treated with Azacitidine and Venetoclax

She has been in complete remission. MRD negative by flow cytometry and molecular studies.

Table 1 Comparison of methods for measurable residual disease detection

Method	Sensitivity	Advantages	Disadvantages
Flow cytometry (LAIP+DFN)	10^{-3} to 10^{-5}	Fast (within few hours) High applicability Relatively inexpensive Information at cellular level Potential detecting phenotypic shift	Requires fresh sample and viable cells Requires high level of expertise Limited standardization
RT-qPCR for gene fusions	10^{-4} to 10^{-5}	Sensitive Relatively simple Standardized No need of patient-specific (PS) primers	Limited applicability Risk of cross contamination Can't detect small subclones or clonal evolution
RT-qPCR for IG/TCR gene rearrangements	10^{-4} to 10^{-5}	Sensitive Standardized with consensus guidelines	Requires diagnostic sample and PS primers Time consuming, labor-intensive, expensive Can't detect small subclones or clonal evolution
Digital PCR	10^{-3} to 10^{-5}	Sensitive Absolute quantification No need of standard curve Not affected by PCR inhibitors	Lack of standardization May require PS design Can't detect small subclones or clonal evolution
Next generation sequencing	10^{-6}	Highly sensitive No need of PS primers Wide applicability Potential to track small subclones and clonal evolution	Requires pretreatment specimen No standardization Requires high degree of informatics expertise Expensive

DFN, different from normal; LAIP, leukemia/lymphoma associated immunophenotype; PS, patient specific; RT-qPCR, real-time quantitative PCR

Chapter 5, Measurable Residual Disease Testing in Acute Leukemia: Technology and Clinical Significance. Leukemia, Li W, editor. Brisbane (AU). Exxon publications. 2022

Discussion

- qRT-PCR and flow cytometry are commonly used methods for MRD detection in t(8;21) AML
- According to a study, the sensitivity of MFC in detecting MRD in AML t(8;21) patients is between 0.01% and 0.1%.
- In a study carried out by same authors at the time of post-induction for first time diagnosis AML, a total of 54 patients had a MFC study performed on morphologic CR BM
- qRT-PCR was also carried out in 59 patients treated for first time AML and had a morphologic CR BM at the time of post-induction

Quyang J, Goswami M, Peng Ji, Zuo Zhang et al. Comparison of Multiparameter Flow cytometry Immunophenotypic Analysis and Quantitative RT-PCR for the detection of minimal residual disease of core binding factor acute myeloid Leukemia. Am J Clin Pathol. 2016; 145: 769-777.

Measurable residual diseaseMRD for t(8;21) AML – qRT-PCR vs flow – efficacy, predictive value

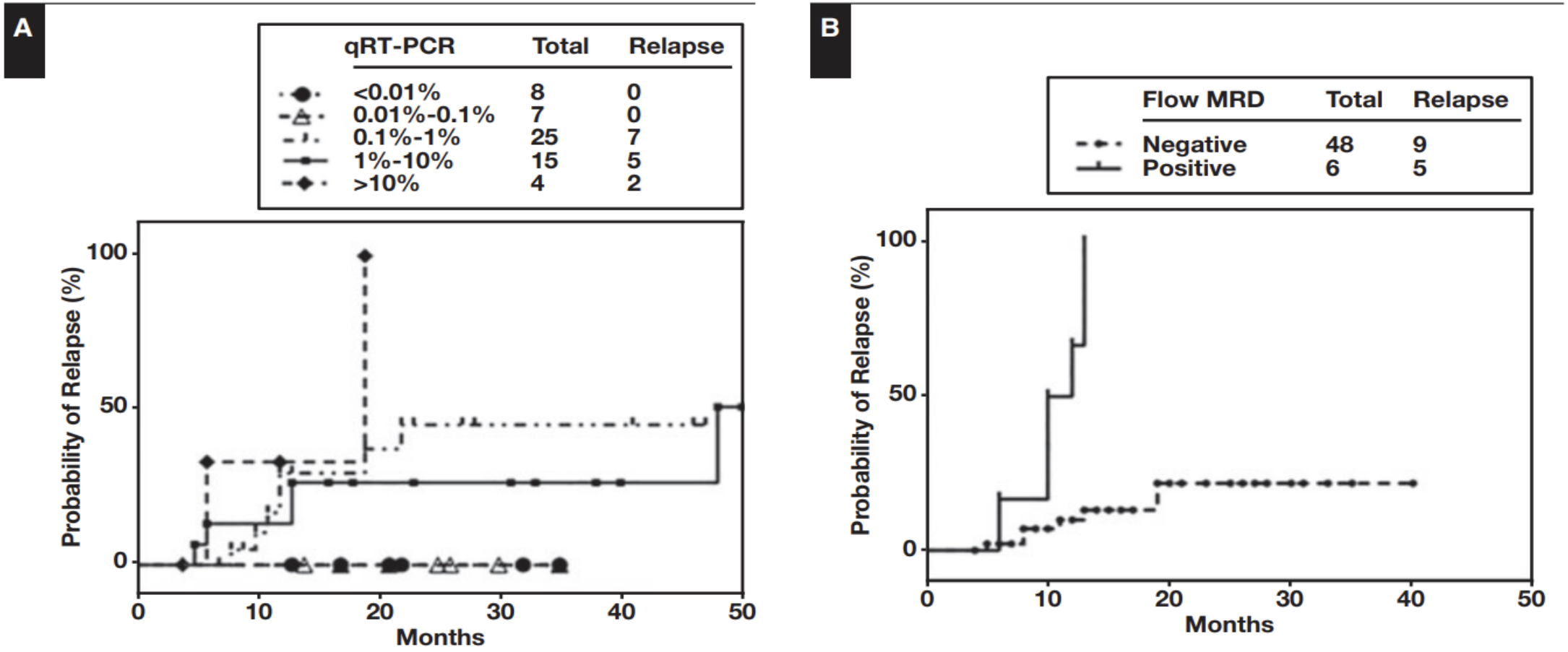


Figure 2 Probability of acute myeloid leukemia relapse by minimal residual disease (MRD) status as detected by quantitative reverse transcription polymerase chain reaction (qRT-PCR) (**A**) ($P = .035$) and multiparameter flow cytometry (**B**) ($P < .001$).

Quyang J, Goswami M, Peng Ji, Zuo Zhang et al. Comparison of Multiparameter Flow cytometry Immunophenotypic Analysis and Quantitative RT-PCR for the detection of minimal residual disease of core binding factor acute myeloid Leukemia. Am J Clin Pathol. 2016; 145: 769-777.

Conclusion

- Flow cytometry-based analysis is a rapid method for evaluating AML MRD in patients with t(8;21) AML.
- Flow cytometry can detect immunophenotypic shift or an emerging new disease with different immunophenotype.
- Flow cytometry and qRT-PCR provide complementary information for assessment of MRD in patients with core binding factor AML.

References

1. Quyang J, Goswami M, Peng Ji, Zuo Zhang et al. Comparison of Multiparameter Flow cytometry Immunophenotypic Analysis and Quantitative RT-PCR for the detection of minimal residual disease of core binding factor acute myeloid Leukemia. Am J Clin Pathol. 2016; 145: 769-777.

2. Chapter 5, Measurable Residual Disease Testing in Acute Leukemia: Technology and Clinical Significance. Leukemia, Li W, editor. Brisbane (AU). Exxon publications. 2022

Thank you