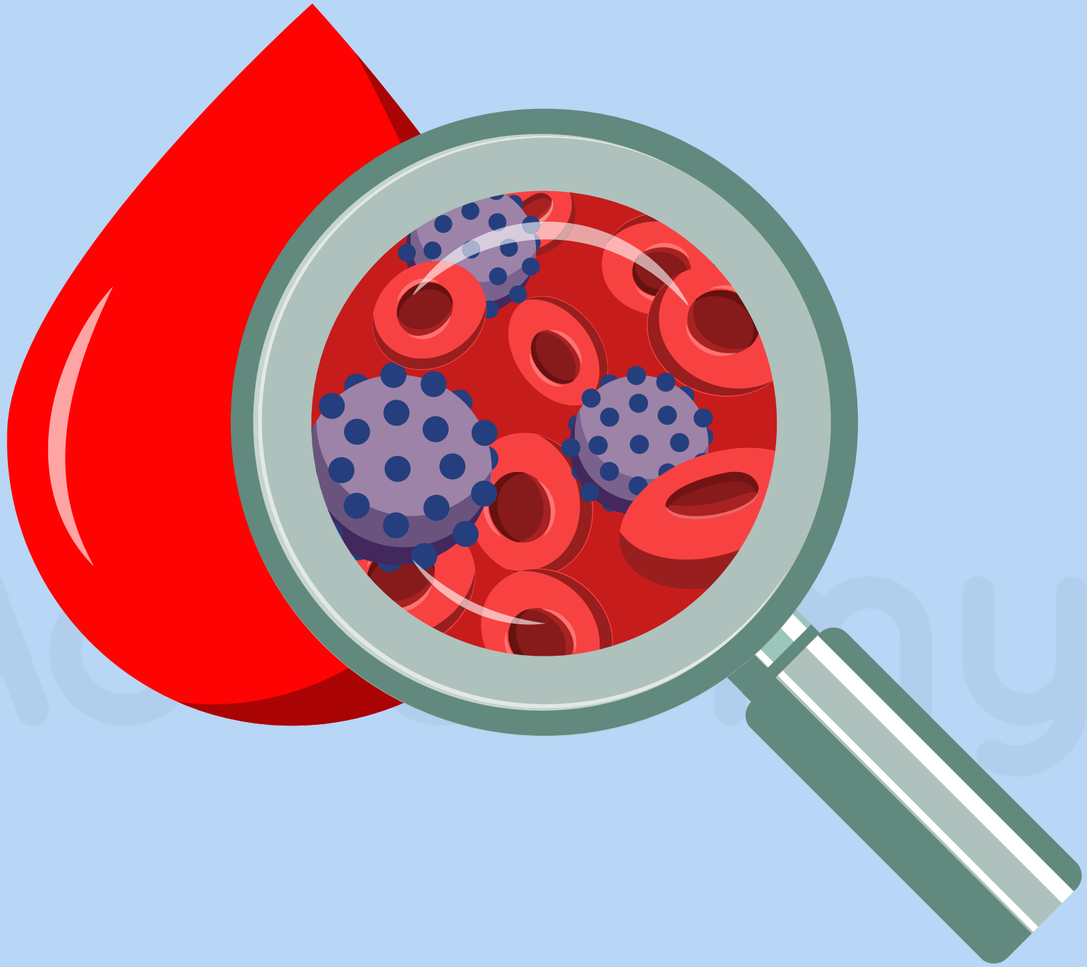
ADVANCED MORPHOLOGY COURSE

SESSION 2

Cases 1 - 4

Blood Academy



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Case 1

PATIENT

89-year-old male

CLINICAL DETAILS

Unwell

PATIENT AUTOMATED BLOOD COUNT

Haemoglobin	101 g/L
White cell count	4.1 x 10^9/L
Platelet count	231 x 10^9/L

QUESTION 1

Describe the main features of the blood and bone marrow aspirate.

QUESTION 2

What is the most likely diagnosis?

QUESTION 3

What makes this diagnosis unique?

QUESTION 4

How can you confirm the diagnosis?

Learning points

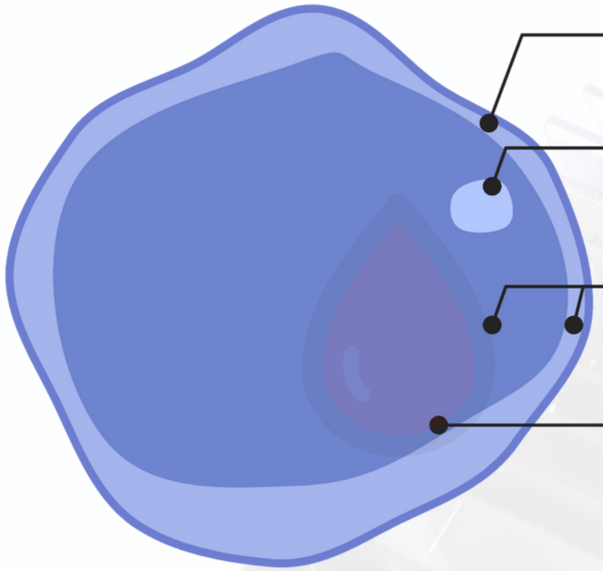
MORPHOLOGICAL FEATURES

- Dysplasia
- Bone marrow infiltration and fibrosis - teardrop red cells, nucleated red cell
- Blast cell (%)
- Auer rods

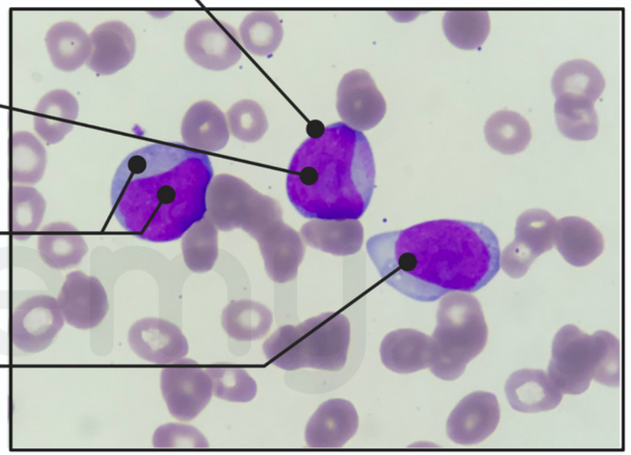
CLINICAL FEATURES

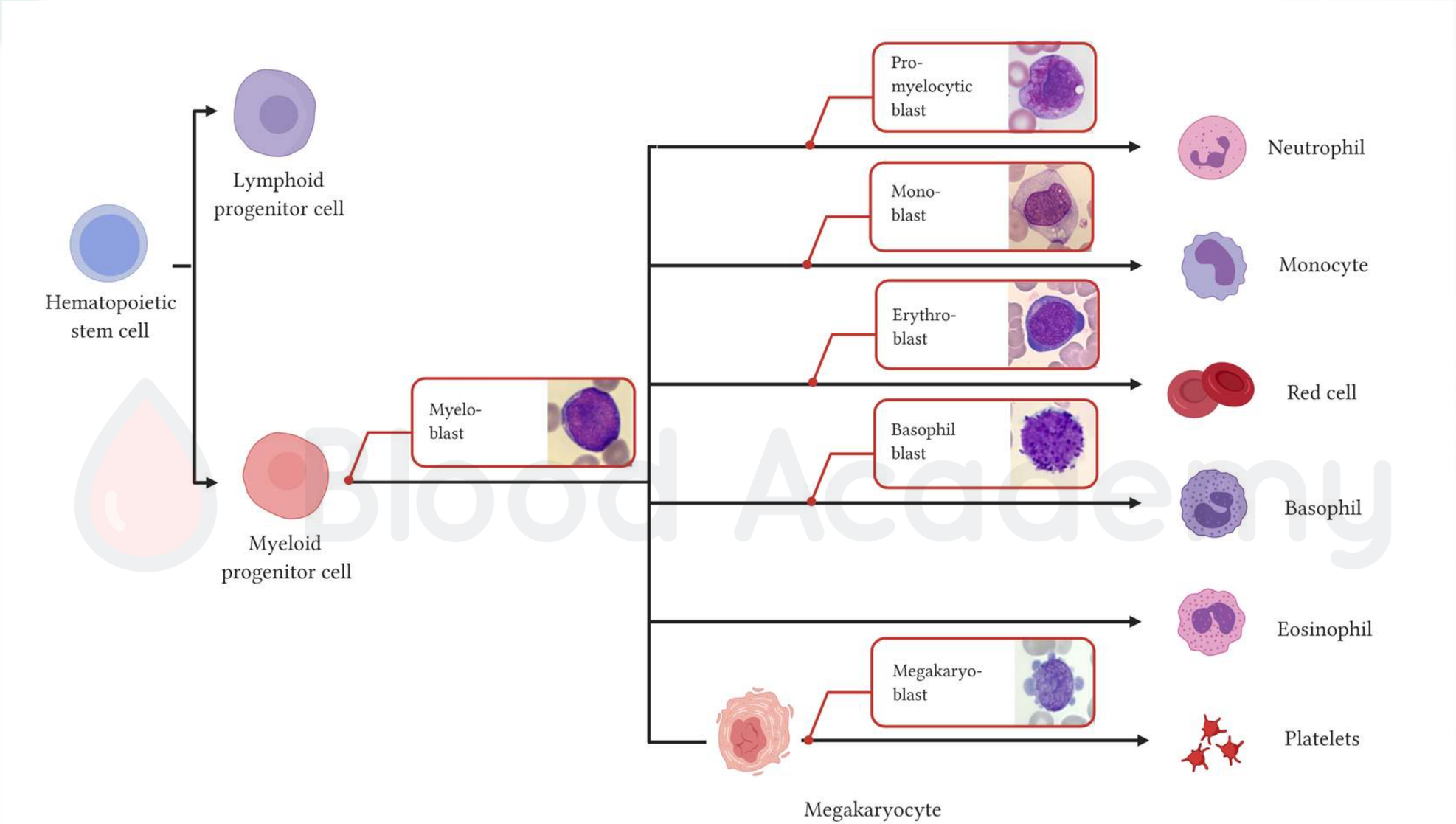
- Previous chemotherapy
- Previous radiotherapy
- History of MDS or MPN
- Bleeding
- Medication
- Infection
- Other malignancy

General characteristics of blast cells



1. Large size, often 2-6 times the size of a mature red cell
2. Presence of nucleoli*
3. High nucleus - cytoplasmic ratio
4. Delicate (open), diffuse chromatin pattern
5. Large number of homogenously appearing cells



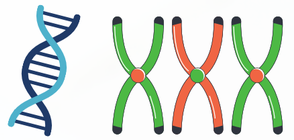

Hematopoietic stem cell
Lymphoid progenitor cell
Myeloid progenitor cell
Myelo-blast
Pro-myelocytic blast
Mono-blast
Erythro-blast
Basophil blast
Megakaryo-blast
Megakaryocyte
Neutrophil
Monocyte
Red cell
Basophil
Eosinophil
Platelets

WHO classification of acute myeloid leukaemia (2022)

Morphology

Pre-existing condition e.g. MDS

Post cytotoxic therapy



Genetics e.g. PCR, karyotype

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 leukaemia with NPM1 mutation
- Acute myeloid leukaemia with CEBPA mutation*
- Acute myeloid leukaemia, myelodysplasia-related
- Acute myeloid leukaemia with other defined genetic alterations

All entities defined irrespective of blast % except those designated *

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

Secondary myeloid malignancies

- Myeloid neoplasms post cytotoxic therapy
- Myeloid neoplasms associated with germline predisposition

Myeloid sarcoma

Defined as the presence of one or more cytogenetic or molecular abnormalities listed in * and ** and/or history of MDS or MDS/MPN

Defining cytogenetic abnormalities*

- Complex karyotype (≥3 abnormalities)
- 5q deletion or loss of 5q due to unbalanced translocation
- Monosomy 7, 7q deletion, or loss of 7q due to unbalanced translocation
- 11q deletion
- 12p deletion or loss of 12p due to unbalanced translocation
- Monosomy 13 or 13q deletion
- 17p deletion or loss of 17p due to unbalanced translocation
- Isochromosome 17q
- idic(X)(q13)

Defining somatic mutations**

- ASXL1
- BCOR
- EZH2
- SF3B1
- SRSF2
- STAG2
- U2AF1
- ZRSR2

ICC (2022) classification of acute myeloid leukaemia

Entities requiring blast ≥10%

- Acute promyelocytic leukemia (APL) with t(15;17)(q24.1;q21.2)/*PML::RARA*
- APL with other *RARA* rearrangements
- AML with t(8;21)(q22;q22.1)/*RUNX1::RUNX1T1*
- AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22)/*CBFB::MYH11*
- AML with t(9;11)(p21.3;q23.3)/*MLLT3::KMT2A*
- AML with other *KMT2A* rearrangements
- AML with t(6;9)(p22.3;q34.1)/*DEK::NUP214*
- AML with inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2)/*GATA2; MECOM*(*EVI1*)
- AML with other *MECOM* rearrangements
- AML with other rare recurring translocations
- AML with mutated *NPM1* ≥10%
- AML with in-frame bZIP *CEBPA* mutations ≥10%

Entities requiring variable blast %

- AML not otherwise specified (NOS) 10-19% (MDS/AML) and ≥20% (AML)
- AML with t(9;22)(q34.1;q11.2)/*BCR::ABL1* ≥20%
- AML and MDS/AML with mutated *TP53* 10-19% (MDS/AML) and ≥20% (AML)
- AML and MDS/AML with myelodysplasia-related gene mutations 10-19% (MDS/AML) and ≥20% (AML)
  - Defined by mutations in *ASXL1*, *BCOR*, *EZH2*, *RUNX1*, *SF3B1*, *SRSF2*, *STAG2*, *U2AF1*, or *ZRSR2*
- AML with myelodysplasia-related cytogenetic abnormalities 10-19% (MDS/AML) and ≥20% (AML)
  - Defined by detecting a complex karyotype (≥3 unrelated clonal chromosomal abnormalities in the absence of other class-defining recurring genetic abnormalities), del(5q)/t(5q)/add(5q), -7/del(7q), +8, del(12p)/t(12p)/add(12p), i(17q), -17/add(17p) or del(17p), del(20q), and/or idic(X)(q13) clonal abnormalities

Myeloid sarcoma

Diagnostics qualifiers for MDS, AML (or MDS/AML diagnosis)

Therapy-related	Progressing from myelodysplastic syndrome	Progressing from MDS/MPN (specify)	Germline predisposition
Prior chemotherapy, radiotherapy, immune interventions	MDS should be confirmed by standard diagnostic	MDS should be confirmed by standard diagnostic	

Learning points

WHO

- 20% blast threshold remains (excluding notable exceptions)
- AML NOS removed
- Myelodysplasia-related changes, now called AML, myelodysplasia-related (AML-MR)
  - removal of morphology alone as a diagnostic premise to make a diagnosis of AML-MR
  - defining mutations: SRSF2, SF3B1, U2AF1, ZRSR2, ASXL1, EZH2, BCOR, STAG2

ICC

- 10-19% blasts = MDS/AML group
- AML NOS remains
- Myelodysplasia-related mutations
  - ASXL1, BCOR, EZH2, **RUNX1**, SF3B1, SRSF2, STAG2, U2AF1, or ZRSR2

BOTH

- AML with CEBPA mutations

FINAL DIAGNOSIS

Acute myeloid leukaemia with normal karyotype and ASXL1 mutation

Case 2

PATIENT

41-year-old male

CLINICAL DETAILS

Widespread brusing

PATIENT AUTOMATED BLOOD COUNT

Haemoglobin	98 g/L
White cell count	3.4 x 10^9/L
Platelet count	48 x 10^9/L

QUESTION 1

Report the blood film and bone marrow aspirate

QUESTION 2

What would you expect to see on immunophenotyping (flow cytometry) of this sample?

QUESTION 3

Give three main priorities in the management of this patient?

Learning points

BLOOD FILM

- Blasts
- Granular, large, bilobed nuclei, nucleoli
- Some fine azurophilic granules
- Nucleated red cell (rare)

Conclusion - acute leukaemia, APML

BONE MARROW ASPIRATE

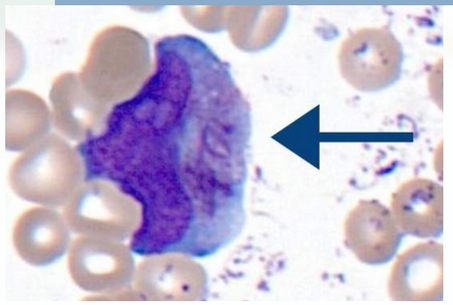
- Blasts
- Granular, large, bilobed nuclei, nucleoli
- Some fine azurophilic granules
- Nucleated red cell (rare)

Conclusion - acute leukaemia, APML

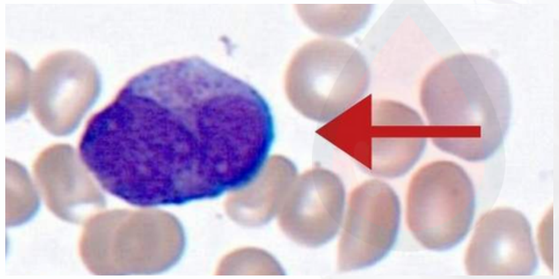
Learning points

- Admit patient
- Assess and manage coagulopathy
- Start ATRA
- Confirm diagnosis (FISH/PCR APL::RARA)

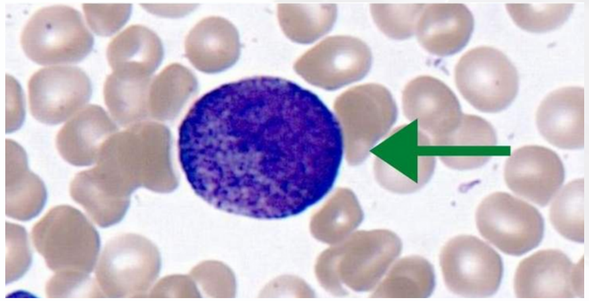
Learning points



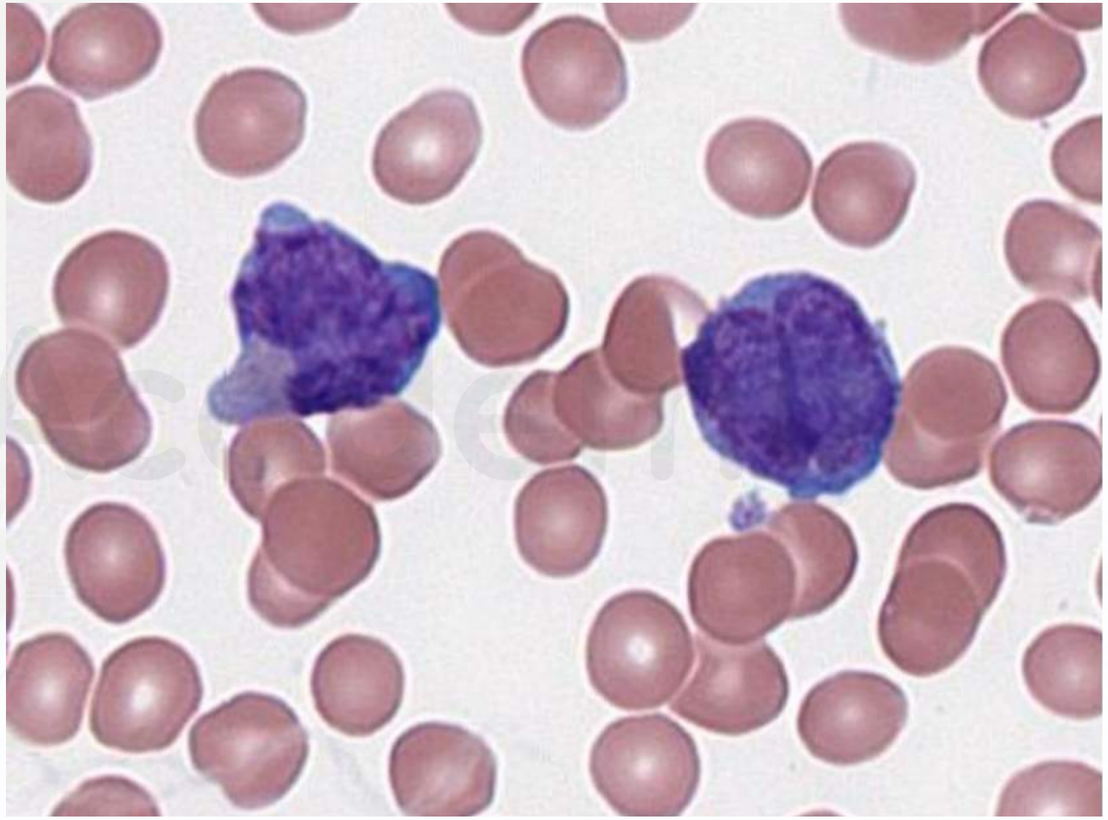
Faggot cell



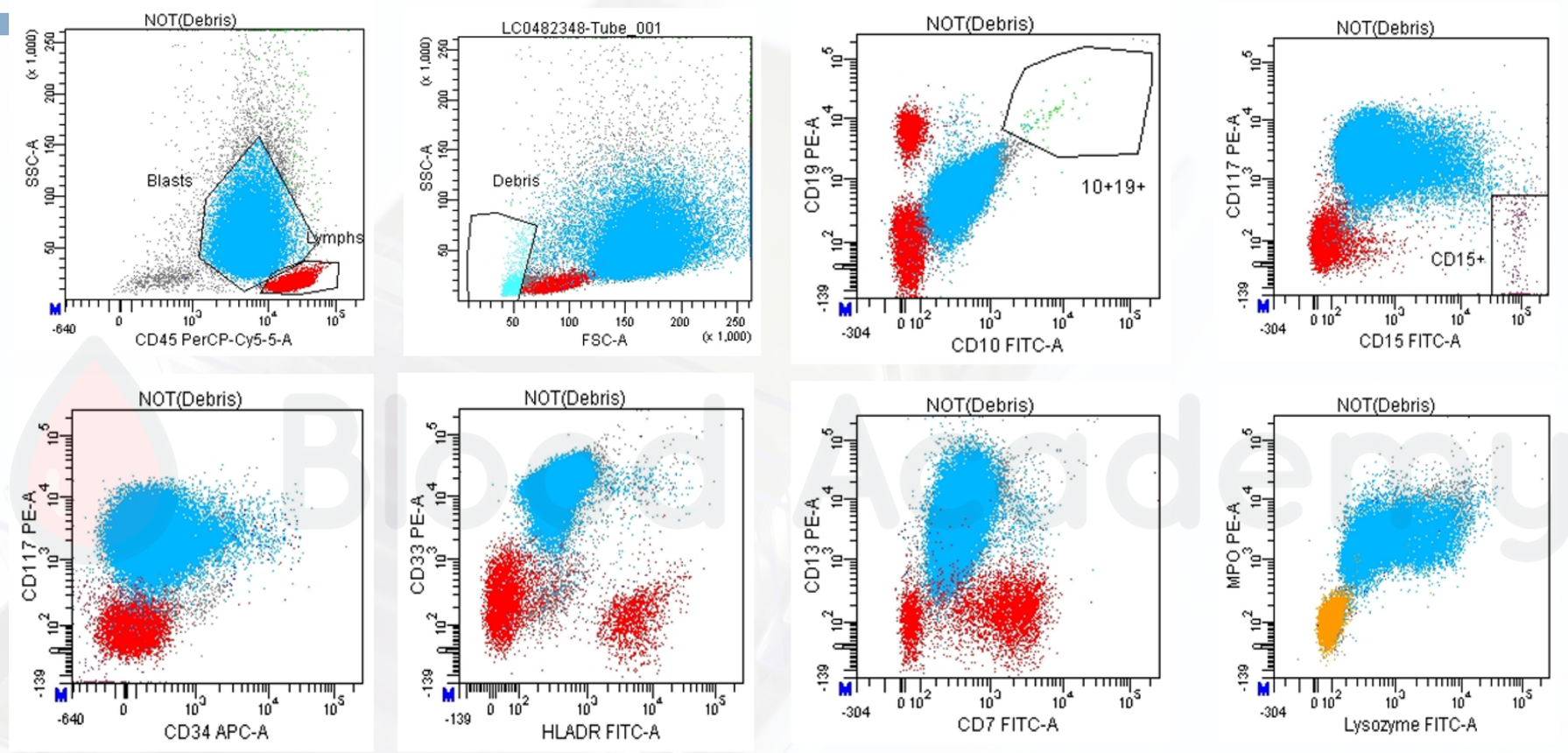
bilobed
nucleus



heavy granulated
cytoplasm



Learning points

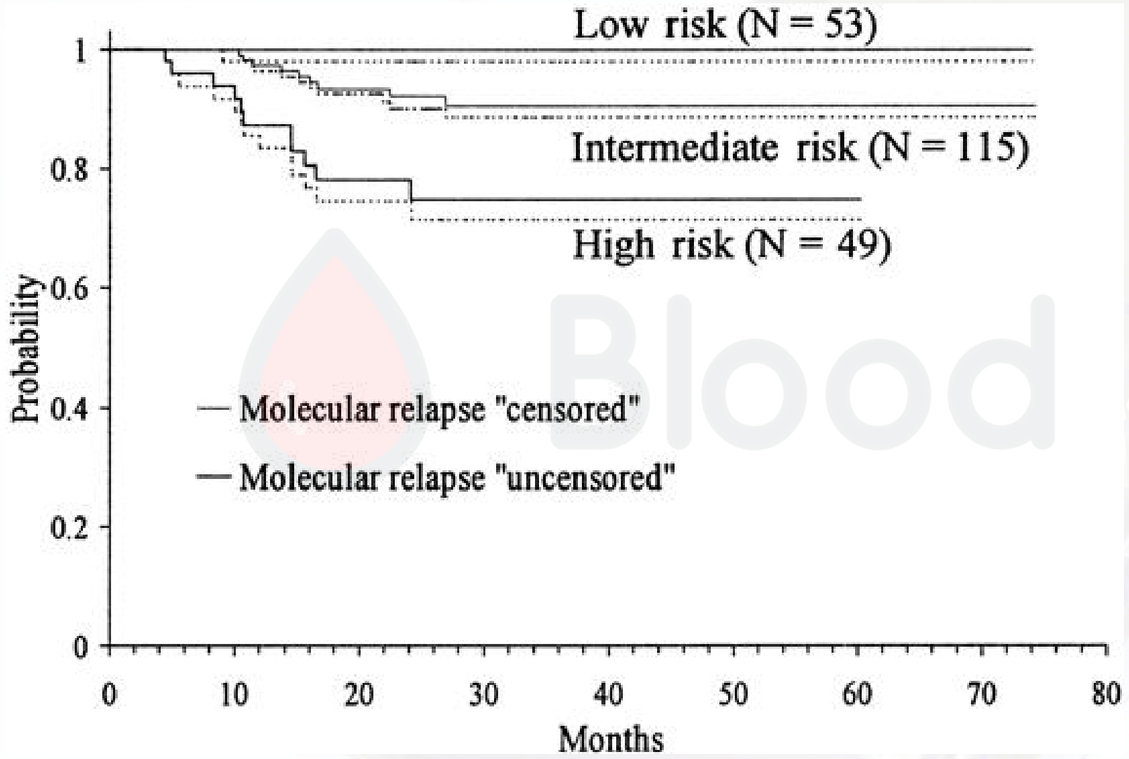


Low side scatter

Positive: MPO, CD33 (bright), CD117 (weak), CD13 (variable)

Negative: HLA-DR, CD34, CD15

Learning points



Low risk
WBC ≤10
Platelets >40

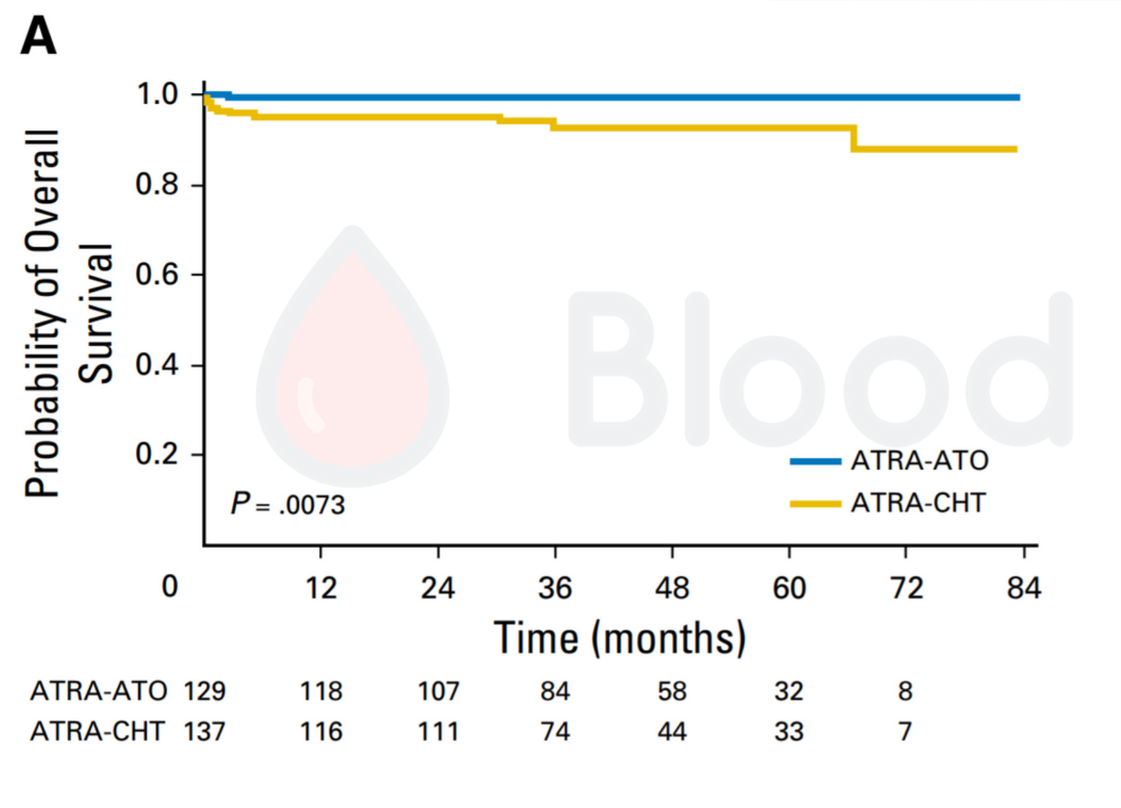
Intermediate
WBC ≤10
Platelets ≤40

High risk
WBC >10

Italian GIMEMA and the Spanish PETHEMA trials
(risk free survival)

Blood. 2000;96(4):1247.

Learning points



Low and Intermediate risk

One group in era of Arsenic Trioxide + ATRA

J Clin Oncol. 2017;35(6):605

FINAL DIAGNOSIS

Acute promyelocytic leukaemia with PML::RARA fusion

Case 3

PATIENT

24-year-old male

CLINICAL DETAILS

Fatigue and fever

PATIENT AUTOMATED BLOOD COUNT

Haemoglobin	103 g/L
White cell count	28.9 x 10^9/L
Platelet count	62 x 10^9/L

QUESTION 1

Describe the main features of the blood and bone marrow aspirate.

QUESTION 2

What is the most likely diagnosis?

QUESTION 3

What makes this diagnosis unique?

QUESTION 4

How can you confirm the diagnosis?

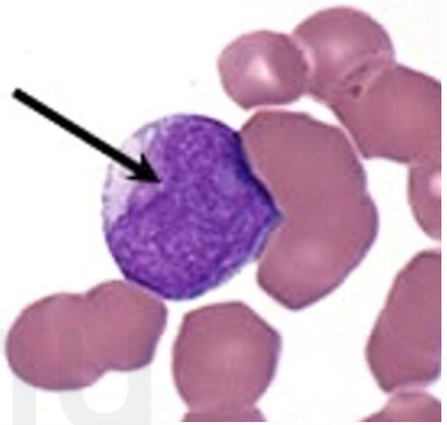
Learning points

BLOOD FILM

- Blasts
- Auer rods
- Myeloid maturation ? dysplasia

BONE MARROW ASPIRATE

- Blasts
  - Auer rods
  - Granules
  - Peri-nuclear thumbprinting/clearing
- Dysplastic neutrophils
- No erythroid/megakaryocytic elements



Learning points

QUESTION 2

What is the most likely diagnosis?
AML with t(8;21)

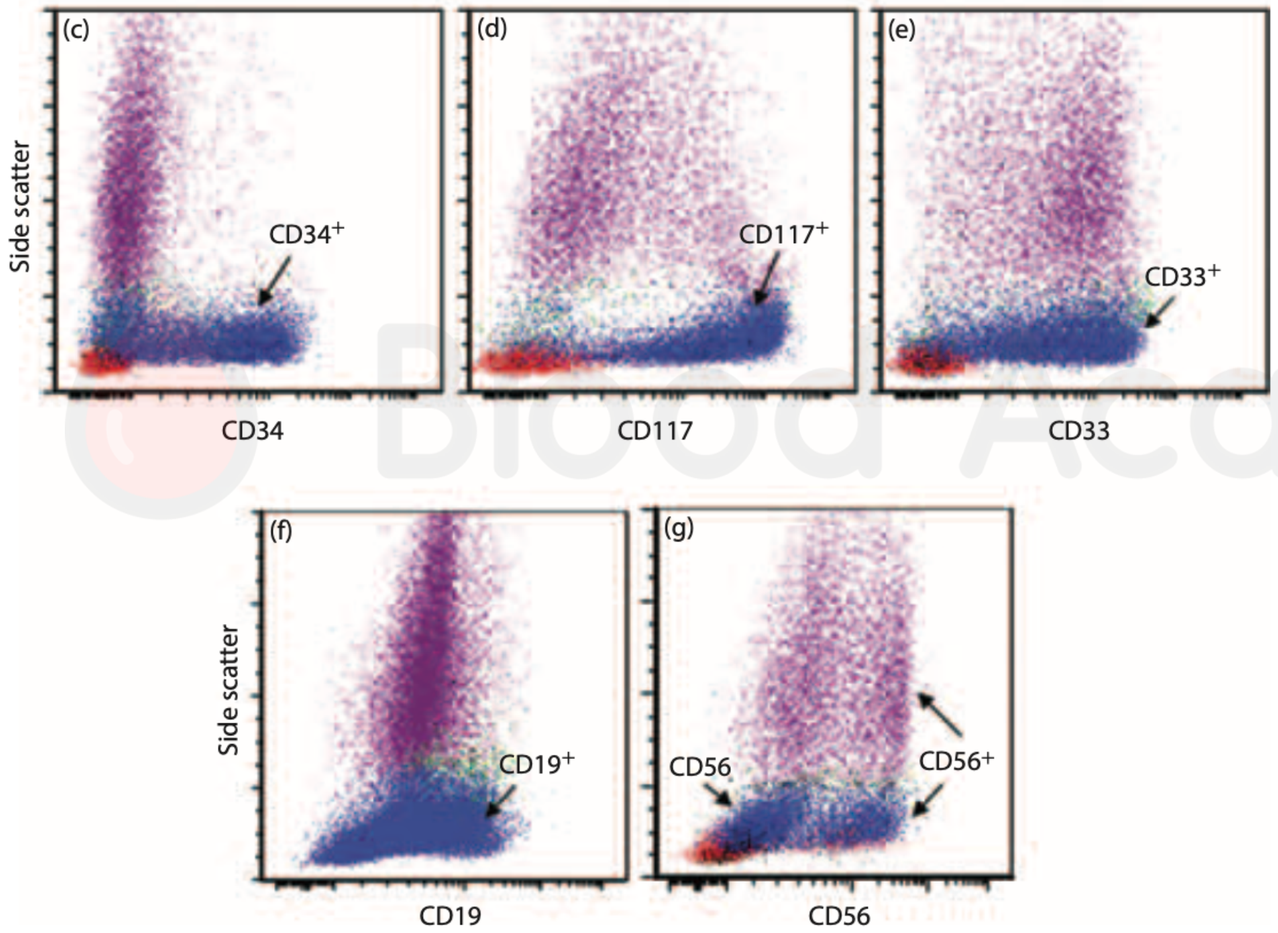
QUESTION 3

What makes this diagnosis unique?
Blast % <20% = AML

QUESTION 4

How can you confirm the diagnosis?
Karyotype, FISH, PCR

Learning points



FLOW CYTOMETRY

- Aberrant expression of CD19
- Often express CD56

Ref: Flow cytometry in neoplastic hematology

Learning points

QUESTION 2

What is the most likely diagnosis?
AML with t(8;21)

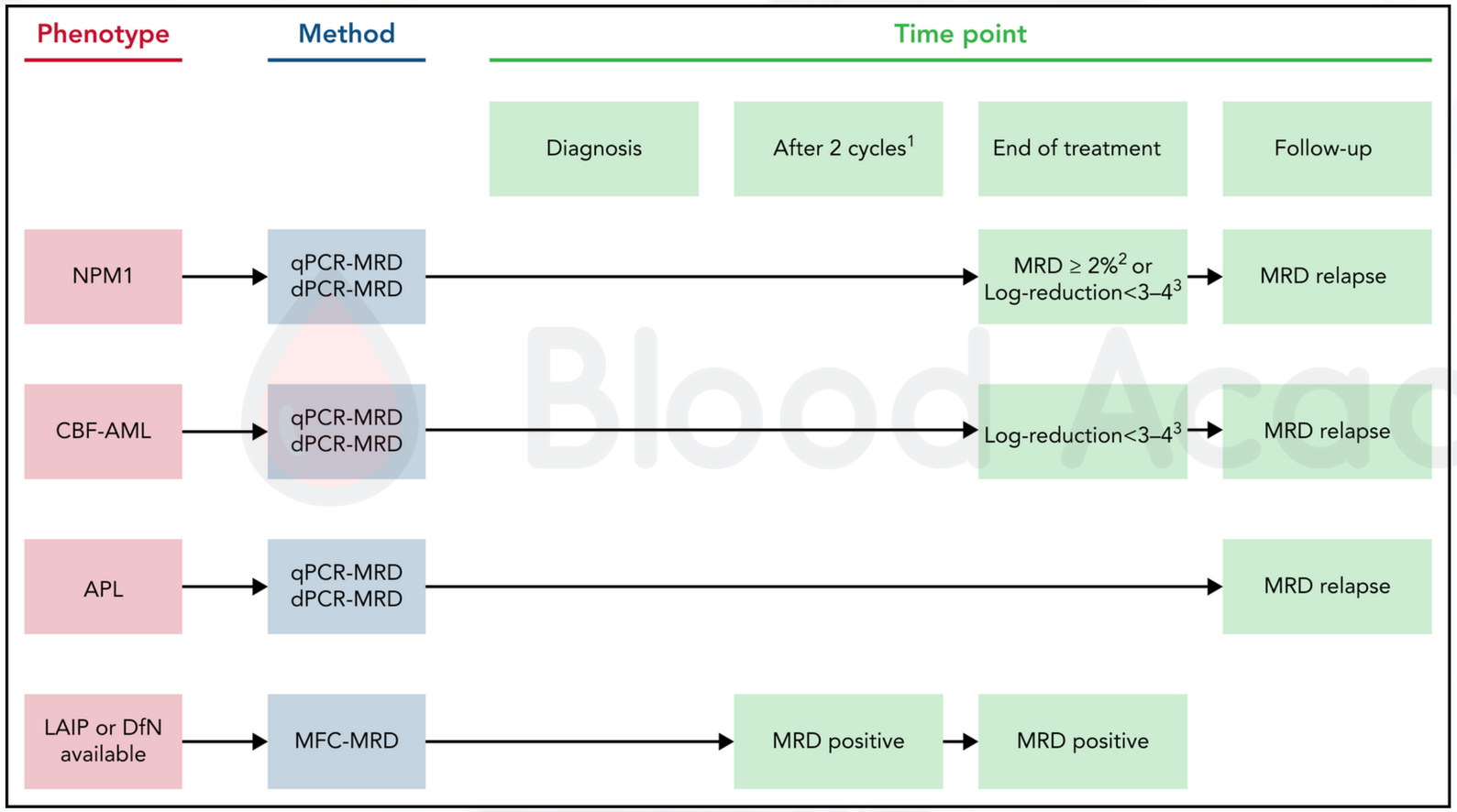
QUESTION 3

What makes this diagnosis unique?
Blast % <20% = AML

QUESTION 4

How can you confirm the diagnosis?
Karyotype, FISH, PCR

Learning points



MRD ASSESSMENT

- ELN recommendations

Heuser, M., et al. (2021). 2021 Update on MRD in acute myeloid leukemia: a consensus document from the European LeukemiaNet MRD Working Party. Blood, The Journal of the American Society of Hematology, 138(26), 2753-2767.

FINAL DIAGNOSIS

Acute myeloid leukaemia with t(8;21) RUNX1::RUNX1T1

Case 4

PATIENT

34-year-old male

CLINICAL DETAILS

Fatigue and mouth ulcers

PATIENT AUTOMATED BLOOD COUNT

Haemoglobin	104 g/L
White cell count	19.2 x 10^9/L
Platelet count	56 x 10^9/L

QUESTION 1

Provide a combined report the blood film, bone marrow aspirate and immunophenotyping results

QUESTION 2

What are the likely associated genetic changes?

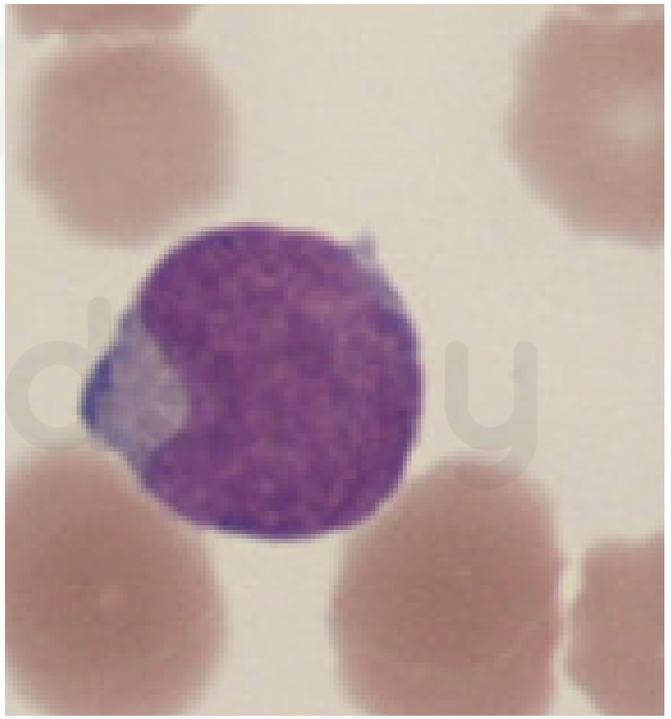
QUESTION 3

Provide a brief management plan regarding therapy and monitoring?

Learning points

MORPHOLOGY

- Prominent nuclear invagination
- Cup-like or fish mouth blasts
- Due to indentation of the nucleus by bits of cytoplasm



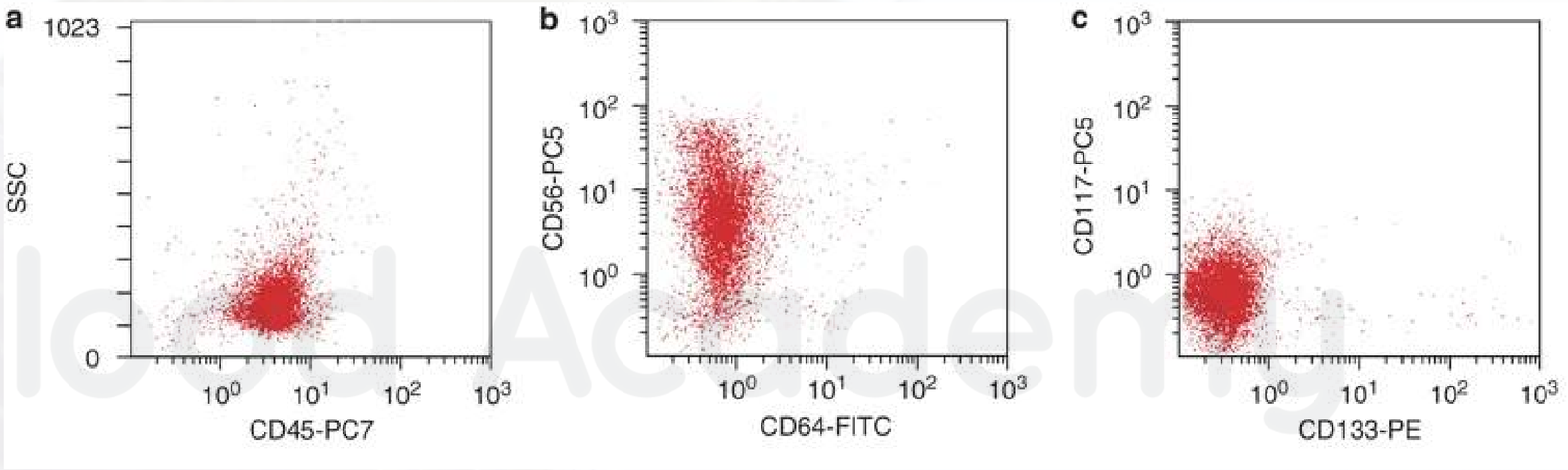
Learning points

QUESTION 2

- Other markers
  - Positive expression - MPO, CD33
  - Negative expression - CD34, HLA-DR

- HLA-DR, CD34 negative Myeloblastic
- Monocytic differentiation (CD64, CD14, and/or CD11b)
- Confused with APML (hypogranular variant)

NPM1 +/- FLT3



Learning points

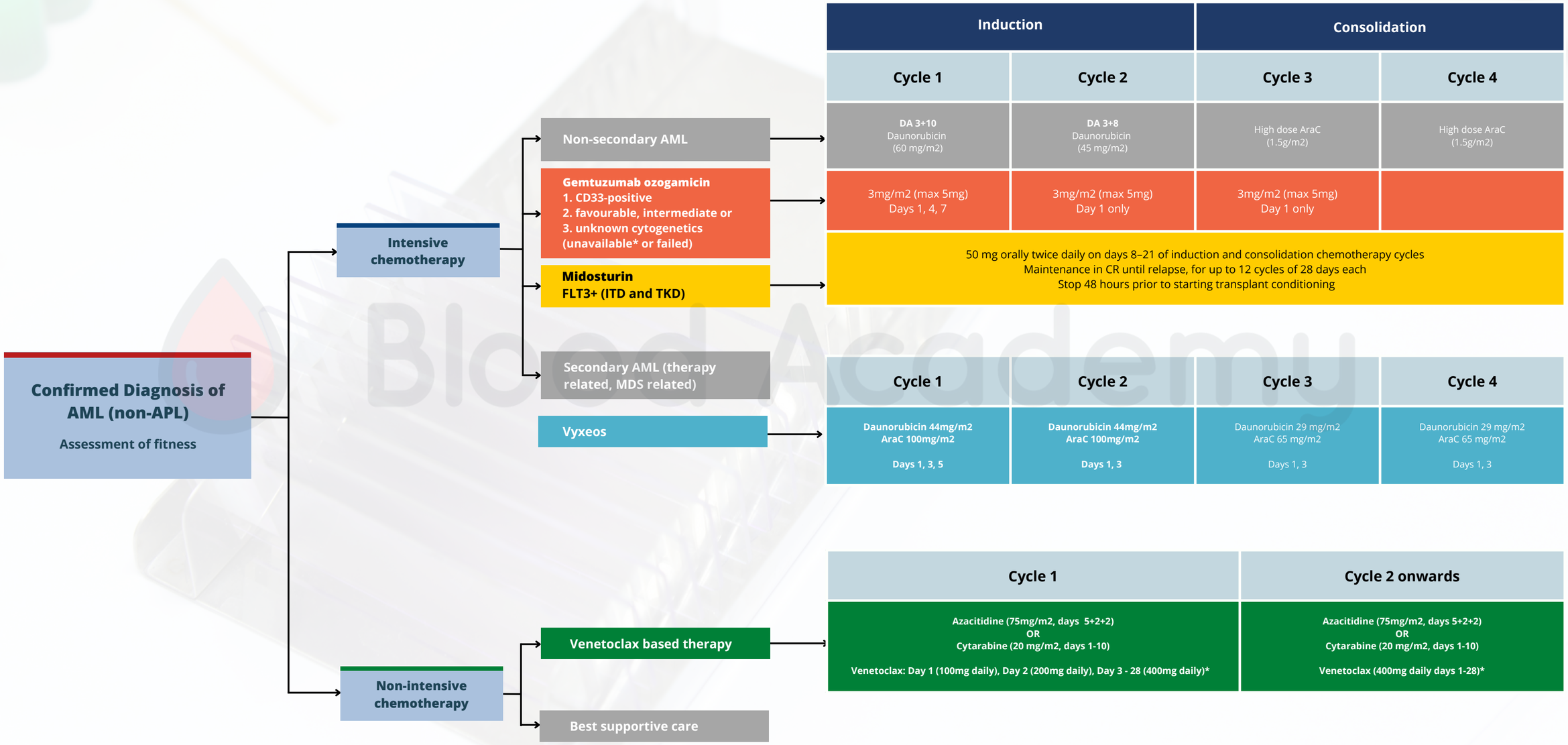
IMPORTANT MORPHOLOGICAL ENTITIES

Subtype	**Morphological features**	Immunophenotype
APL with t(15; 17)(q24.1;q21.2); PML-RARA	Hypergranular blasts with bilobed nuclei and multiple Auer rods (faggot cell). Hypogranular variant lacks cytoplasmic granules.	High side scatter, CD117+, CD33++, CD34-,HLA-DR-
AML with inv(16)(p13.1q22) or t(16; 16)(p13.1;q22); CBFB-MYH11	Monoblasts with eosinophilia including eosinophilic precursors especially in the bone marrow	CD34+, HLA-DR+, CD117+. May show distinct populations: CD13, CD33, CD65, MPO (myeloblasts), CD4, CD14, CD64 (monoblasts)
AML with t(8; 21)(q22;q22.1); *RUNX1-RUNX1T1*	Neutrophilia, blasts with indented nuclei (hot), Auer rods	CD34+, CD117+, CD13+, CD33+, MPO+. Aberant CD19, CD56
AML with NPM1 Mutation	Monoblastic leukaemia, blasts show cup-shaped nuclei	CD34-, HLA-DR-. CD33+, CD117+, MPO+. Monocytic differentiation with CD64+, CD14+, and/or CD11b+. CD56 may be +
AML with t(9; 11)(p21.3;q23.3); KMT2A-MLLT3	Monoblastic leukaemia	Variable immunophenotype. Often CD34-, CD15+, CD14-, CD33+, CD13-
AML with t(6; 9)(p23;q34.1); DEK-NUP214	Monoblastic leukaemia, sometimes with basophilic differentiation	Variable immunophenotype. CD34+, CD117+, CD33+, CD13+, CD9+, CD38+
AML with inv(3)(q21.3q26.2) or t(3; 3)(q21.3;q26.2); *GATA2, MECOM*	Dysplastic megakaryocytes, erythroid and granulocytic dysplasia often present	CD34+, CD117+, CD33+, CD13+, MPO-, megakaryocytic differentiation (CD41+, CD42+, and/or CD61+)
Acute Megakaryoblastic Leukaemia with t(1; 22)(p13.3;q13.1); *RBM15-MKL1*	Megakaryoblasts with basophilic staining cytoplasmic blebs	CD41+, CD42+, and/or CD61+
Myeloid Proliferations Associated with Down's Syndrome	Megakaryoblasts with giant and hypogranular platelets	CD41+, CD42+, and/or CD61+

2022 European LeukemiaNet (ELN) risk classification by genetics at initial diagnosis

Risk Category	Genetic Abnormality
Favorable	• t(8;21)(q22;q22.1)/RUNX1::RUNX1T1 • inv(16)(p13.1q22) or t(16;16)(p13.1;q22)/CBFB::MYH11 • Mutated NPM1 without FLT3-ITD • bZIP in-frame mutated CEBPA
Intermediate	• Mutated NPM1 with FLT3-ITD • Wild-type NPM1 with FLT3-ITD • t(9;11)(p21.3;q23.3)/MLLT3::KMT2A • Cytogenetic and/or molecular abnormalities not classified as favorable or adverse
Adverse	• t(6;9)(p23;q34.1)/DEK::NUP214 • t(v;11q23.3)/KMT2A-rearranged • t(9;22)(q34.1;q11.2)/BCR::ABL1 • t(8;16)(p11;p13)/KAT6A::CREBBP • inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2)/GATA2, MECOM(EVI1) • t(3q26.2;v)/MECOM(EVI1)-rearranged • -5 or del(5q); -7; -17/abn(17p) • Complex karyotype,h monosomal karyotype • Mutated ASXL1, BCOR, EZH2, RUNX1, SF3B1, SRSF2, STAG2, U2AF1, or ZRSR2j • Mutated TP53

Currently approved treatments for first line management of acute myeloid leukaemia (non-APL) in the UK



FINAL DIAGNOSIS

Acute myeloid leukaemia with mutated NPM1

ADVANCED MORPHOLOGY COURSE

SESSION 2

Cases 1 - 4

Blood Academy

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