EHA-GBMTA-AHA Hematology Tutorial: New aspects in diagnostic choices and treatment options of hematological malignancies

Self-assessment case

Session: Acute Myeloid Leukemia

20th October 2024, Margarita Guenova

Clinical history

A 30-years old woman was referred to the Hematology Hospital because of:

- Persistent neutropenia and anemia
- Fatique and weakness
- Febrile episodes up to 38^0C

since Covid-19 infection 6 months ago

Medical history:

- Profession - accountant
- Unremarkable family history
- No history of chemical/physical agents exposure
- No previous diseases (except Covid)

https://www.behance.net/gallery/11769999/FREE-EPS-Beautiful-Young-Woman-Face-Black-Ink-Sketch

Physical examination

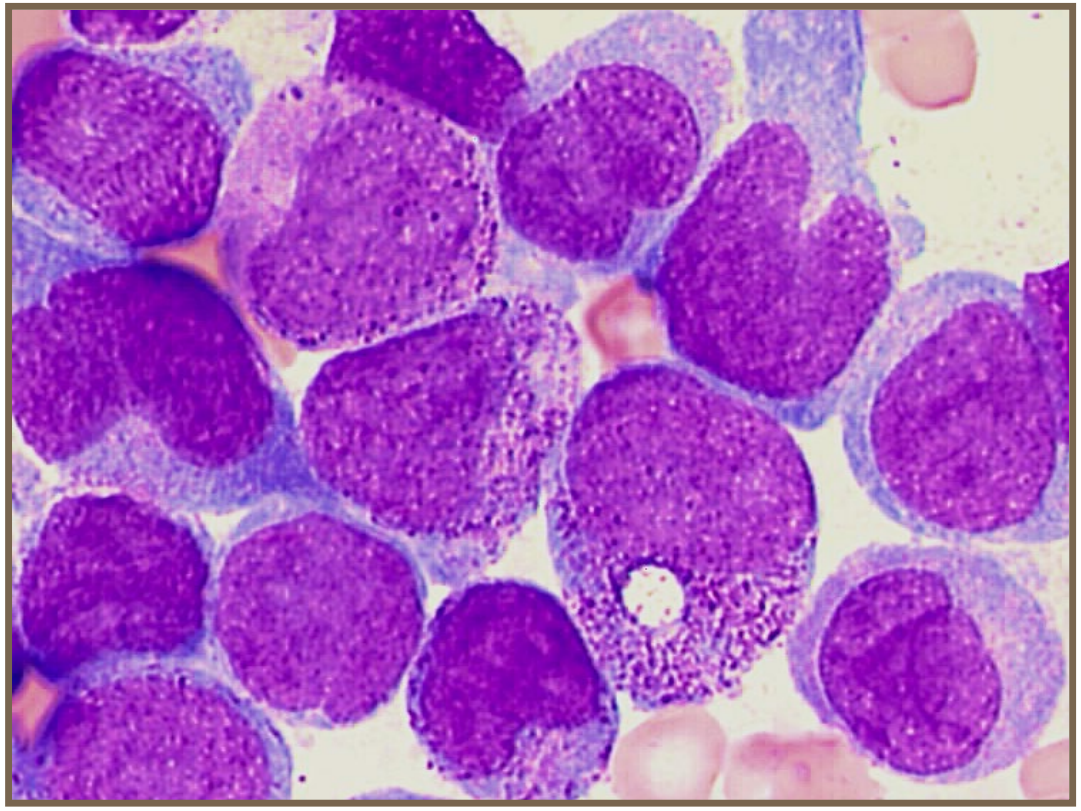
- Skin pallor
- No hemorrhagic diatheses
- Small palpable submandibular lymph nodes < 1 cm
- No organomegaly
- Vital signs: temperature 37.9°C; pulse 100 bpm
- ECOG performance status 0-1

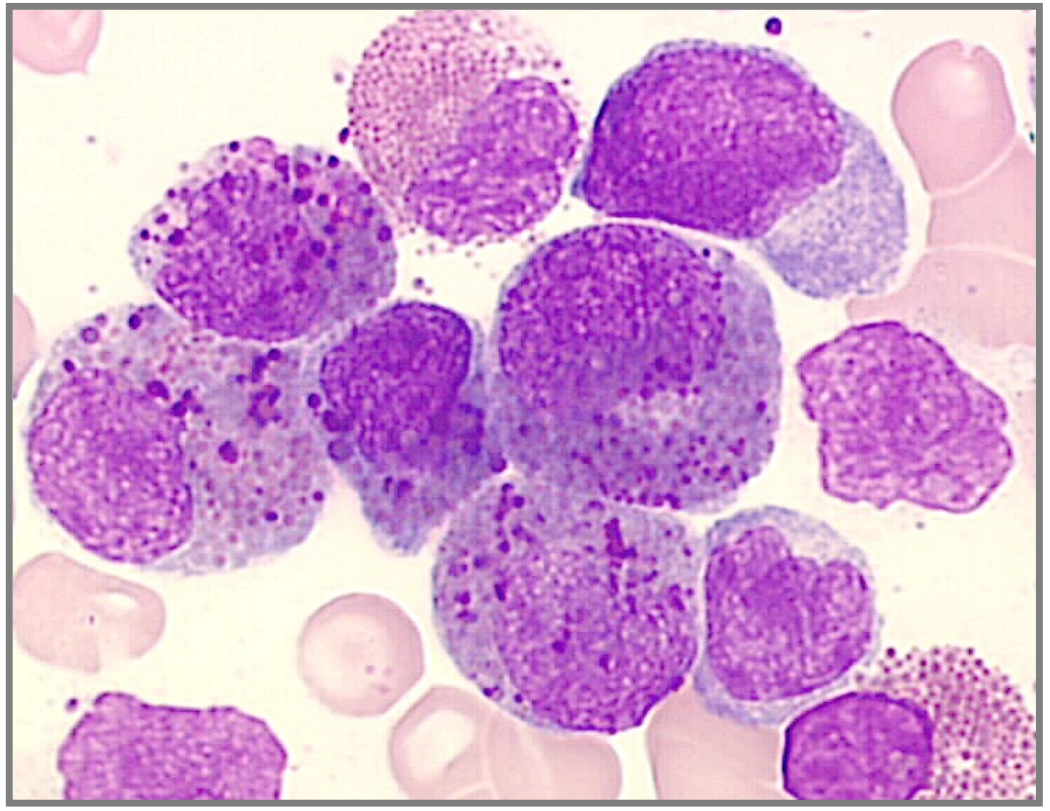
Laboratory Findings

Parameter	Patient	Reference values
WBC	1.56 x 10^9/l	4 - 11
▪ Neutrophils	▪ 0.19 x 10^9/l	1.5 - 7.0
▪ Lymphocytes	▪ 1.27 x 10^9/l	1.5 - 4.0
▪ Monocytes	▪ 0.09 x 10^9/l	<0.8
▪ Eosinophils	▪ 0.01 x 10^9/l	0.04 - 0.4
RBC	3.39 x 10^{12}/l	3.5 - 5.0
Hb	110 g/l	120 - 160
MCV	94 fL	80 - 96
PLT	258 x 10^9/l	150 - 400

Laboratory Chemistry	
ASAT, ALAT, GGT, AP, Bil	Within reference ranges
Creatinine, urea, uric acid	
Albumin, Total protein	
Fe, Transferrin, Vit B12, Folate etc.	

Bone marrow aspirate





- Hypercellular
- 52% myeloid blast-equivalents *
- 11% eosinophils *

Flow cytometry:

- CD45dim; Myeloperoxidase+; CD13+; CD15+; CD33+a
- CD34+; CD38+; CD117+; HLA DR+ /CD56+; CD123+

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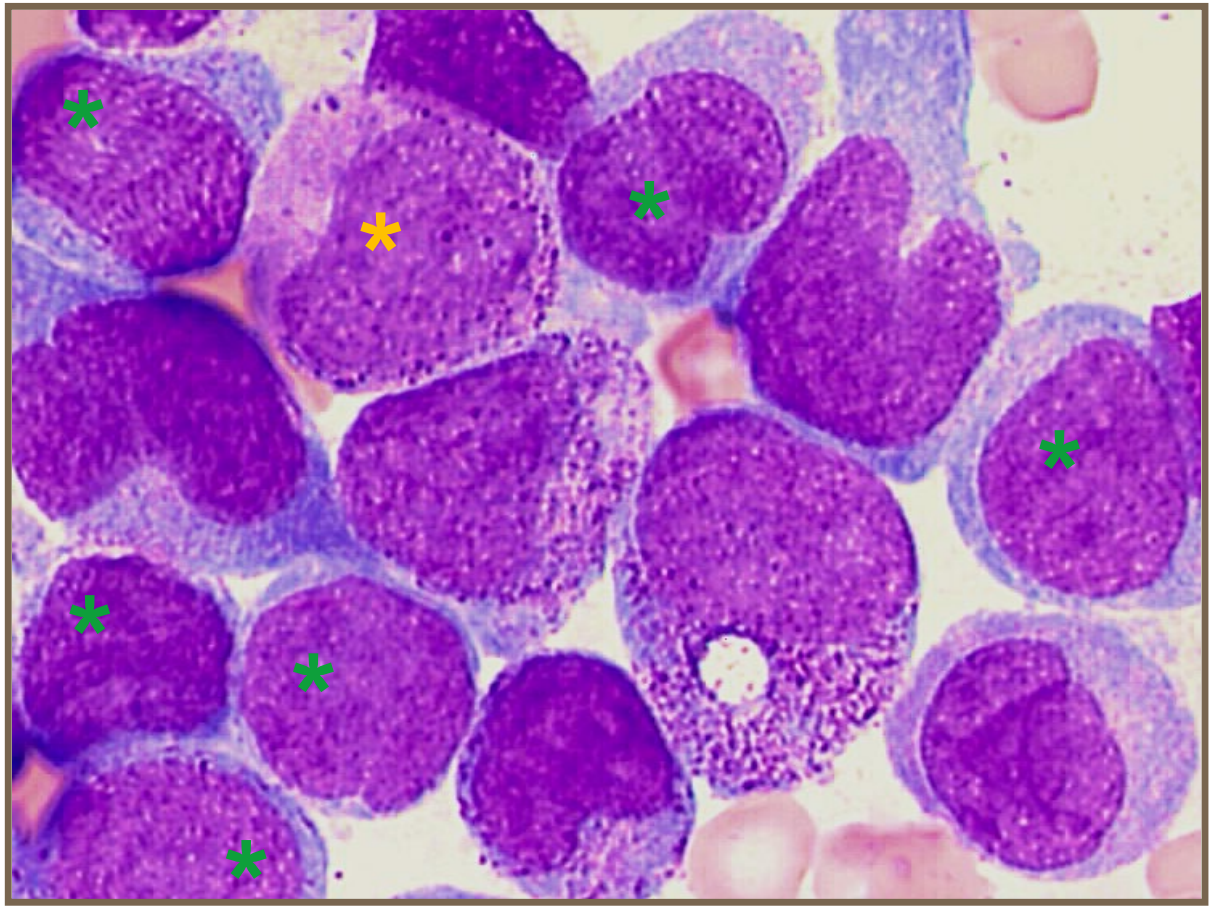
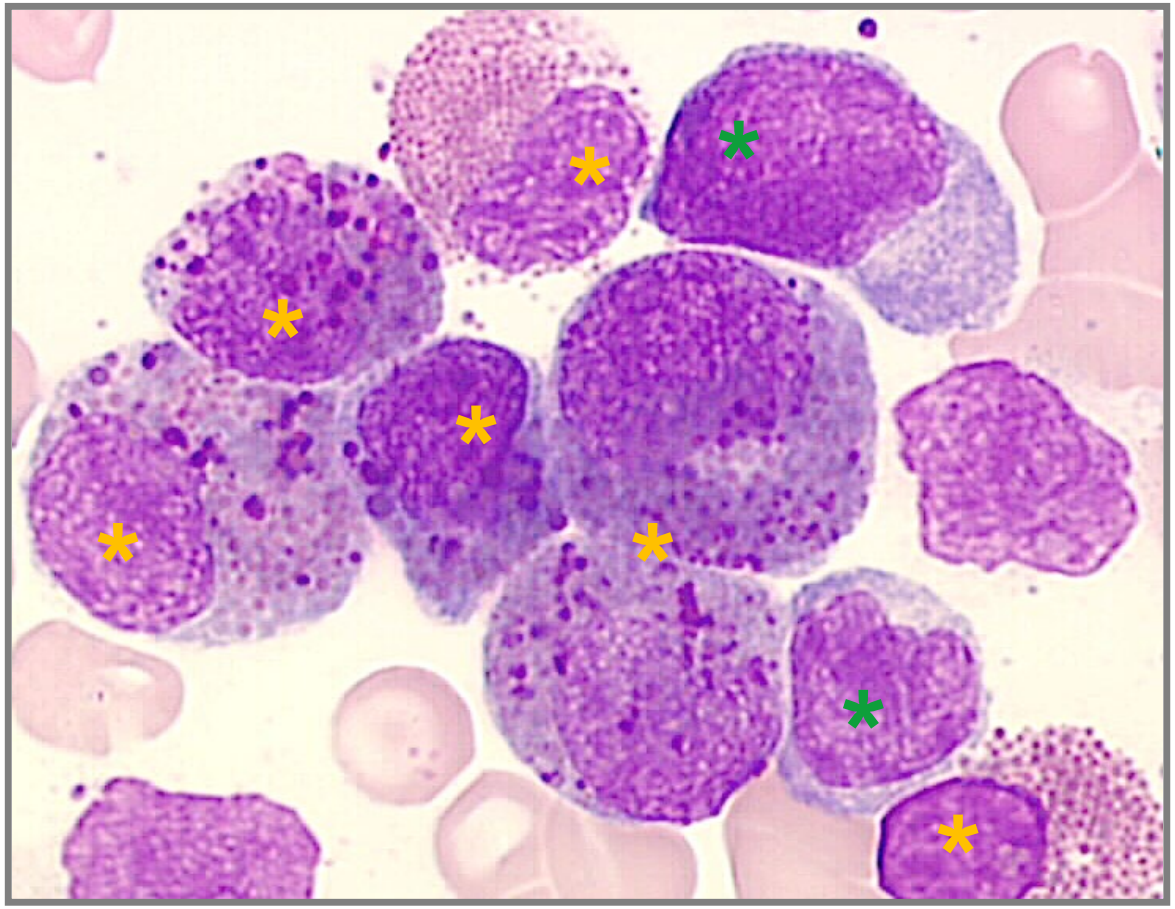
Bone marrow aspirate





- Hypercellular
- 52% myeloid blast-equivalents *
- 11% eosinophils *

Q1. Can we predict the most likely genetic abnormality associated with the morphological findings?

1) t(5;14)(q31.1;q32.1)/ *IGH::IL3*

2) t(7;12)(q22;p13)/ *ETV6* rearranged

3) t(8;21)(q22;q22)/ *RUNX1::RUNX1T1*

4) t(15;17) (q24;q21)/ *PML::RARA*

5) inv(16) (p13;q22)/ *CBFB::MYH11*

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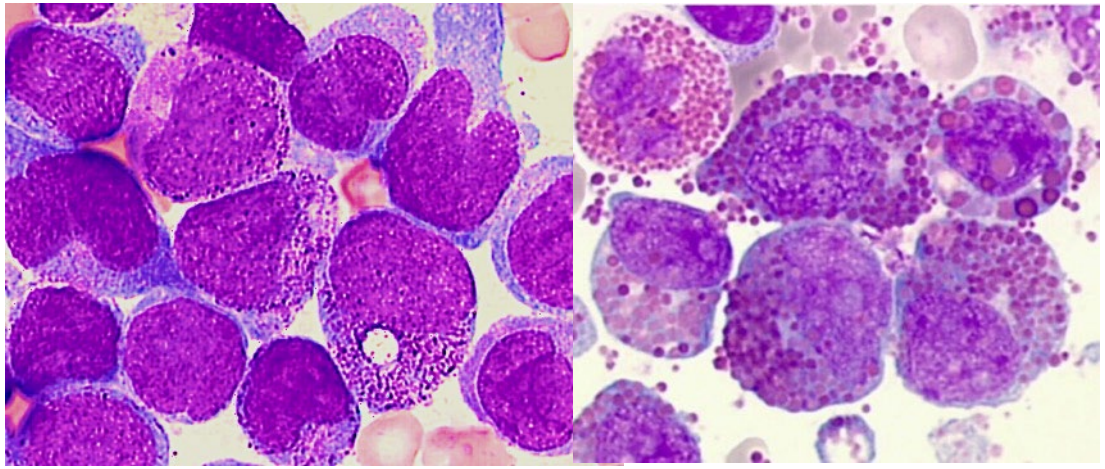
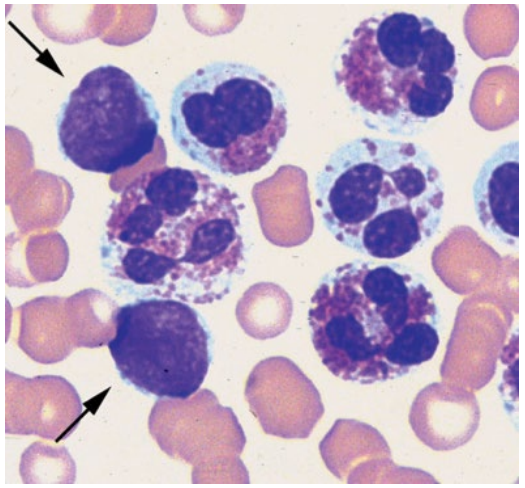
Can we predict genetics from morphology?

ALL

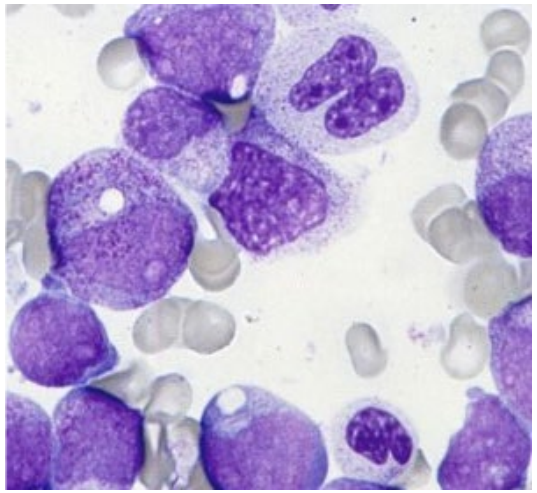


AML: inv(16) /CBFB::MYH11

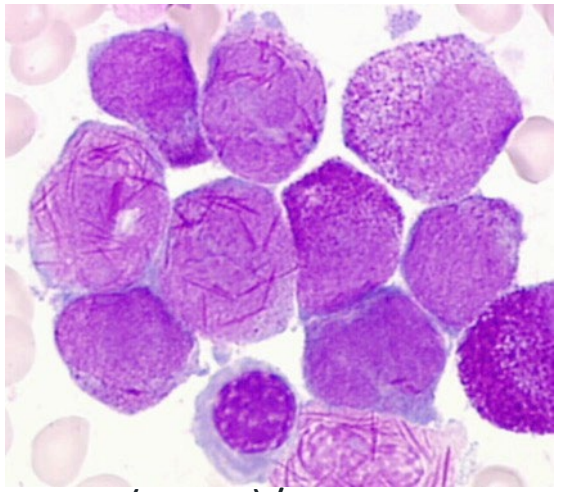


B-ALL-Eo: t(5;14)/IGH::IL3

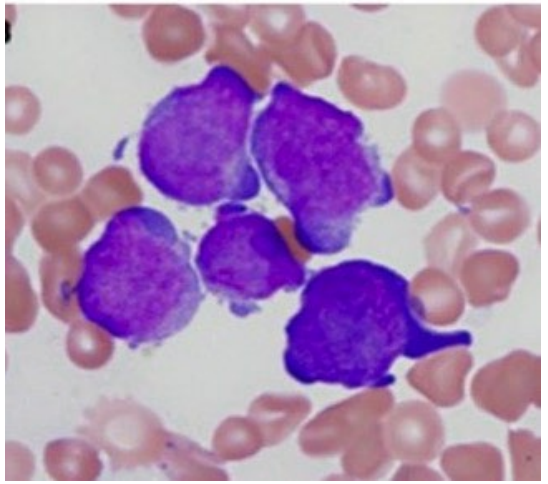
AML



AML: t(8;21)



APL: t(15;17)/PML::RARA



infant AML: t(7;12)/ETV6 rearr

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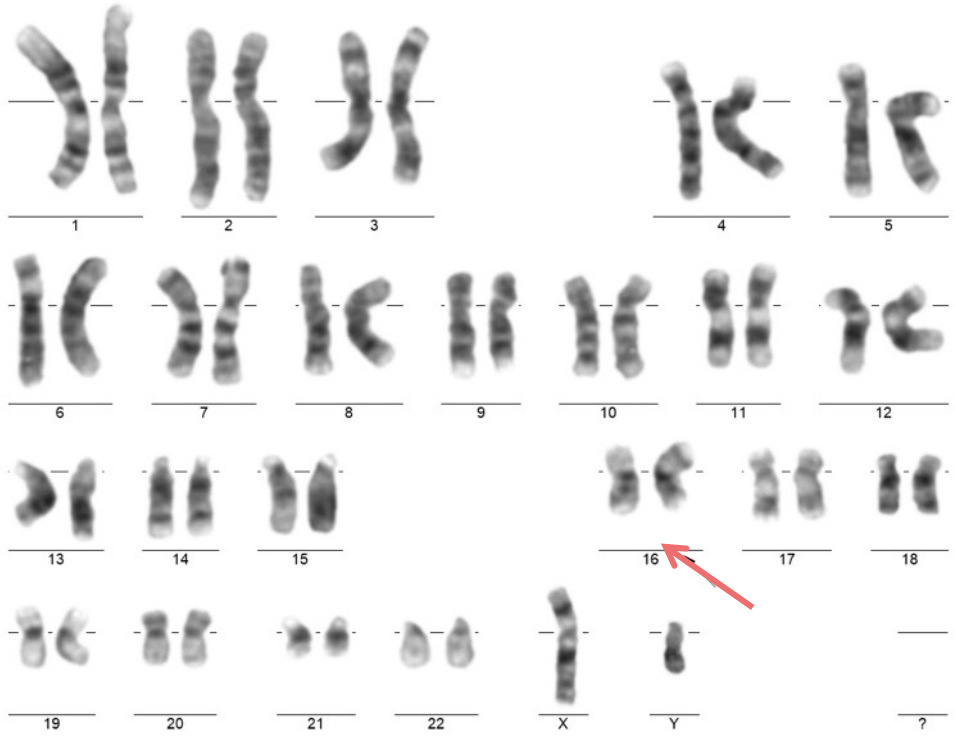
Cytogenetic and molecular findings

Cytogenetics

- 46,XX, **inv(16)(p13.1q22)**[20]



Courtesy prof. G.Balatzenko

Molecular testing by PCR

- *PML::RARA (-) neg*
- *RUNX1::RUNX1T1* (*AML1::ETO*) (-) neg
- ***CBFb::MYH11* (+) pos**
- *FLT3-ITD* (-) neg
- *FLT3-TKD* (-) neg
- *NPM1*^{mut} (-) neg
- *JAK2 V617F* (-) neg
- *BCR::ABL* (-) neg

Molecular testing by NGS

- ***KIT*^{mut} c.1255_1257delGAC [15.3%]**

Q2. What is the diagnosis according to WHO Classification 2022?

1) Acute myeloid leukaemia, myelodysplasia-related
2) Acute myeloid leukaemia defined by differentiation
3) Acute myeloid leukaemia with defining genetic abnormalities
4) Acute myeloid leukaemia with other defined genetic alterations
5) Acute leukaemia of ambiguous lineage with defining genetic abnormalities

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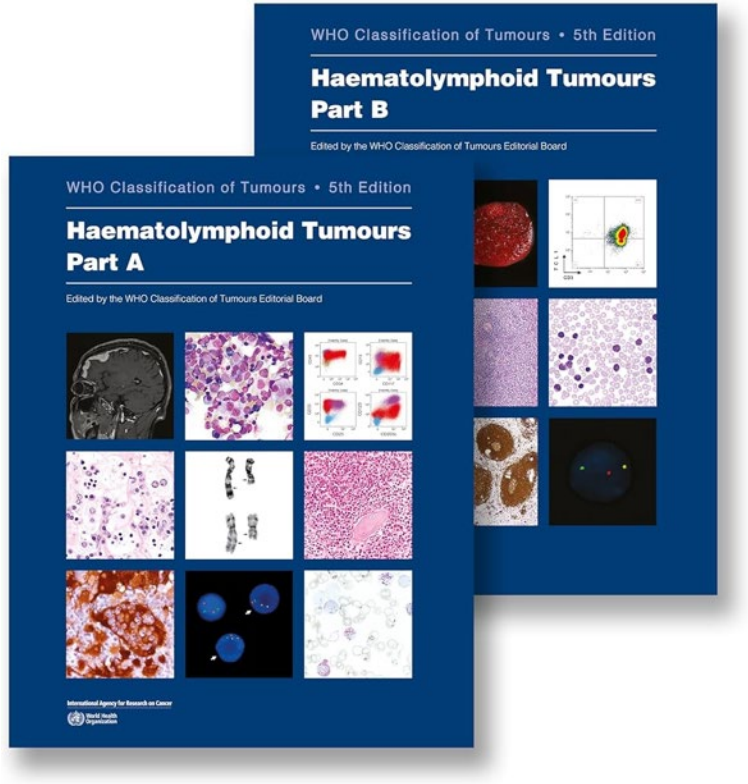
AML in WHO-HAEM5[2022]



Acute myeloid leukaemia

- Acute myeloid leukaemia: Introduction

Acute myeloid leukaemia with defining genetic abnormalities

- Acute promyelocytic leukaemia with PML::RARA fusion
- Acute myeloid leukaemia with RUNX1::RUNX1T1 fusion
- Acute myeloid leukemia with CBFb::MYH11 fusion
- Acute myeloid leukaemia with DEK::NUP214 fusion
- Acute myeloid leukaemia with RBM15::MRTFA fusion
- Acute myeloid leukaemia with BCR::ABL1 fusion
- Acute myeloid leukaemia with KMT2A rearrangement
- Acute myeloid leukaemia with MECOM rearrangement
- Acute myeloid leukaemia with NUP98 rearrangement
- Acute myeloid leukaemia with NPM1 mutation
- Acute myeloid leukaemia with CEBPA mutation
- Acute myeloid leukaemia, myelodysplasia-related
- Acute myeloid leukaemia with other defined genetic alterations
  - 9861/3 Acute myeloid leukaemia with *CBFA2T3*::*GLIS2* fusion
  - 9861/3 Acute myeloid leukaemia with *KAT6A*::*CREBBP* fusion
  - 9861/3 Acute myeloid leukaemia with *FUS*::*ERG* fusion
  - 9861/3 Acute myeloid leukaemia with *MNX1*::*ETV6* fusion
  - 9861/3 Acute myeloid leukaemia with *NPM1*::*MLF1* fusion

Acute myeloid leukaemia defined by differentiation

- Acute myeloid leukaemia with minimal differentiation
- Acute myeloid leukaemia without maturation
- Acute myeloid leukaemia with maturation
- Acute basophilic leukaemia
- Acute myelomonocytic leukaemia
- Acute monocytic leukaemia
- Acute erythroid leukaemia
- Acute megakaryoblastic leukaemia

Myeloid sarcoma

- Myeloid sarcoma

References: WHO Classification of Tumours Editorial Board. Haematolymphoid Tumours. 5th ed. Lyon (France): IARC; 2022. WHO Classification of Tumours Series, Vol. 11.; Khoury *et al.* The 5th edition of the WHO Classification of Haematolymphoid Tumours: Myeloid and Histiocytic/Dendritic Neoplasms. *Leukemia* **36**, 1703–1719 (2022).

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KIT mutations and CBF-AML

AML with ***CBFB::MYH11***

NRAS ~40%
KIT ~35%
FLT3-TKD ~20%
KRAS ~15%

- Somatic mutations are detected in > 90% of cases

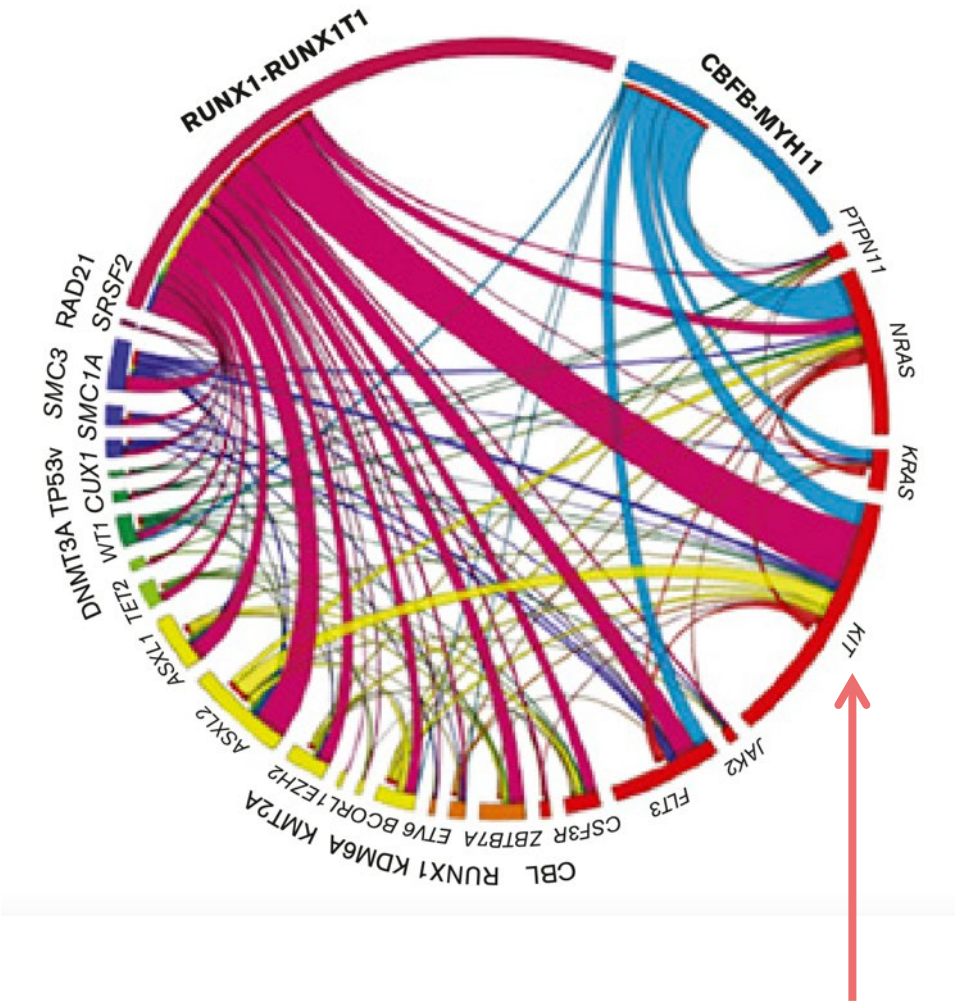
AML with ***RUNX1::RUNX1T1***

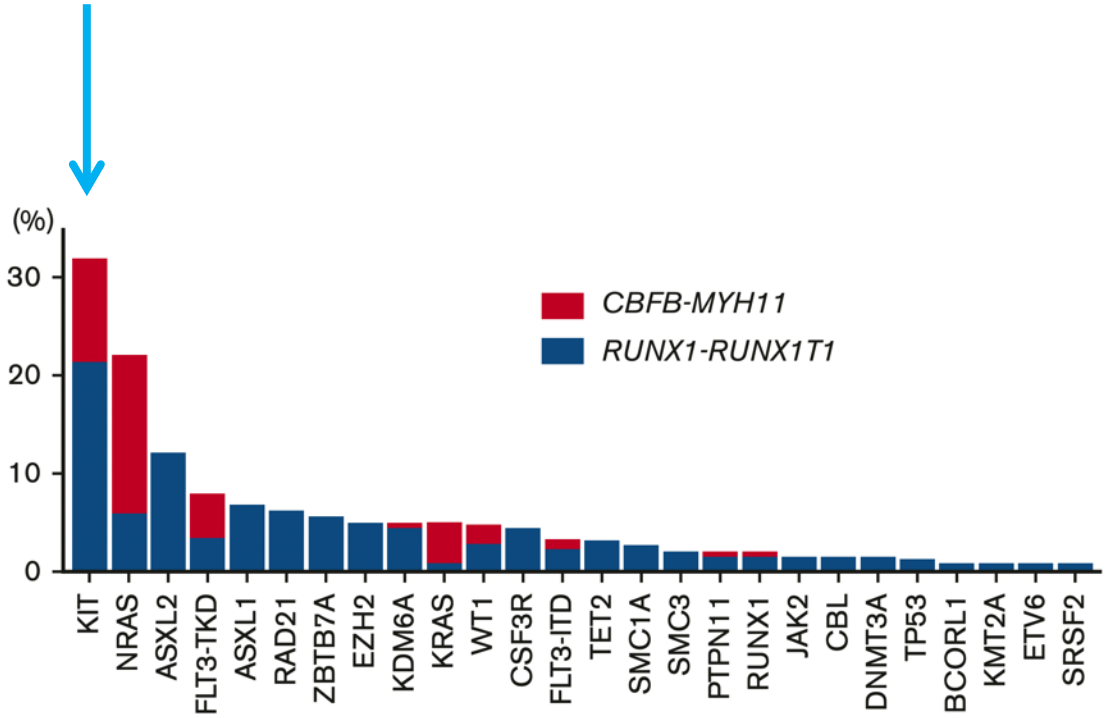
KIT ~25%	*NRAS* ~20%
Cohesin ~20%	*ASXL2* 20%
ZBTB7A ~20%	*ASXL1* ~10%
EZH2 ~5%	*KDM6A* ~5%
MGA ~5%	*DHX15* ~5%

- The most common *KIT* mutations in inv(16) AML occur in exon 17, particularly the D816 codon

Döhner et al Blood. 2017 Jan 26;129(4):424-447.; Paschka et al.. Blood. 2013 Jan 3;121(1):170-7.; Duployez et al.. Blood. 2016 May 19;127(20):2451-9. ; Opatz et al. Leukemia. 2020 Jun;34(6):1553-1562.

Mutation landscape of CBF-AML





- Circos plots illustrate the association of mutated genes in AML with RUNX1::RUNX1T1 or CBFB::MYH11.
- The width of the arches indicates the percentage of mutations.

Ishikawa et al. Blood Adv. 2020 Jan 14;4(1):66-75.

Q3. What is the risk category according to ELN Classification 2022?

1) Very low
2) Favourable
3) Intermediate
4) Adverse
5) Very high

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ELN^{2022} risk classification by

Risk category	Genetic abnormality
Favourable	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 (without adverse-risk genetic lesions)
	t(9;11)(p21.3;q23.3)/MLLT3::KMT2A†,¶
	Cytogenetic and/or molecular abnormalities not classified as favorable or adverse
Abverse	t(6;9)(p23.3;q34.1)/DEK::NUP214
	t(v;11q23.3)/KMT2A-rearranged#
	t(9;22)(q34.1;q11.2)/BCR::ABL1
	t(8;16)(p11.2;p13.3)/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, and/or ZRSR2‡‡
	Mutated TP53a

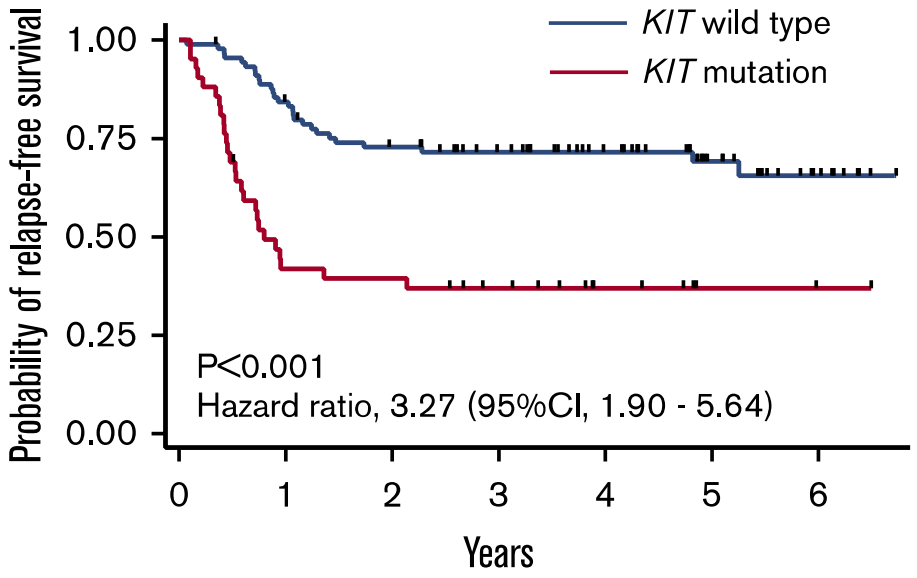
‡Concurrent KIT and/or FLT3 gene mutation does not alter risk categorization.

Döhner et al. Diagnosis and management of AML in adults: 2022 recommendations from an international expert panel on behalf of the ELN. Blood. 2022 Sep 22;140(12):1345-1377.

Prognostic impact of KIT mutations

KIT^{mut} is a poor prognostic factor in AML with RUNX1::RUNX1T1, but not in those with *CBFB::MYH11*

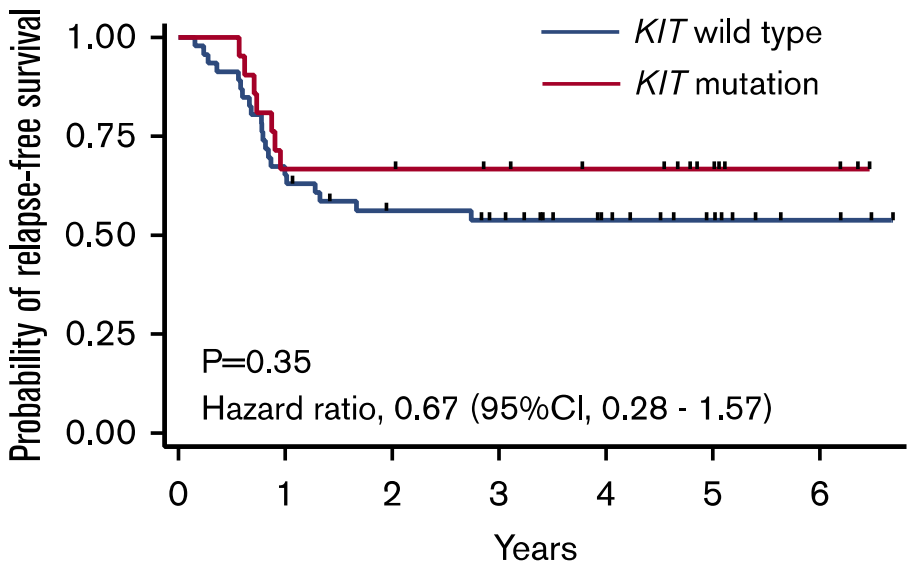
RUNX1-RUNX1T1



No. at risk

wild type	90	74	62	52	40	22	8
mutation	42	17	16	12	6	2	1

CBFB-MYH11



No. at risk

wild type	46	30	23	20	13	8	3
mutation	21	14	14	12	10	5	3

Ishikawa Y, et al. Blood Adv. 2020 Jan 14;4(1):66-75.

Q4. Which would be the most appropriate first-line induction therapy?

1) Daunorubicin or idarubicin and cytarabine «7+3» induction

2) Daunorubicin or idarubicin and cytarabine «7+3» induction + Gemtuzumab ozogamicin

3) Daunorubicin and cytarabine liposomal formulation «CPX-351»

4) Azacitidine or decitabine and venetoclax

5) Fludarabine; cytarabine; idarubicin; G-CSF «FLAG-IDA»

Q4. Which would be the most appropriate first-line therapy?

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Patients fit for intensive therapy

ELN 2022 Recommendations - Induction therapy

Anthracyclines and cytarabine remain the backbone of intensive chemotherapy.

- Daunorubicin or idarubicin and cytarabine «7+3» induction
  - + kinase inhibitor [**midostaurin or quizartinib**] for patients with FLT3-mutant AML.
  - + **Gemtuzumab-ozogamicin** (GO) [humanized anti-CD33 Ab linked to a calicheamicin-based cytotoxic warhead] in favourable genetic risk AML (CBF, $NPM1^{mut}$)
- CPX-351 [dual-drug liposomal formulation of cytarabine/ daunorubicin in a 5:1 fixed molar ratio] in t-AML, a history of MDS or CMML, or de novo AML with myelodysplasia-related cytogenetic abnormalities, 60-75 yrs of age.

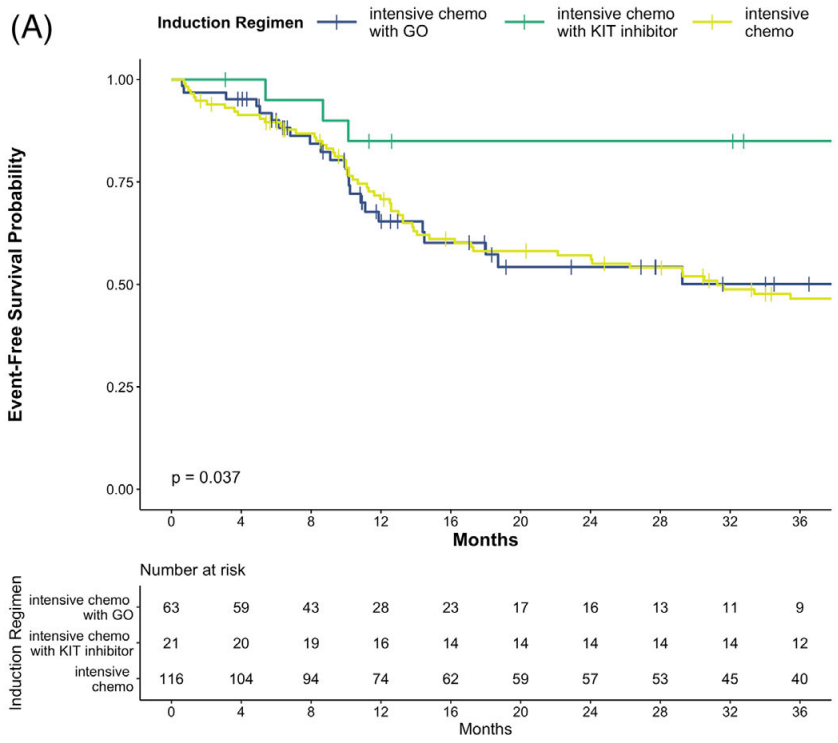
Alternative - fludarabine, cytarabine, G-CSF, and idarubicin (FLAG-IDA) and mitoxantrone-based cytarabine regimens

Döhner et al. Diagnosis and management of AML in adults: 2022 recommendations from an international expert panel on behalf of the ELN. Blood. 2022 Sep 22;140(12):1345-1377.

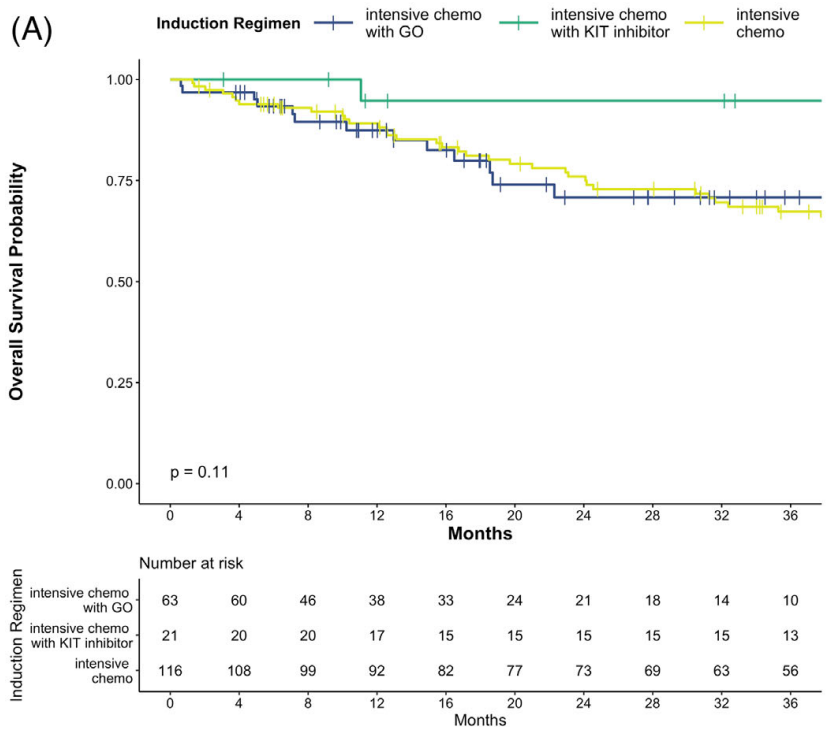
Intensive chemotherapy in CBF-AML

Real-world outcomes

Event-free survival outcomes



Overall survival outcomes



Rojek AE, et al. Real-world outcomes of intensive induction approaches in core binding factor acute myeloid leukemia. EJHaem. 2024 Jul 24;5(4):728-737.

Induction X 2

Daunorubicin 60 mg/m^2 IV d1-3

Cytarabine 200 mg/m^2/d CIV d1-7

GO 3 mg/m2 IV, d1

Consolidation X 3

IDAC 1000 mg/m2 IV

[GO 3 mg/m2 on d1 – C1 and C2]

CRi

BM blasts < 5%

PB no blasts

ANC 2.3 x 10^9/L

PLT 85 X 10^9/L

BM - FCM MRD -neg <0.1%

PB - qPCR MRD-neg

BM - FCM MRD = 0.14%

BM - qPCR MRD= 0.12%

Q5. Which would be the most appropriate next step?

1) Two additional consolidations with IDAC and GO and if MRD-neg stop therapy and follow up

2) Two additional consolidations with IDAC and GO followed by allogeneic HSCT

3) Two additional consolidations with IDAC followed by allogeneic HSCT

4) Consolidation with allogeneic HSCT

5) Send a second sample of bone marrow for qPCR-MRD testing ASAP

Q5. Which would be the most appropriate next step?

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3) Consolidation with allogeneic HSCT

4) Send a second sample of bone marrow for qPCR-MRD testing ASAP

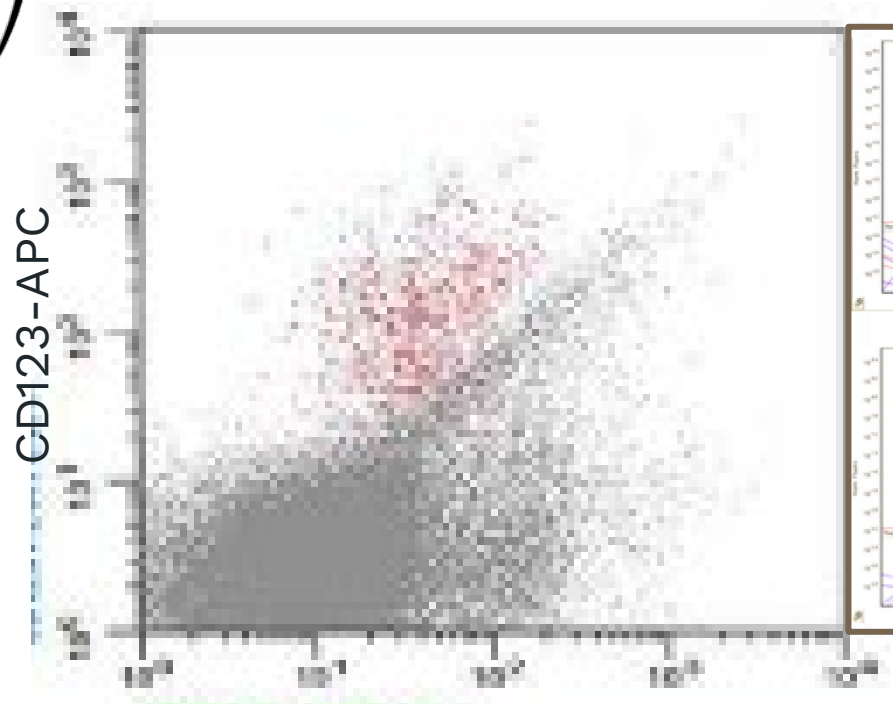
ELN^{2022} response criteria

Category	Definition	Comment
Treatment failure (if including assessment of MRD)§ MRD relapse (after CR, CRh or CRi without MRD)	1. Conversion from MRD negativity to MRD positivity, independent of method, or 2. Increase of MRD copy numbers $\geq$ 1 $\log_{10}$ between any two positive samples in patients with $CR_{MRD\text{-}LL}$, $CRh_{MRD\text{-}LL}$ or $CRi_{MRD\text{-}LL}$ by qPCR The result of 1. or 2. should be rapidly confirmed in a second consecutive sample from the same tissue source	Test methodology, sensitivity of the assay, and cutoff values used must be reported; analyses should be done in experienced laboratories (centralized diagnostics)

Döhner et al. Diagnosis and management of AML in adults: 2022 recommendations from an international expert panel on behalf of the ELN. Blood. 2022 Sep 22;140(12):1345-1377. ; Heuser M, et al. 2021 Update on MRD in acute myeloid leukemia: a consensus document from the European LeukemiaNet MRD Working Party. Blood. 2021 Dec 30;138(26):2753-2767.

FCM-MRD

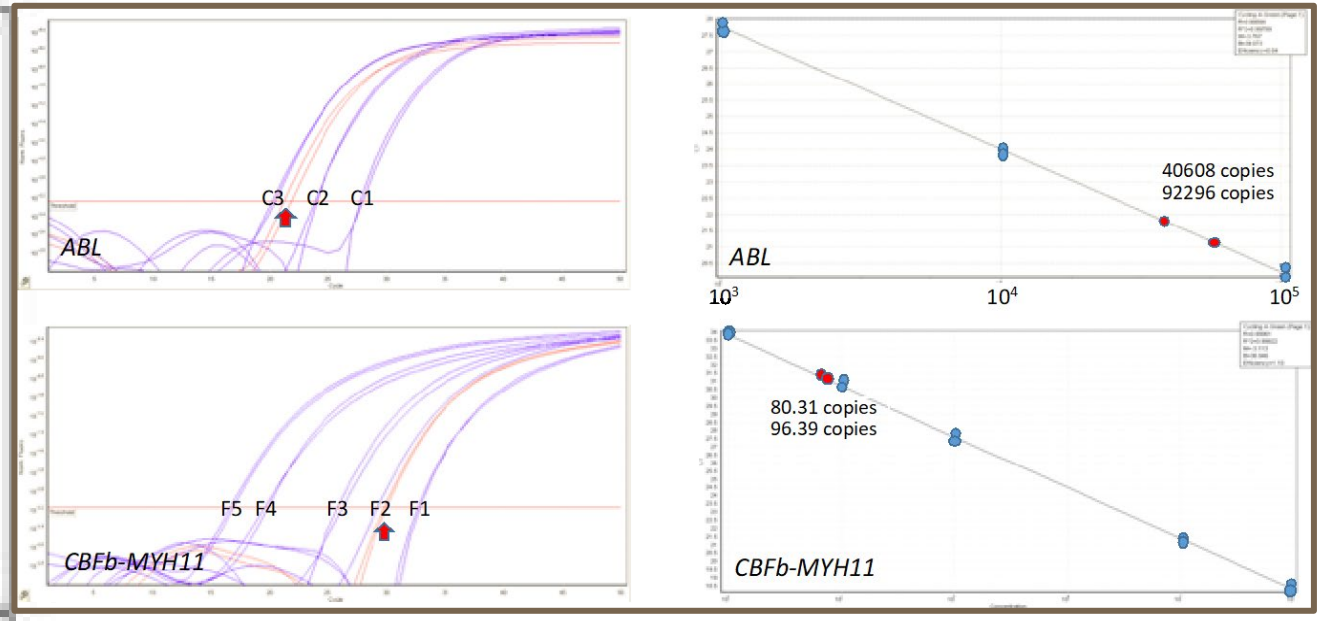
LAIP = 0.11%



Courtesy R.Vladimirova

qPCR-MRD

CBFb-MYH11:ABL = 0.07%



Courtesy prof. G.Balatzen

Q6. Which would be the most appropriate next step?

1) Observation and MRD monitoring

2) Azacitidine maintenance

3) Consolidation with autologous HSCT

4) Consolidation with allogeneic HSCT

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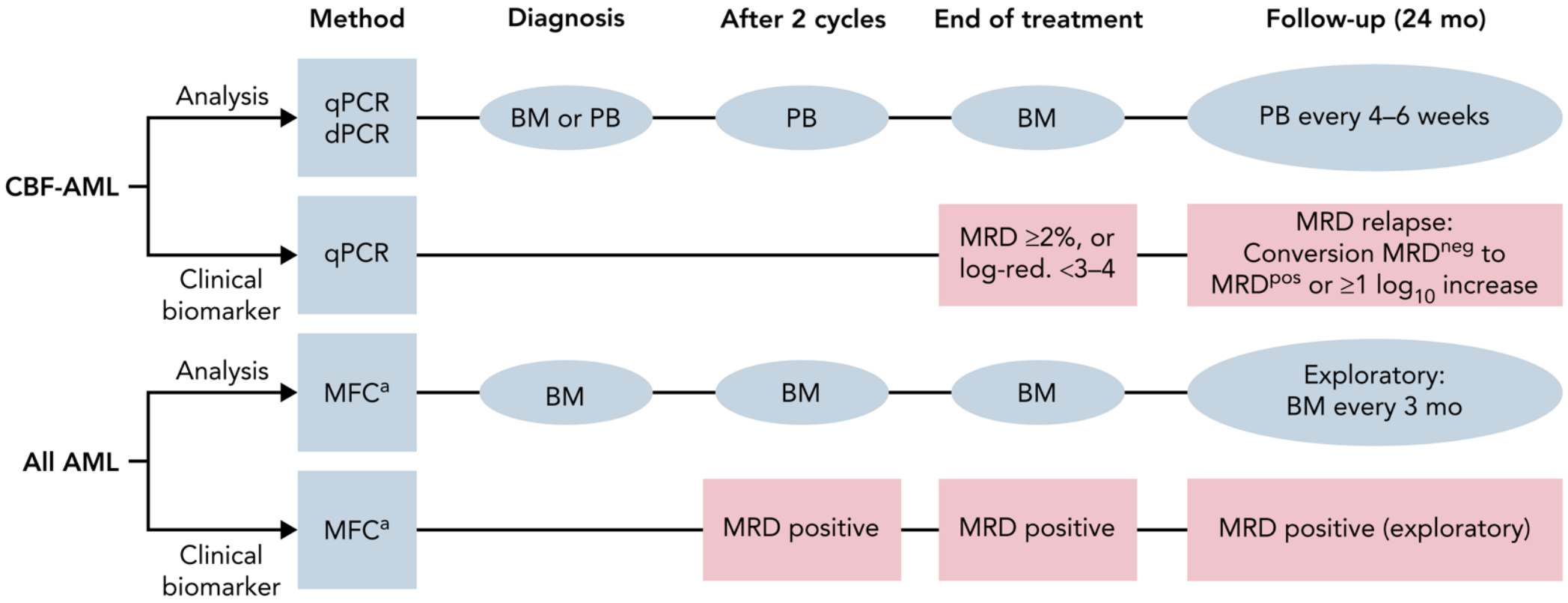
ELN^{2022} algorithm of MRD assessment



In *NPM1*-mutated and **CBF-AML**, CR with molecular MRD detectable at low-level (CR_{MRD-LL}) defined as < 2% is designated as negative for MRD, because when measured at the end of consolidation treatment, is associated with a very low relapse rate.

Döhner et al. Diagnosis and management of AML in adults: 2022 recommendations from an international expert panel on behalf of the ELN. Blood. 2022 Sep 22;140(12):1345-1377.

MRD(+) CBF-AML – still controversial

Chemotherapy	Allo- HSCT
Pros:	Pros:
▪ Achieves high complete remission (CR) rates of around 90% with standard induction therapy ▪ Allows for consolidation with high-dose cytarabine (HDAC), which can help deepen responses ▪ Avoids the risks associated with allo-SCT, such as transplant-related mortality	▪ Significantly reduces relapse risk and improves survival in MRD(+) patients compared to CT alone ▪ Provides a potent graft-versus-leukemia effect that can eradicate residual disease ▪ Recommended for patients with suboptimal MRD response to initial therapies
Cons:	Cons:
▪ Patients with suboptimal MRD response (< 3-log reduction) have high relapse rates of up to 79% with CT alone ▪ Survival is significantly inferior compared to allo-SCT in MRD-positive patients ▪ Additional therapies like HMAs may be needed to convert MRD (+) to (-), but efficacy is limited	▪ Associated with transplant-related mortality and complications, especially in older patients or those with poor performance status ▪ Patients with high MRD levels prior to transplant have inferior outcomes ▪ Requires finding a suitable donor and managing post-transplant complications

Borthakur G, Kantarjian H. Blood Cancer J. 2021 Jun 16;11(6):114; Halaburda K, et al. Haematologica. 2020 Jun;105(6):1723-1730; Al Hamed R, et al. Allogeneic SCT in de novo CBF AML in first complete remission: data from the EBMT. Bone Marrow Transplant. 2024. doi: 10.1038/s41409-024-02373-5.

Allo-SCT in de novo CBF-AML in CR1
Retrospective, multi-national, EBMT-based study

N= 1901 pts [34.4% inv(16)] ASCT vs Allo-SCT = 23% : 77%

- allo-SCT was an independent and significant, negative predictor of non-relapse mortality (NRM) and OS (HR 4.26, $p < 0.0001$ and HR 1.67, $p = 0.003$)
- allo-SCT from matched sibling donors had the best outcomes, comparable to ASCT
- NRM was worse in the allo-SCT group both in MRD(-): 12.9% vs 5.2%, $p = 0.007$; and MRD(+): 10.6% vs 0%, $p = 0.004$.

In conclusion:

- consolidation in CR1 with allo-SCT results in worse outcomes than ASCT.
- whether consolidation with ASCT yields better outcomes than CT alone or CT+GO is yet to be investigated.

33 Al Hamed R, et al. Allogeneic stem cell transplantation in de novo core-binding factor acute myeloid leukemia in first complete remission: data from the EBMT. Bone Marrow Transplant. 2024 Aug 2.

In conclusion

inv(16) (p13q22)/t(16;16)/ *CBFB::MYH11* AML represents a unique subset of AML with specific treatment challenges and monitoring requirements.

- CBFB::MYH11 AML demonstrates a diverse pattern of cooperating molecular events
- CBFB::MYH11 AML is considered a good-risk AML in the context of cytarabine based intensive chemotherapy

- Still, outcome can be improved significantly through risk-stratification, effective implementation of available therapeutic measures and appropriate disease monitoring.

References:

1. WHO Classification of Tumours Editorial Board. Haematolymphoid Tumours. 5th ed. Lyon (France): IARC; 2022. WHO Classification of Tumours Series, Vol. 11.
2. Khoury JD, et al. The 5th edition of the World Health Organization Classification of Haematolymphoid Tumours: Myeloid and Histiocytic/Dendritic Neoplasms. Leukemia. 2022 Jul;36(7):1703-1719.
3. Döhner H, et al. Diagnosis and management of AML in adults: 2022 recommendations from an international expert panel on behalf of the ELN. Blood;140(12):1345-1377.
4. Rojek AE, et al. Real-world outcomes of intensive induction approaches in core binding factor acute myeloid leukemia. EJHaem. 2024;5(4):728-737.
5. Heuser M, et al. 2021 Update on MRD in acute myeloid leukemia: a consensus document from the European LeukemiaNet MRD Working Party. Blood. 2021;138(26):2753-2767.
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7. Paschka P, et al. Secondary genetic lesions in acute myeloid leukemia with inv(16) or t(16;16): a study of the German-Austrian AML Study Group (AMLSG). Blood. 2013;121(1):170-7.
8. Duployez N, et al. Comprehensive mutational profiling of core binding factor acute myeloid leukemia. Blood. 2016;127(20):2451-9.
9. Döhner H, et al. Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel. Blood. 2017;129(4):424-447.
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11. Opatz S, et al. The clinical mutatome of core binding factor leukemia. Leukemia. 2020;34(6):1553-1562.
12. Halaburda K, et al. Allogeneic stem cell transplantation in second complete remission for core binding factor acute myeloid leukemia: a study from the Acute Leukemia Working Party of the European Society for Blood and Marrow Transplantation. Haematologica. 2020;105(6):1723-1730.
13. Borthakur G, Kantarjian H. Core binding factor acute myelogenous leukemia-2021 treatment algorithm. Blood Cancer J. 2021;11(6):114.

EHA-GBMTA-AHA Hematology Tutorial: New aspects in diagnostic choices and treatment options of hematological malignancies

Self-assessment case

Session: Acute Myeloid Leukemia

20th October 2024, Margarita Guenova

Clinical history

A 57-years old man was referred to the Hematology Hospital because of:

- Fatigue and weakness for > 1 week
- Fever > 38^0C for >3-4 days
- Large hematoma on the left thigh >10 cm and several smaller subcutaneous in other area
- Hemoptoe for the last 2 days

Medical history:

- Profession - teacher
- No history of chemical/physical agents exposure
- No previous diseases/drug exposure
- Unremarkable family history

Physical examination

- Skin pallor
- Hematoma on the left thigh 10-12 cm
- Multiple subcutaneous hemorrhages in the abdominal area 1-2 cm
- No organomegaly
- Vital signs: temperature 38.5°C; pulse 100 bpm
- ECOG performance status 1

Laboratory Findings

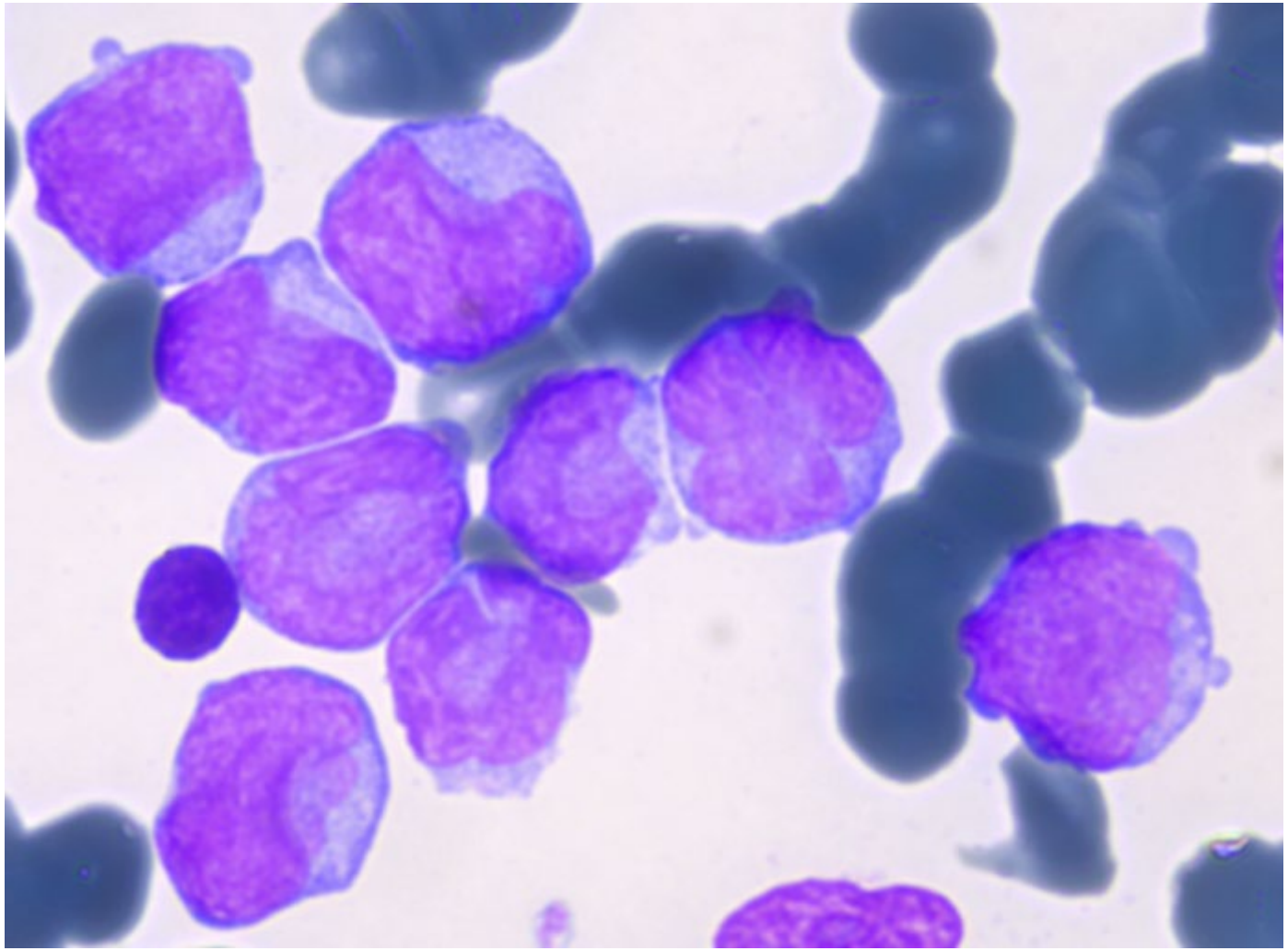
Parameter	Values	Reference values
WBC	109 x 10^9/l	4 – 11 x 10^9/l
▪ Neutrophils	▪ 2%	45 -0 73 %
▪ Lymphocytes	▪ 3%	22 – 40 %
▪ Monocytes	▪ 2%	0.7 - 7.0 %
▪ Blast cells	▪ 93%	-
Hb	108 g/l	130 – 165 g/l
MCV	91 fL	80 – 96 fL
PLT	42 x 10^9/l	150 – 400 x 10^9/l

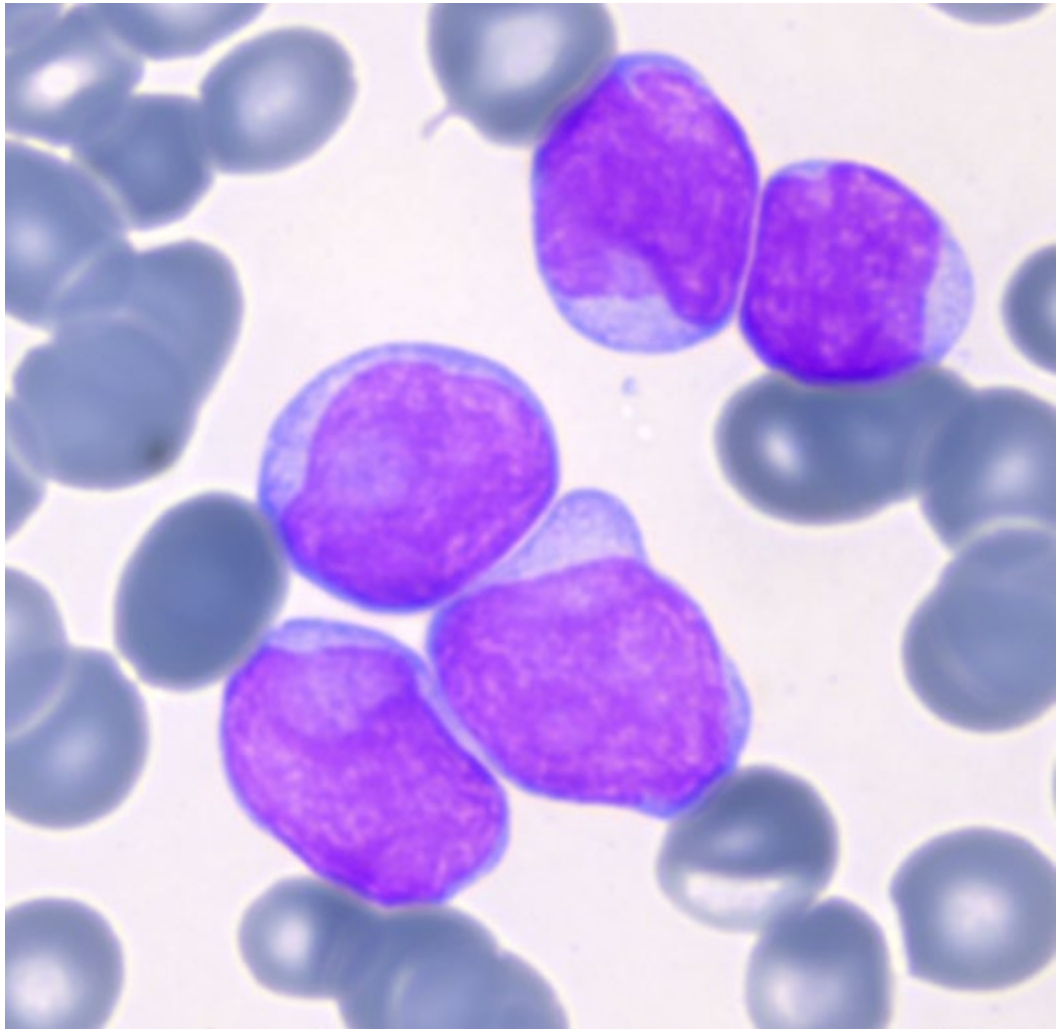
Laboratory Chemistry	Values	Reference values
AST	69 U/l	1-31 U/l
GGT	185 U/l	<50 U/l
LDH	1250 U/l	<250 U/l

Laboratory findings (2

Coagulation panel	Values	Reference values
Prothrombin time (PT)	**17.2 sec**	11.5–15.5 sec
International normalized ratio (INR)	**1.6**	<1.2
Fibrinogen	**0.8 g/l**	1.8 – 5.0 g/l
Activated partial thromboplastin time (aPTT)	30 sec	30-40 sec
D-dimer	**9 mg/l**	<0.5 mg/L

Peripheral blood





Q1. How would you describe the most probable nature of the leukemic neoplastic cells?

1) Immature myeloid
2) Immature monocytic
3) Megakaryoblastic
4) Precursor lymphoid
5) Large cell lymphoma

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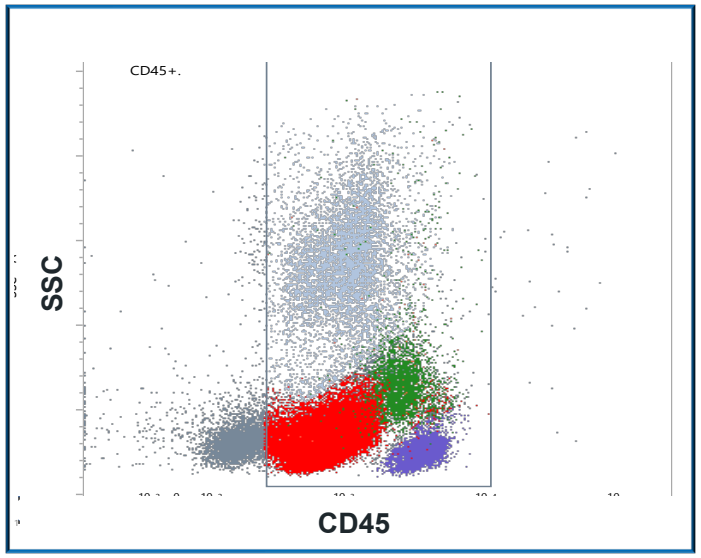
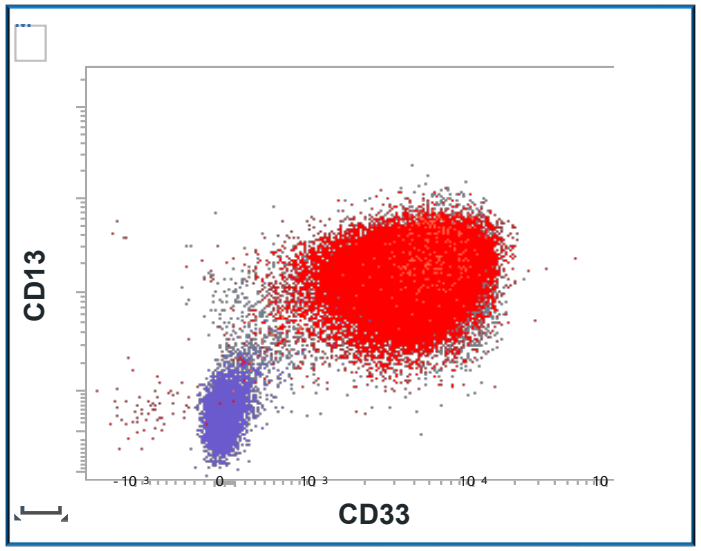
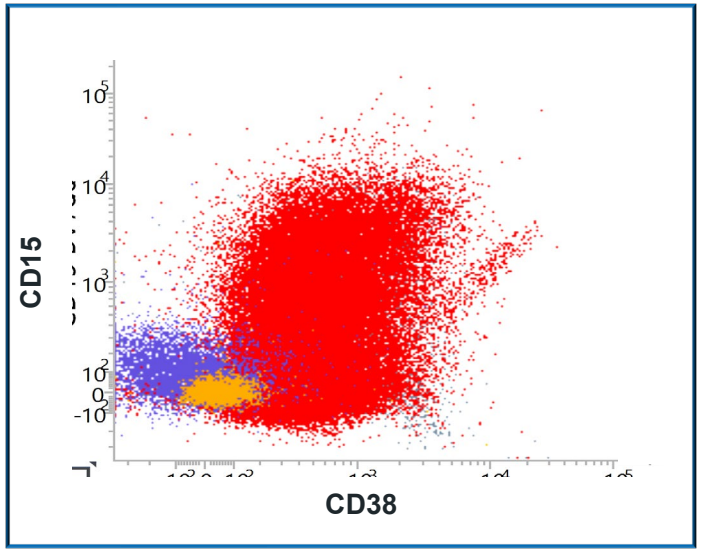
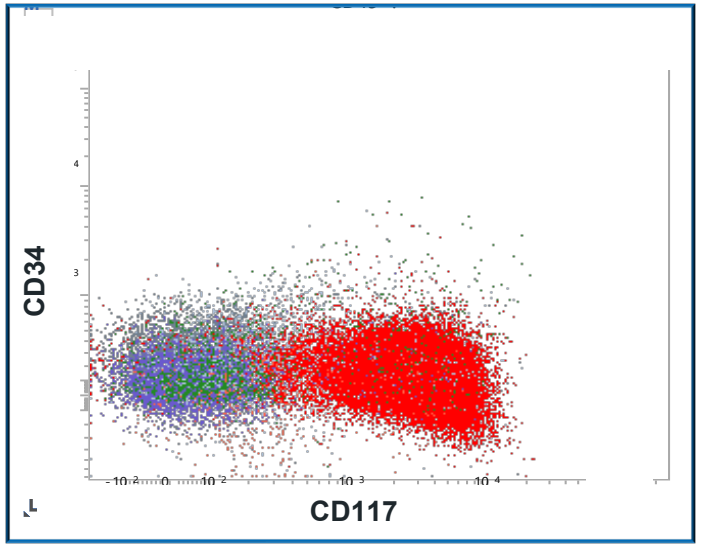
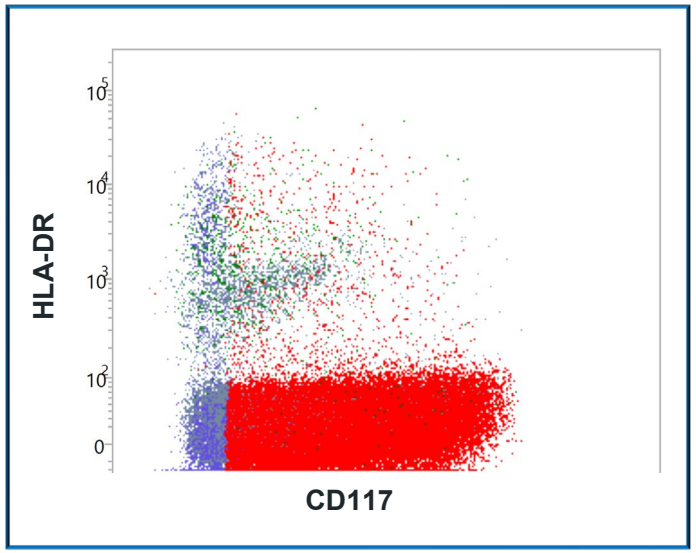
Flowcytometry

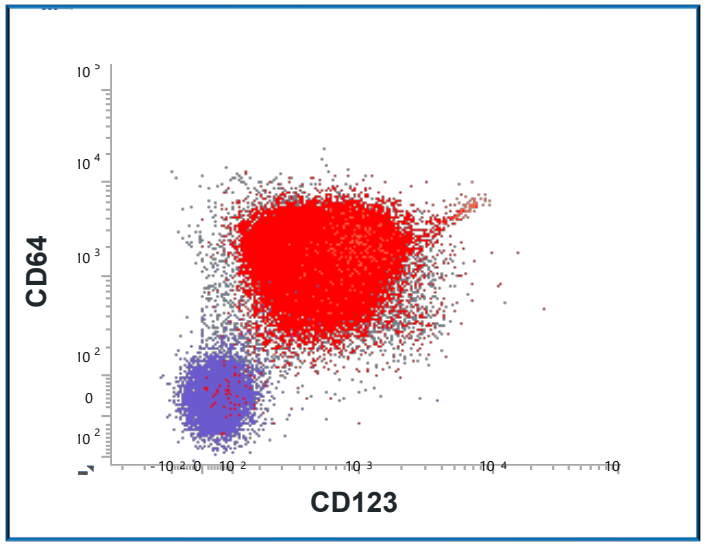












eha

Q2. At this point can we predict the most likely genetic abnormality?

1) *PML::RARA*
2) *RUNX1::RUNX1T1*
3) *KMT2A* rearrangement
4) *NPM1*^{mut}
5) *CEBPA*^{mut}

Q2. At this point can we predict the most likely genetic abnormality?

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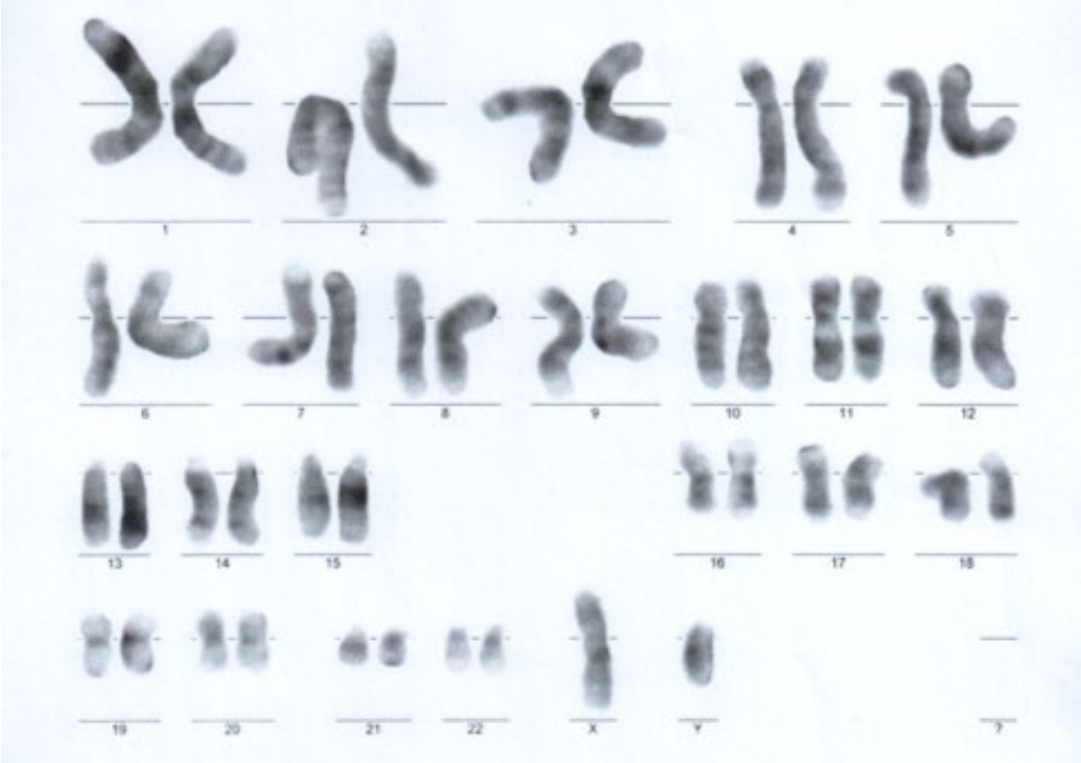
5) *CEBPA*^{mut}

Cytogenetic and molecular findings

Cytogenetics

- 46,XY [20]



Courtesy Svetlana Angelova

Molecular testing by PCR

- *RUNX1::RUNX1T1* (*AML1::ETO*) (-) neg
- *CBFb::MYH11* (-) neg
- *PML::RARA* (-) neg
- ***FLT3-ITD* (+) pos**
- *FLT3-TKD* (-) neg
- ***NPM1*^{mut} (+) pos**
- *IDH1/IDH2* (-) neg
- *JAK2 V617F* (-) neg
- *BCR::ABL* (-) neg

Q3. How do you classify the disease according to WHO-HAEM5^{2022}?

1) Acute myeloid leukaemia with minimal differentiation
2) Acute promyelocytic leukemia
3) Acute myeloid leukaemia, myelodysplasia-related
4) Acute myeloid leukaemia with *NPM1* mutation
5) Acute myeloid leukaemia with other defined genetic alterations

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5) Acute myeloid leukaemia with other defined genetic alterations

The final diagnosis is

- Acute myeloid leukaemia
  - Acute myeloid leukaemia: Introduction
  - *Acute myeloid leukaemia with defining genetic abnormalities*
    - Acute promyelocytic leukaemia with PML::RARA fusion
    - Acute myeloid leukaemia with RUNX1::RUNX1T1 fusion
    - Acute myeloid leukaemia with CBFB::MYH11 fusion
    - Acute myeloid leukaemia with DEK::NUP214 fusion
    - Acute myeloid leukaemia with RBM15::MRTFA fusion
    - Acute myeloid leukaemia with BCR::ABL1 fusion
    - Acute myeloid leukaemia with KMT2A rearrangement
    - Acute myeloid leukaemia with MECOM rearrangement
    - Acute myeloid leukaemia with NUP98 rearrangement
    - **Acute myeloid leukemia with NPM1 mutation**
    - Acute myeloid leukaemia with CEBPA mutation
    - Acute myeloid leukaemia, myelodysplasia-related
    - Acute myeloid leukaemia with other defined genetic alterations
  - *Acute myeloid leukaemia defined by differentiation*
    - Acute myeloid leukaemia with minimal differentiation
    - Acute myeloid leukaemia without maturation
    - Acute myeloid leukaemia with maturation
    - Acute basophilic leukaemia
    - Acute myelomonocytic leukaemia
    - Acute monocytic leukaemia
    - Acute erythroid leukaemia
    - Acute megakaryoblastic leukaemia
  - *Myeloid sarcoma*
    - Myeloid sarcoma

ICD-11 coding

2A60.0 & XH74W8 Acute myeloid leukaemia with recurrent genetic abnormalities & Acute myeloid leukaemia with mutated *NPM1*

References: WHO Classification of Tumours Editorial Board. Haematolymphoid Tumours. 5th ed. Lyon (France): IARC; 2022. WHO Classification of Tumours Series, Vol. 11.; Khoury *et al.* The 5th edition of the WHO Classification of Haematolymphoid Tumours: Myeloid and Histiocytic/Dendritic Neoplasms. *Leukemia* **36**, 1703–1719 (2022).

eha

Acute myeloid leukemia with *NPM1*^{mut}

Morphology:

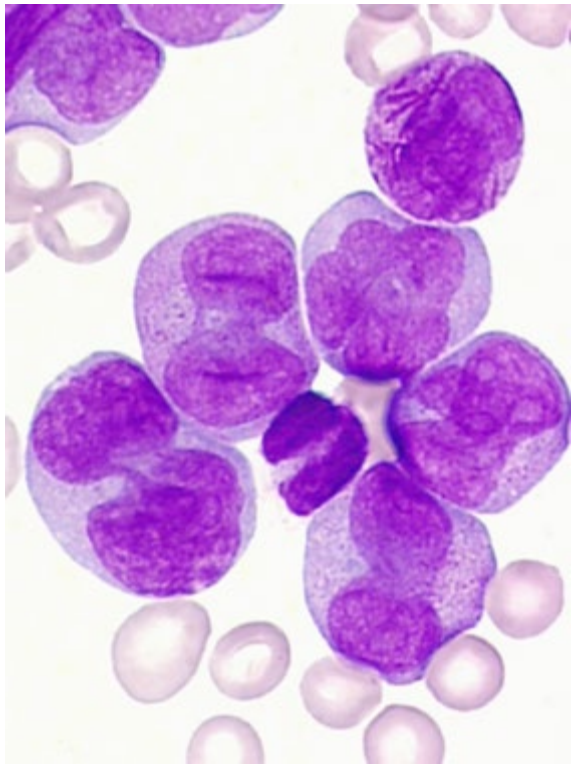
- Identification of cup-like morphology in > 10% of blasts is highly specific for AML with *NPM1* mutation
- Cup-like nuclear morphology is strongly associated with *NPM1*^{mut} + *FLT3-ITD*

Immunophenotype:

- About 80% of cases have an absence of CD34 expression
- CD33, KIT (CD117), and CD123 expression is common
- Three immunophenotypic categories include:
  - predominance of immature myeloid blasts (CD34+ or CD34-, CD117+; HLA-DR+),
  - acute promyelocytic leukaemia-like features (CD34–, HLA-DR–, CD117+),
  - predominance of myelomonocytic/monocytic differentiation (CD14+, CD36+, CD64+)

WHO Classification of Tumours Editorial Board. Haematolymphoid Tumours. 5th ed. Lyon (France): IARC; 2022. WHO Classification of Tumours Series, Vol. 11.; Nomdedeu et al. Leuk Res. 2011 Feb;35(2):163-8.; Loghavi S, et al. Br J Haematol. 2021 Mar;192(6):1054-1063.; Chen et al. Cancer. 2009 Dec 1;115(23):5481-9.

APL-like *NPM1*^{mut} AML

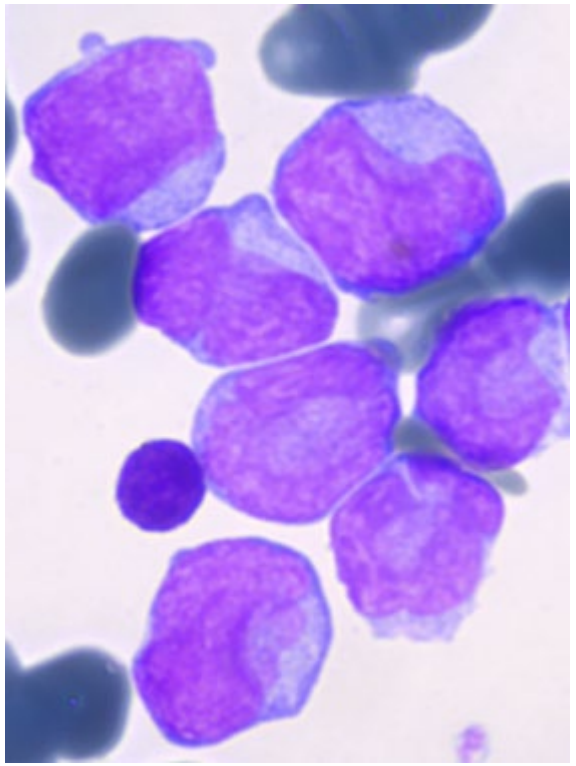


(1) cup-like morphology in >5–10% of blasts

(2) immunophenotype, mostly HLA-DR(-)CD34(-)

(3) normal karyotype

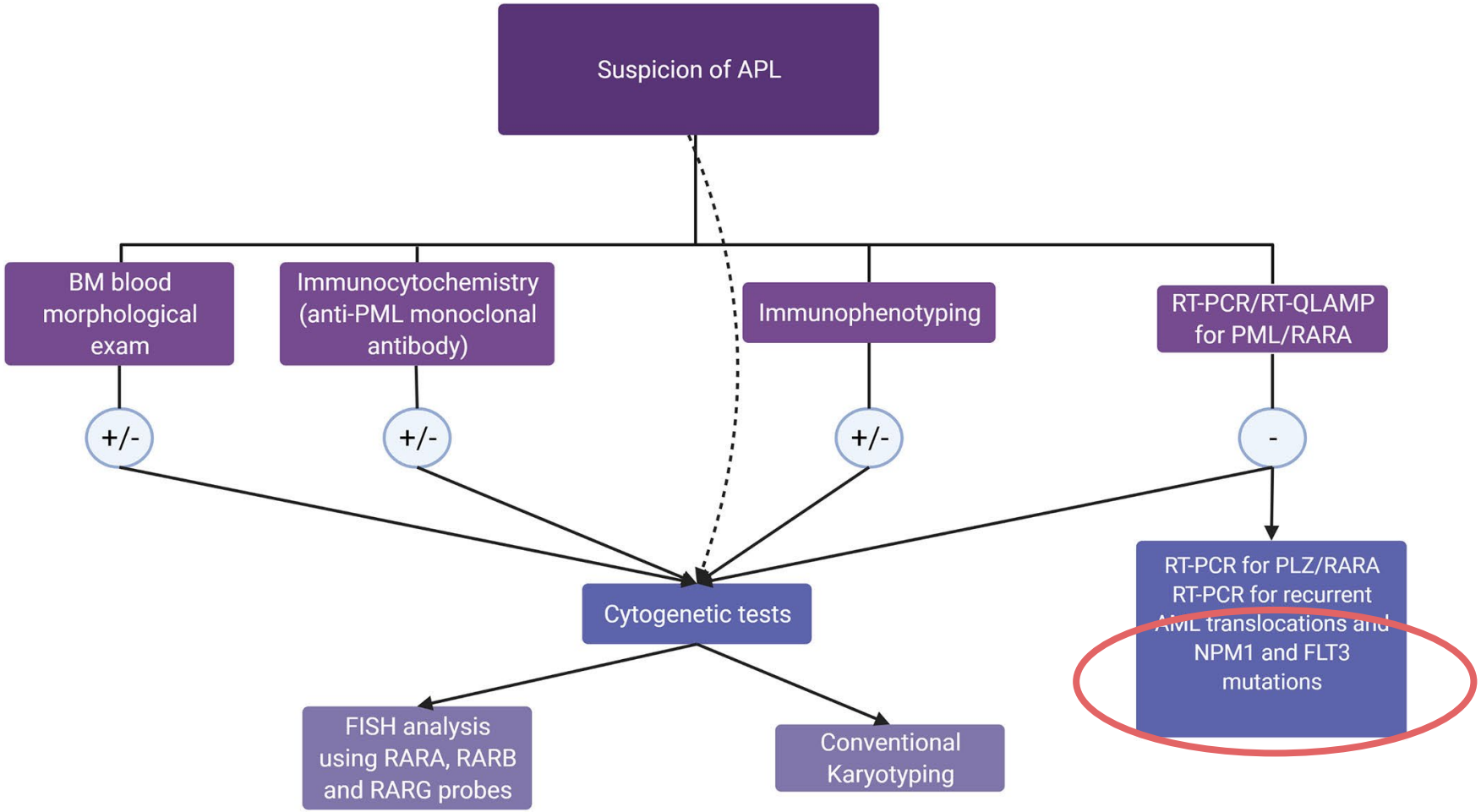
(4) clinical parameters:
- high number of bone marrow blasts
- high WBC counts
- high D-dimer levels



APL_{var} ⟷ **$NPM1^{mut}$ AML**

Arana Rosainz MJ, et al. Int J Lab Hematol. 2021 Apr;43(2):218-226.; Sun J, et al. Anticancer Drugs. 2022 Jan 1;33(1):e813-e817. ; Jalal S, et al. Br J Haematol. 2010 Jan;148(2):182.; Pepper M, Tan B. Blood. 2020 Sep 17;136(12):1467.; Chen et al. Cancer. 2009 Dec 1;115(23):5481-9.

Diagnostic algorithm in the suspicion of APL/APL-like AML



Sanz MA, et al. Blood. 2019 Apr 11;133(15):1630-1643.; Guarnera L, et al. Front Oncol. 2022 Apr 12;12:871590.

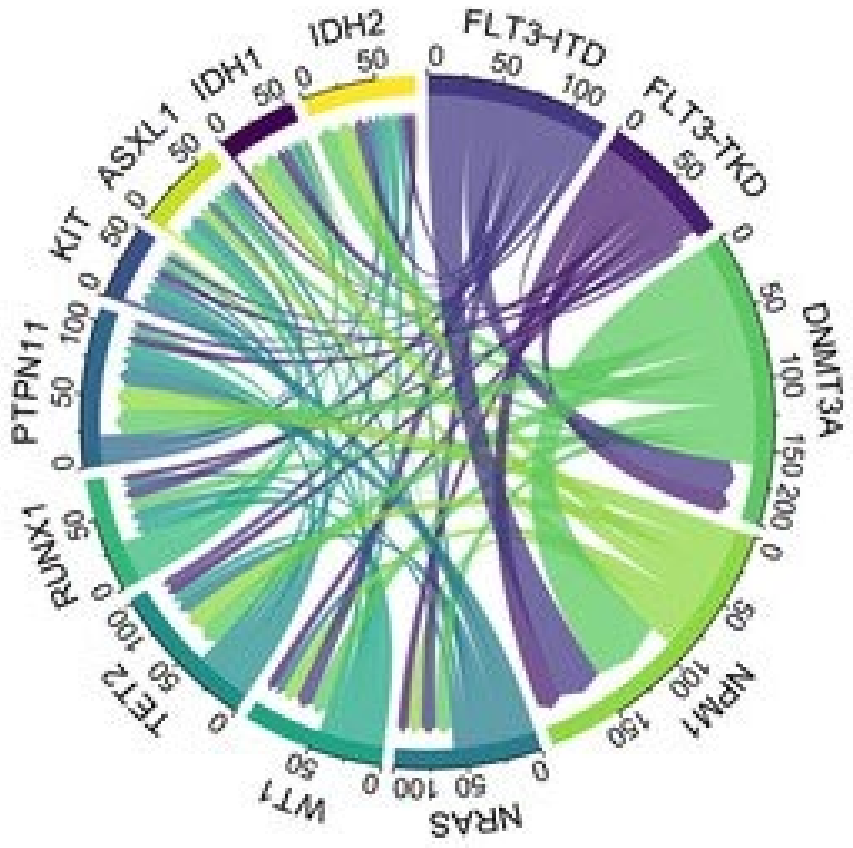
Q4. What is the risk category according to ELN Classification 2022?

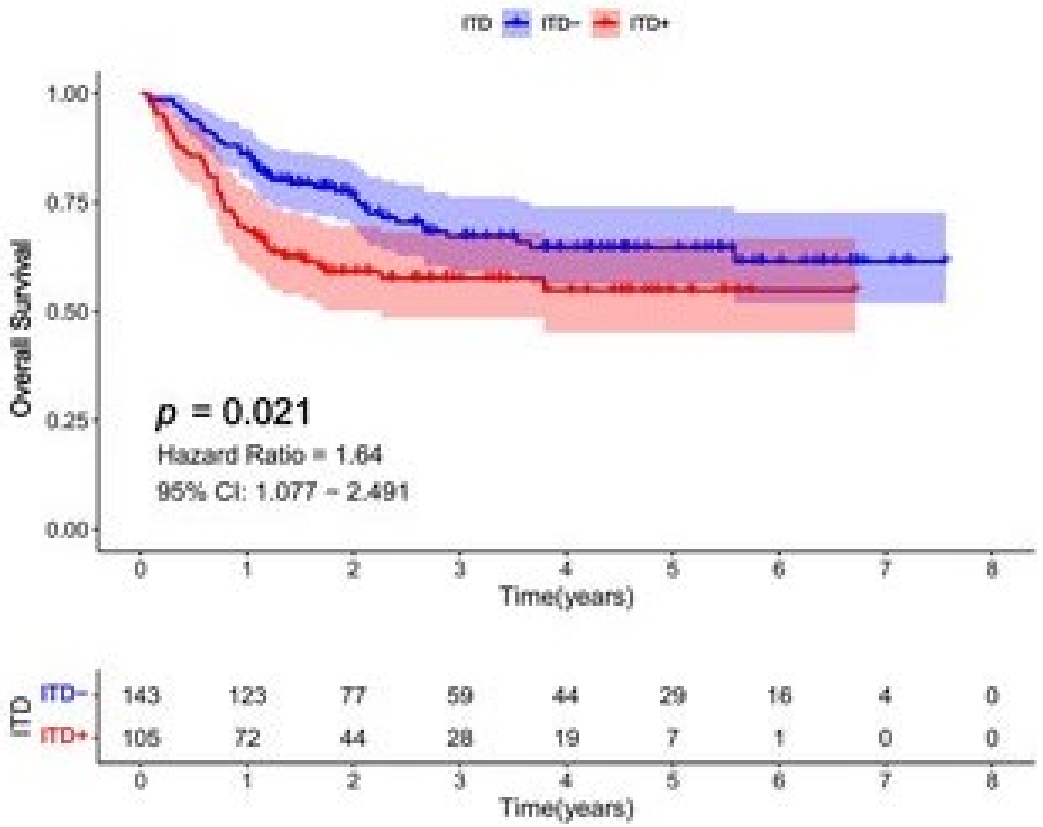
1) Very low
2) Favourable
3) Intermediate
4) Adverse
5) Very high

Q4. What is the risk category according to ELN Classification 2022?

1) Very low
2) Favourable
3) Intermediate
4) Adverse
5) Very high

FLT3^{mut} in *NPM1*^{mut} AML





Pan X, et al. Br J Haematol. 2023 Oct;203(2):212-223.

ELN^{2022} risk classification by genetics

Risk category	Genetic abnormality
Favourable	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 (without adverse-risk genetic lesions)
	t(9;11)(p21.3;q23.3)/MLLT3::KMT2A†,¶
	Cytogenetic and/or molecular abnormalities not classified as favorable or adverse
Abverse	t(6;9)(p23.3;q34.1)/DEK::NUP214
	t(v;11q23.3)/KMT2A-rearranged#
	t(9;22)(q34.1;q11.2)/BCR::ABL1
	t(8;16)(p11.2;p13.3)/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, and/or ZRSR2‡‡
	Mutated TP53a

§ Mainly based on results observed in intensively treated patients. Initial risk assignment may change during the treatment course based on the results from **analyses of MRD.**

Döhner et al. Diagnosis and management of AML in adults: 2022 recommendations from an international expert panel on behalf of the ELN. Blood. 2022 Sep 22;140(12):1345-1377.

DIC in non-APL AML

ISTH-DIC score 2018 calculation

Points	0	1	2	3	Patient
Platelet count ($x10^9$/l)	≥100	50-99	**<50**		$42x10^9$/l
Fibrinogen (mg/dl)	≥100	**<100**			80 mg/dl
Prothrombin time (sec) *	<16	16-19	**<19**		17.2 sec
D-dimer (ng/mL)	<3000		3000-7000	**>7000**	9000 ng/mL

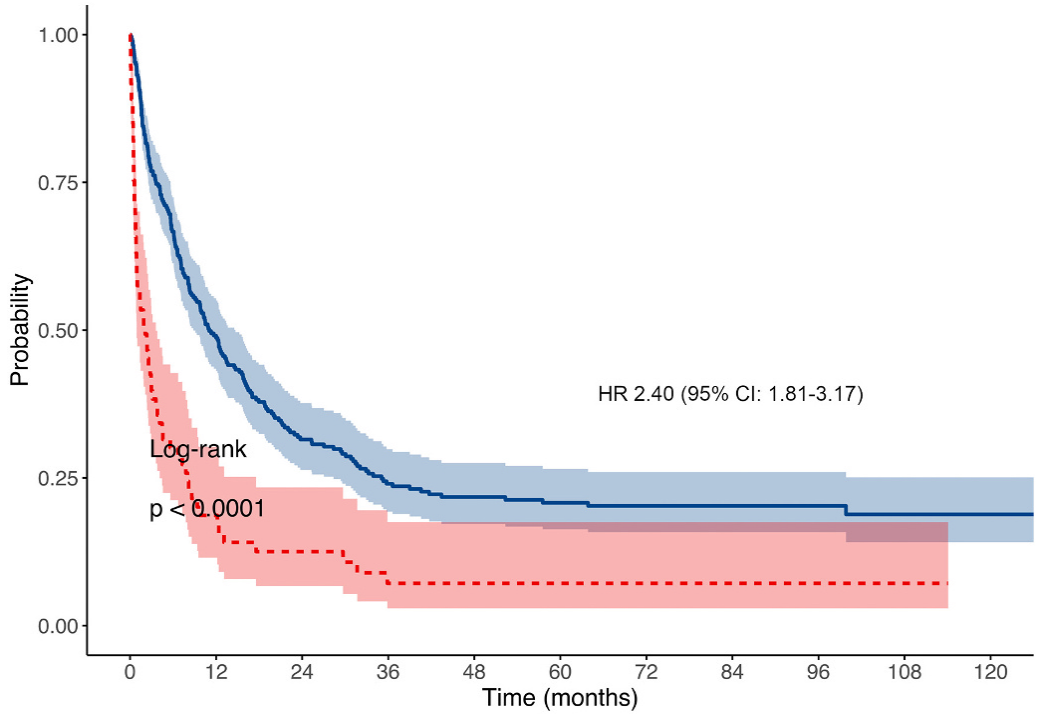
*A score of ≥4 was defined as an overt DIC. Patient score =8

- Overt DIC was present in 21 % of non-APL AML cases
- Associated with advanced age, comorbidities, poor performance status, hyperleukocytosis, LDH levels, *NPM1* mutations, *FLT3-ITD*, CD33(+), CD4(+), CD34(-)

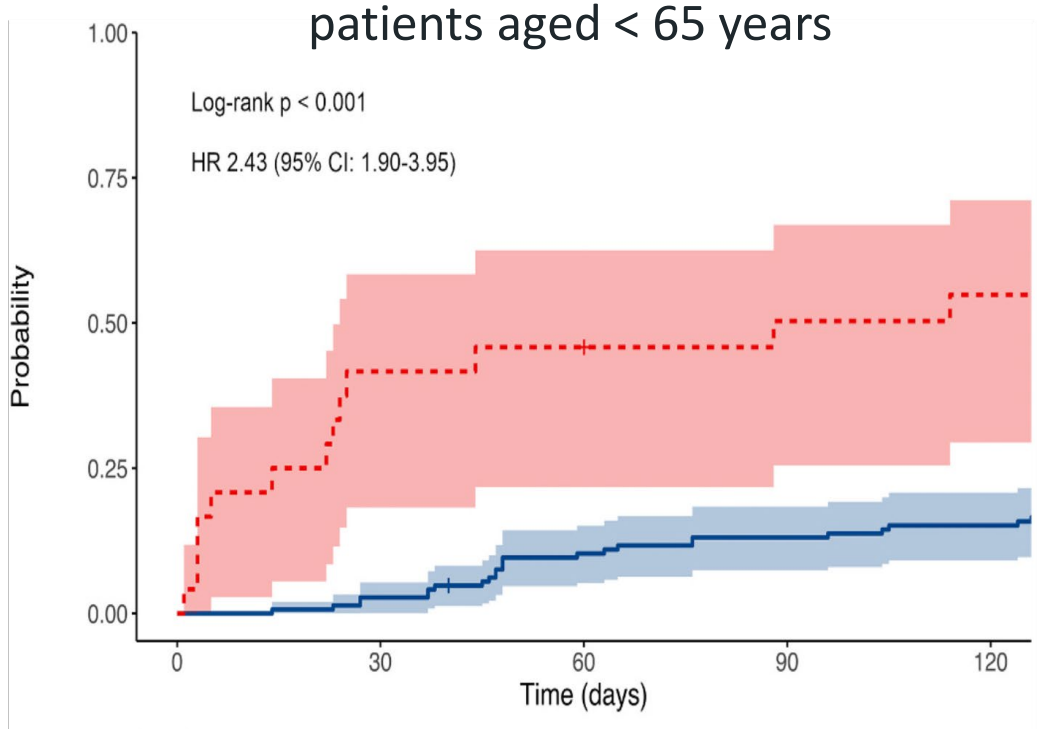
Paterno G, et al. The ISTH DIC-score predicts early mortality in patients with non-promyelocitic acute myeloid leukemia. Thromb Res. 2024 Apr;236:30-36.

DIC in non-APL AML
a potential unfavorable prognostic marker

Overall Survival



Cumulative overall mortality
patients aged < 65 years



Paterno G, et al. The ISTH DIC-score predicts early mortality in patients with non-promyelocitic acute myeloid leukemia. Thromb Res. 2024 Apr;236:30-36.

DIC in non-APL AML
the importance of early recognition

- The prevalence and clinical relevance of DIC in non-promyelocytic AML is not negligible
- Potential as an unfavorable prognostic marker
- Patients with ISTH DIC-score ≥ 4 might be candidates for:
  - a more aggressive support therapy aimed at reversing the coagulopathy, similarly to what recommended for APL
  - a more aggressive antileukemic treatment initiation in order to promptly mitigate the leukemia-associated coagulopathy
  - thereby reducing the risk of early mortality

Paterno G, et al. The ISTH DIC-score predicts early mortality in patients with non-promyelocitic acute myeloid leukemia. Thromb Res. 2024 Apr;236:30-36.
Ten Cate H, Leader A. Management of Disseminated Intravascular Coagulation in Acute Leukemias. Hamostaseologie. 2021 Apr;41(2):120-126.

ATRA

+plasma to maintain fibrinogen >1 g/l

+ platelet transfusions

7+3

Gilteritinib

CR

Iyer SG, et al. The treatment of acute promyelocytic leukemia in 2023: Paradigm, advances, and future directions. Fr[illegible]oncol. 2023 Jan 18;12:1062524.
Falini B, Brunetti L, Martelli MP. How I diagnose and treat NPM1-mutated AML. Blood. 2021 Feb 4;137(5):[illegible]89-599.

Q5. Which is the best biomarker to monitor MRD in this patient?

1) *FLT3-ITD*
2) *NPM1*
3) *NPM1* and *FLT3-ITD*
4) *WT1*
5) Panel-Based NGS (DNA) for somatic mutations

Q5. Which is the best biomarker to monitor MRD in this patient?

1) *FLT3-ITD*

2) *NPM1*

3) *NPM1* and *FLT3-ITD*

4) *WT1*

5) Panel-Based NGS (DNA) for somatic mutations

ELN approved MRD biomarkers in AML

Genetic change	Recommended by ELN 2021 and ELN 2022	Comments
NPM1	Yes	Essential to inform postremission therapy
Signaling pathway genes: *FLT3, KIT, RAS*, others	Possibly – not true MRD markers	Useful if positive but relapse is possible in test-negative subjects
WT1, EVI1	Disfavoured	Expression-based assays may be highly variable
“DTA”genes: *DNMT3A, TET2, ASXL1*	Specifically recommended against	These may be found in ARCH and should be excluded from consideration. Further research is needed to be able to differentiating CHIP-like mutations from mutations with oncogenic potential

Cappelli LV, et al. Leukemia. 2022 Feb;36(2):394-402.; Moritz J, et al. Biomedicines. 2024 Mar 7;12(3):599. ; Blachly JS, et al. Haematologica. 2022 Dec 1;107(12):2810-2822.

NPM1^{mut} is a (nearly) ideal molecular MRD target

Key factors:

- Prevalence and Specificity
- Stability at Relapse
- Quantitative Monitoring
- Prognostic Value
- Guidance for Therapy

Schnittger S, et al. Blood. 2009 Sep 10;114(11):2220-31; Falini B, Brunetti L, Martelli MP. How I diagnose and treat NPM1-mutated AML. Blood. 2021 Feb 4;137(5):589-599.; Falini B, Dillon R. Criteria for Diagnosis and Molecular Monitoring of NPM1-Mutated AML. Blood Cancer Discov. 2024 Jan 8;5(1):8-20.

NPM1^{mut} is stable at relapse and tracks disease

- >90% of NPM1-AML patients maintain detectable levels of the mutation during relapse
- *NPM1*^{WT} relapse in *NPM1*-AML is uncommon
- *NPM1*^{mut} remains reliable indicators of disease status throughout the treatment process

Cocciardi S, et al. Clonal evolution patterns in acute myeloid leukemia with NPM1 mutation. Nat Commun. 2019 May 2;10(1):2031.

NPM1^{mut} can be quantified

***NPM1* mutations can be quantified by molecular techniques that allow for sensitive detection and measurement of these mutations:**

- Real-Time Quantitative Polymerase Chain Reaction (RQ-PCR)
- Fully automated direct qPCR without extraction
- Allele-Specific Oligonucleotide Real-Time Quantitative PCR (ASO-RQ-PCR)
- High-Resolution Melting Analysis (HRM)
- Droplet Digital PCR (ddPCR)
- Next-Generation Sequencing (NGS)

Interlaboratory validation NB!

- The impact of reverse transcriptase and NGS for false (+)
- Future studies of the potential of digital PCR to reduce interlaboratory variations

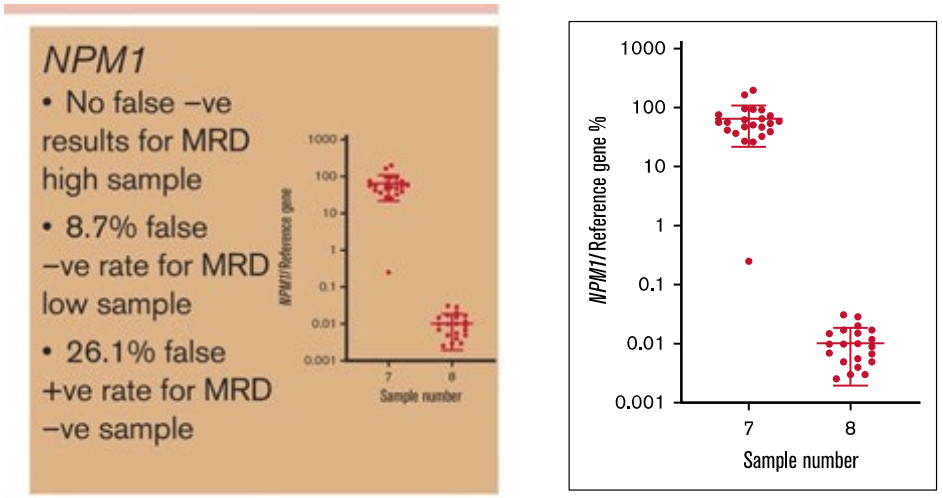


Figure 4. % normalized ratio returned by all participants reporting *NPM1* MRD levels in samples 7 and 8. Long horizontal line represents average. Short horizontal line represents standard deviation.

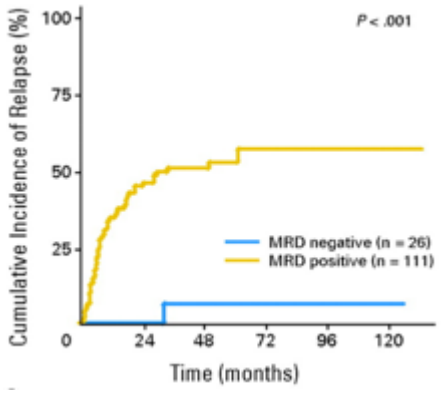
Chin L, et al. Targeting and Monitoring Acute Myeloid Leukaemia with Nucleophosmin-1 (*NPM1*) Mutation. Int J Mol Sci. 2023 Feb 5;24(4):3161.; Scott S, et al. Assessment of acute myeloid leukemia molecular measurable residual disease testing in an interlaboratory study. Blood Adv. 2023 Jul 25;7(14):3686-3694.

NPM1^{mut} predicts relapse and survival

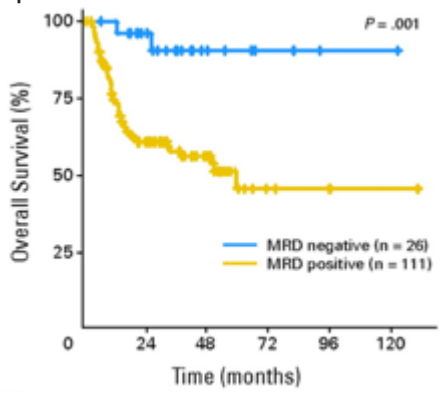
MRD at Th completionMRD after induction

German-Austrian AML study group

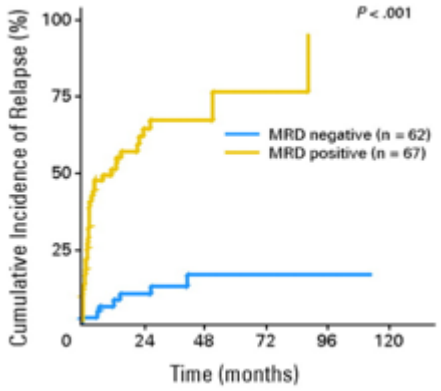
Cummulative risk of relapse



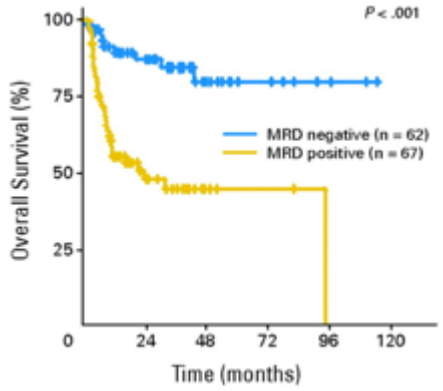
Overall survival



Cummulative risk of relapse



Overall survival



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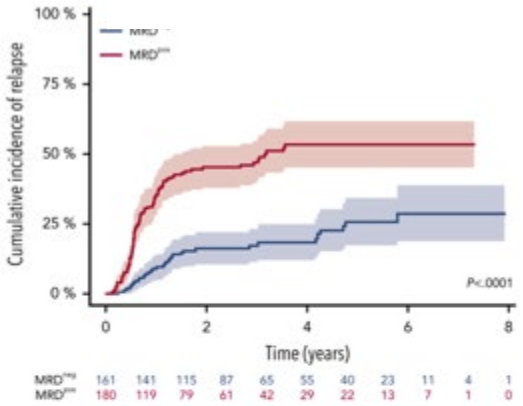
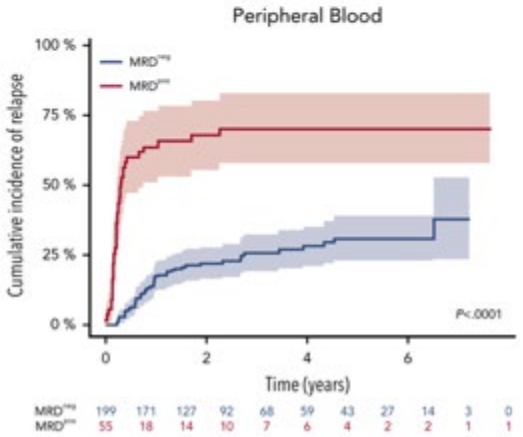
AMLSG 09-09 trial of GO

Cummulative risk of relapse



Peripheral Blood



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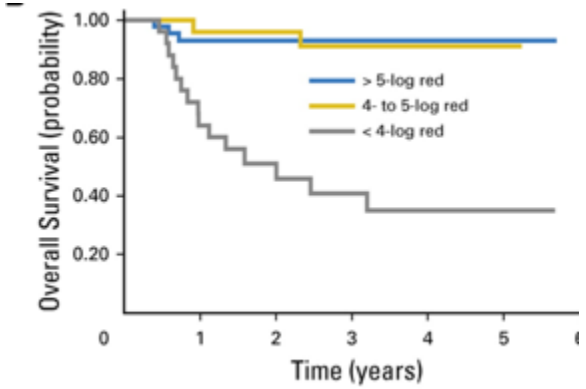
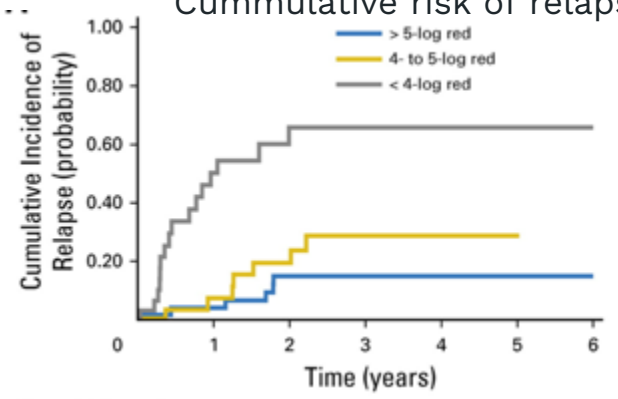
ALFA-0702 trial

Overall survival



Cummulative risk of relapse



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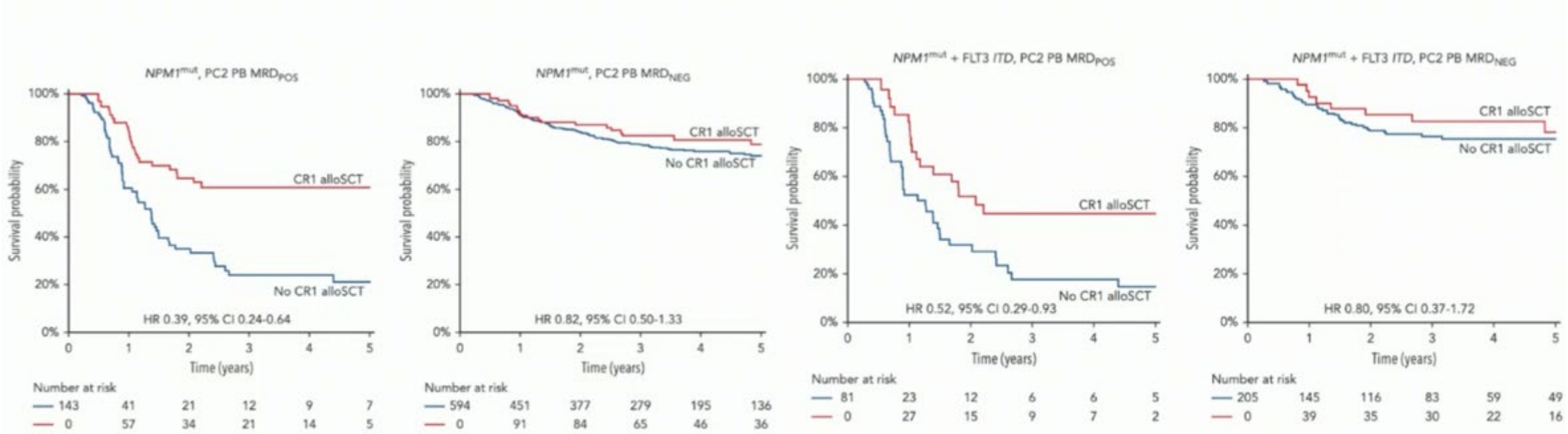
NPM1^{mut} can guide therapy

United Kingdom National Cancer Research Institute AML17 and AML19 studies

- Postinduction molecular MRD(+) reliably identifies those patients who benefit from allogeneic HSCT in CR1
- Patients achieving MRD negativity in blood after second induction show no survival benefit from CR1 transplant, even if *FLT3*-ITD co-mutated.



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In conclusion, AML with *NPM1* mutation

- Exhibits unique molecular, pathological, and clinical features, which led to its recognition as distinct entity in the WHO classification.
- Although diagnostic criteria are well established, its distinction from other AML entities may be difficult.
  - Awareness of APL-like presentation will guide antileukemic and supportive therapy thereby reducing the risk of early mortality.
- Determining the mutational status of *NPM1* together with *FLT3* is mandatory for accurate risk assessment.
- *NPM1* mutations are ideal targets for MRD monitoring, since they are AML specific, stable, quantifiable and provide prognostic information.
- MRD monitoring by qPCR of *NPM1*-mutant transcripts, combined with ELN genetic-based risk stratification, can guide therapeutic decisions.

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