Acute Leukemie

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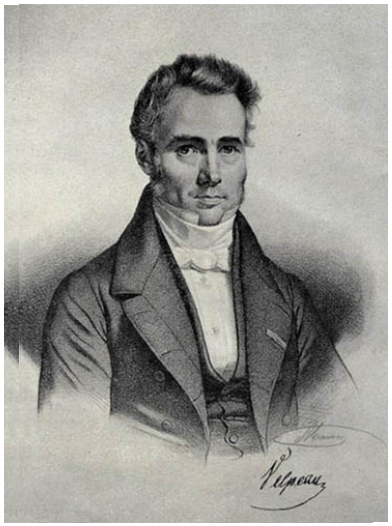
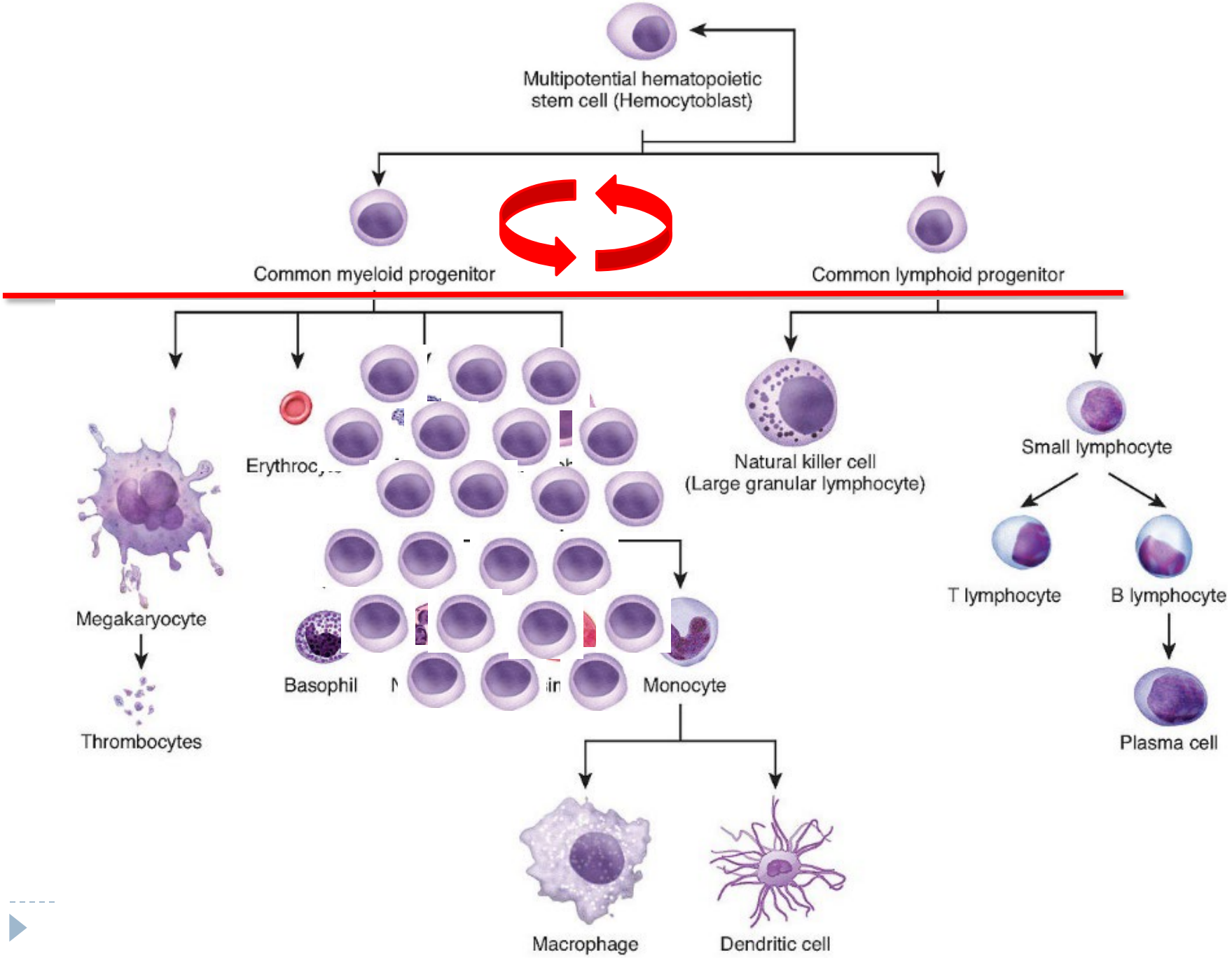
Klinisch Chemicus, Bijzondere Hematologie UMCG

Historie leukemie

- 1827 Velpeau, 1845 Virchow, 1855 Neuman

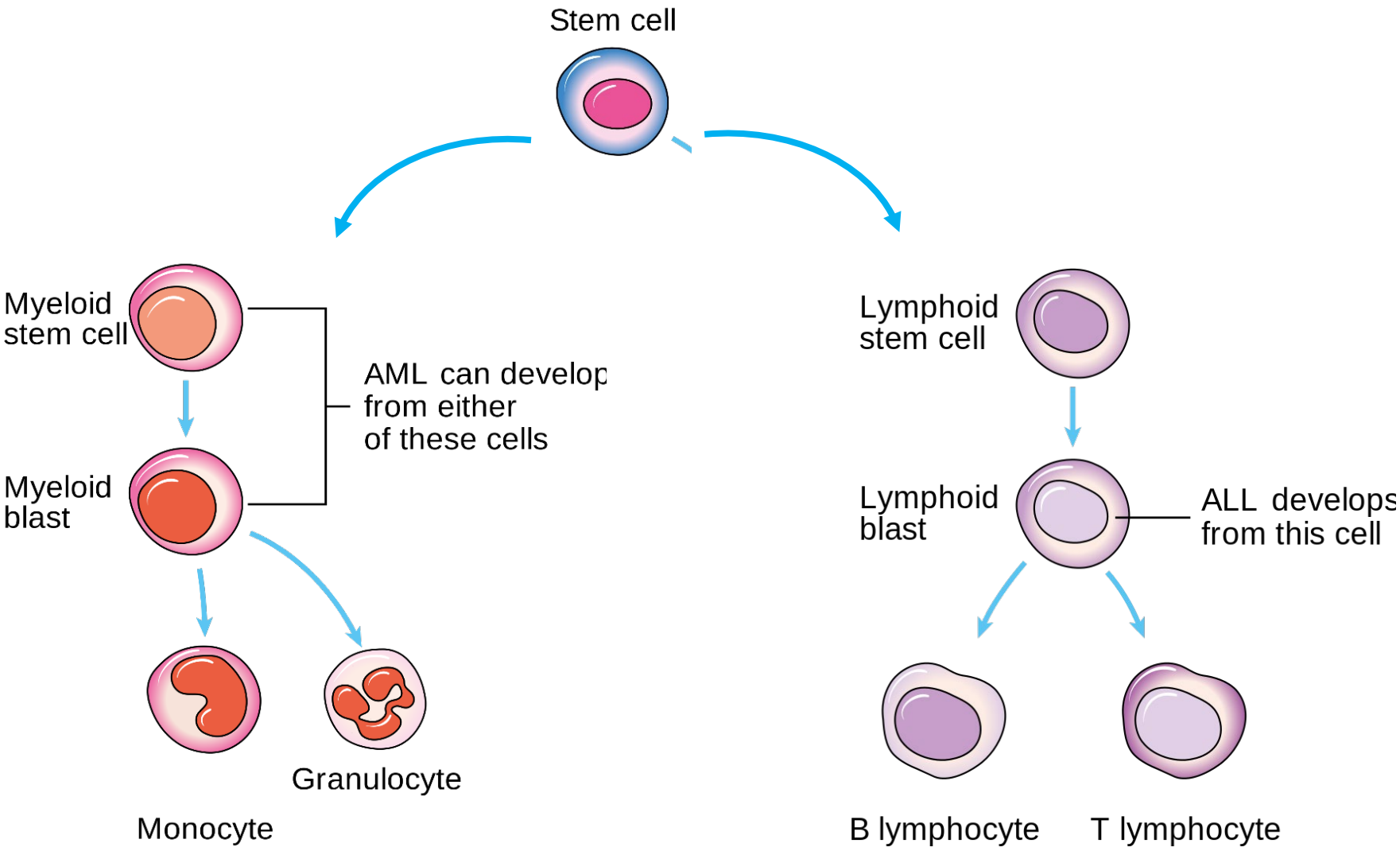


- Afwijkende hoeveelheid witte bloedcellen
- Leukemie *Grieks Leukos (wit) en haima (bloed)*
- Beenmerg is aangedaan bij acute leukemie

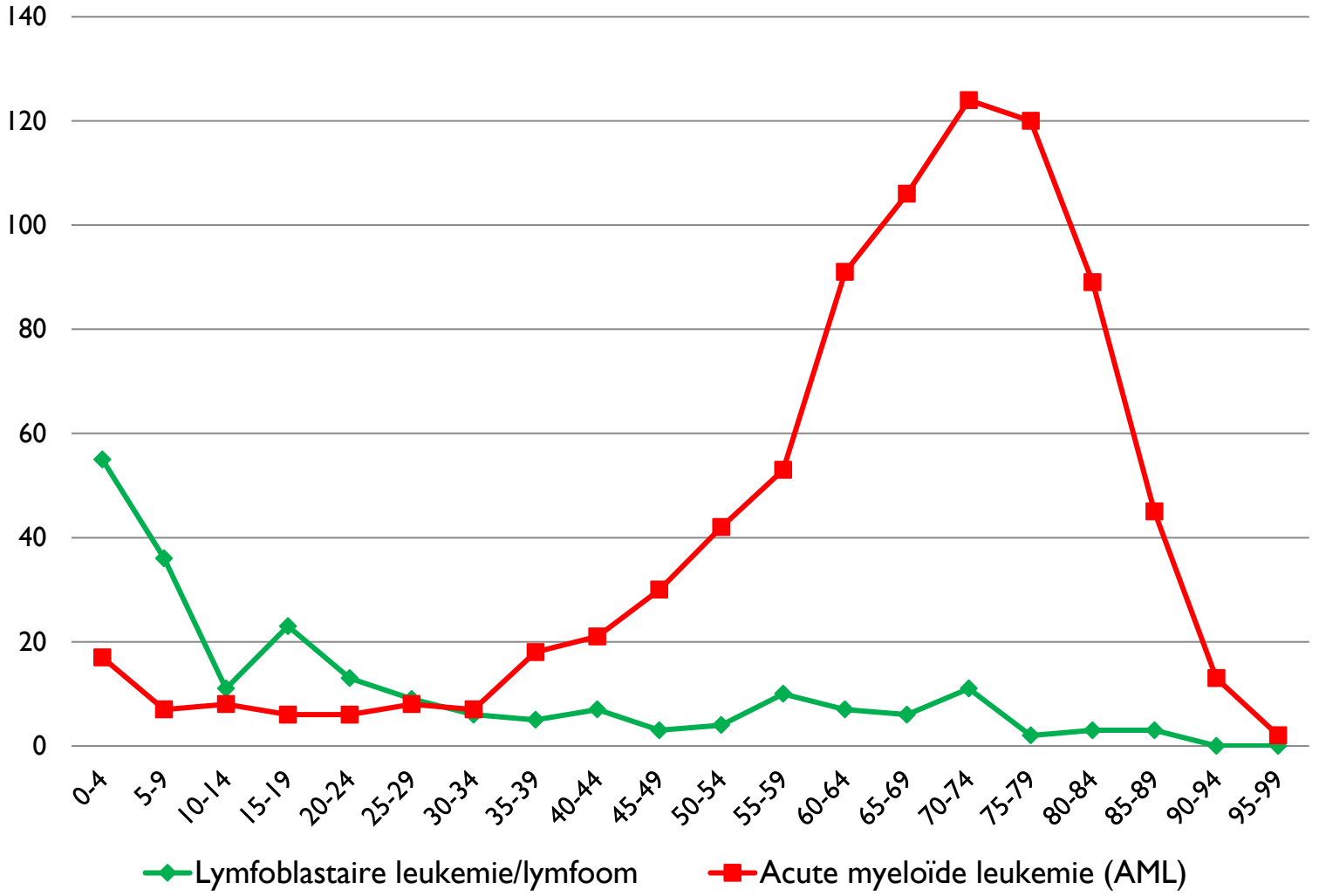

Multipotential hematopoietic stem cell (Hemocytoblast)
Common myeloid progenitor
Common lymphoid progenitor
Megakaryocyte
Thrombocytes
Erythroc
Basophil
Monocyte
Natural killer cell (Large granular lymphocyte)
Small lymphocyte
T lymphocyte
B lymphocyte
Plasma cell
Macrophage
Dendritic cell

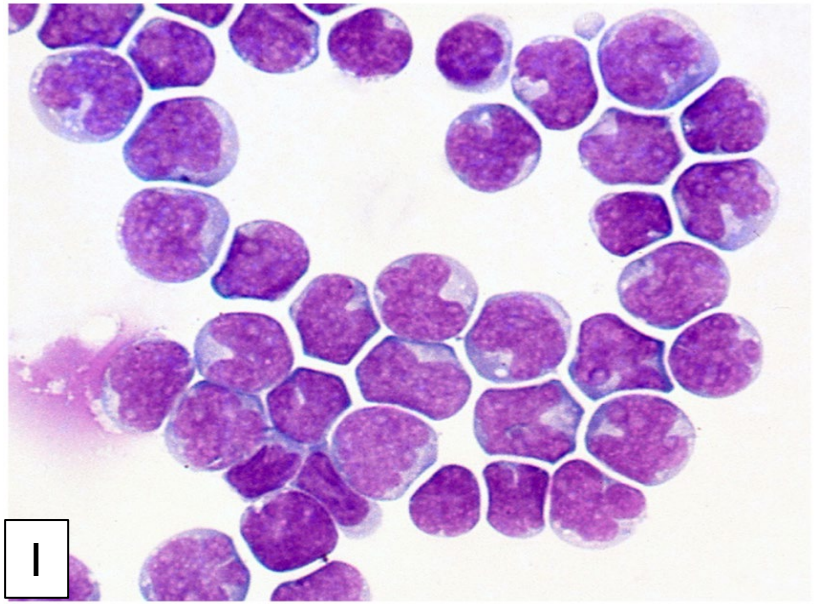
Acute myeloïde leukemie

Acute lymfatische leukemie



Incidentie ALL/AML 2022

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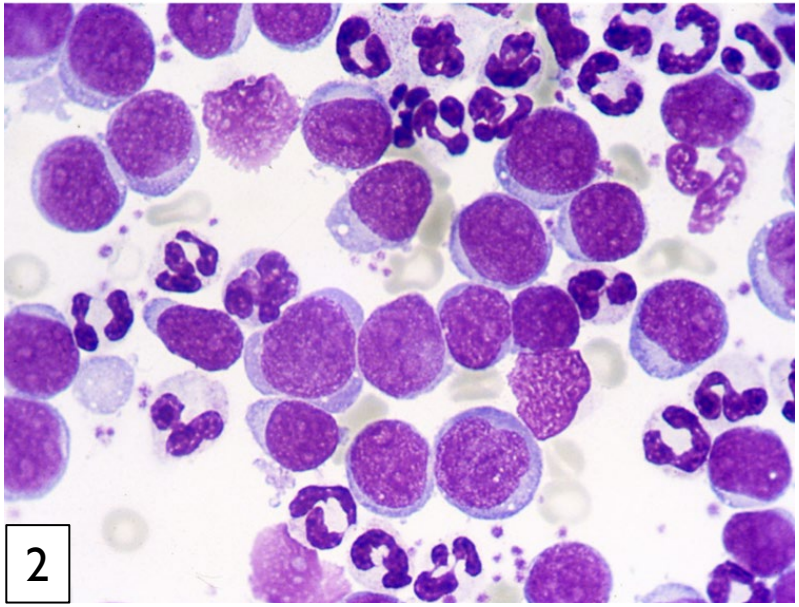
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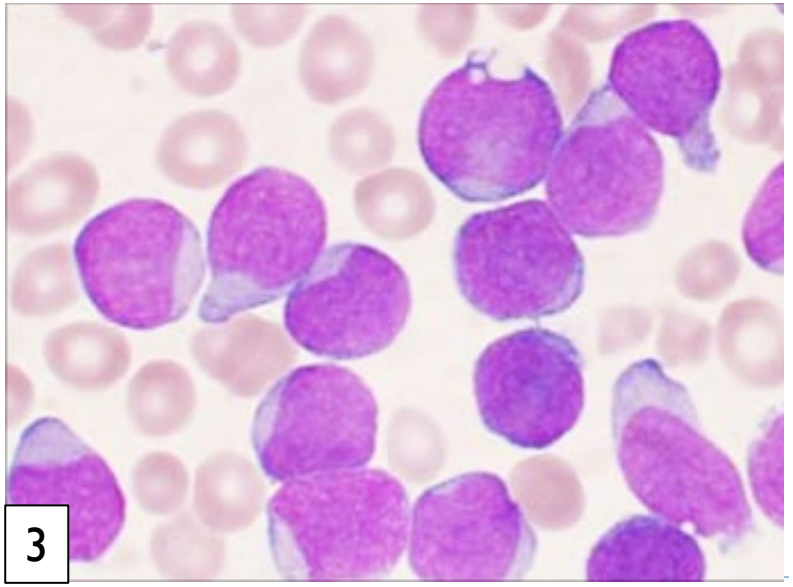
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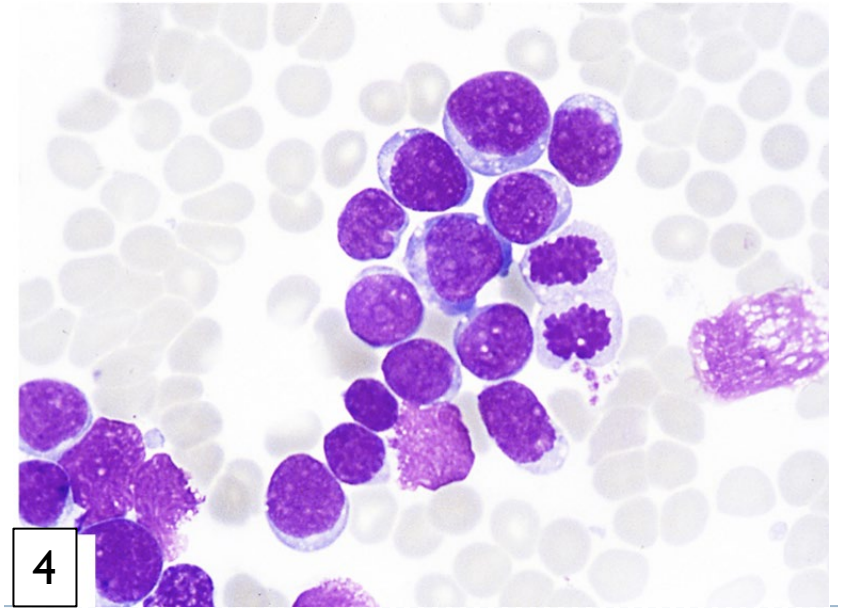
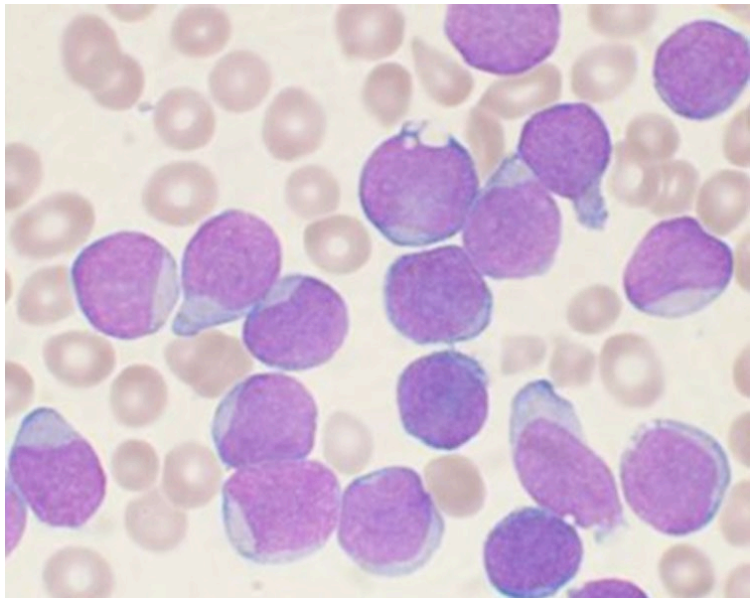
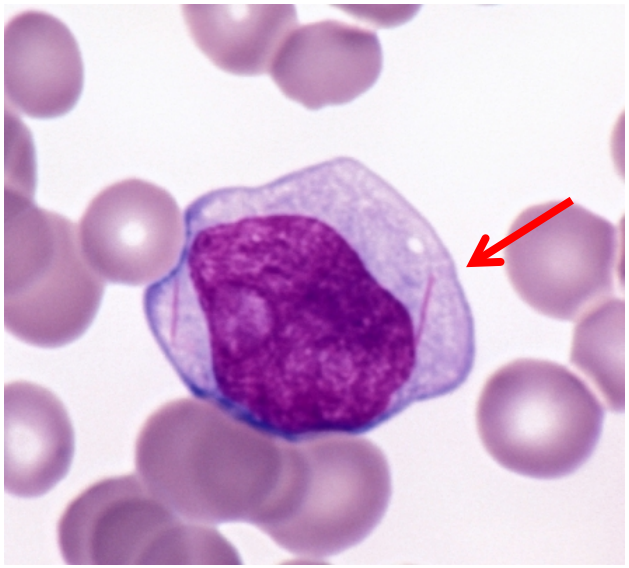

4

Morfologie lymfatisch vs myeloid



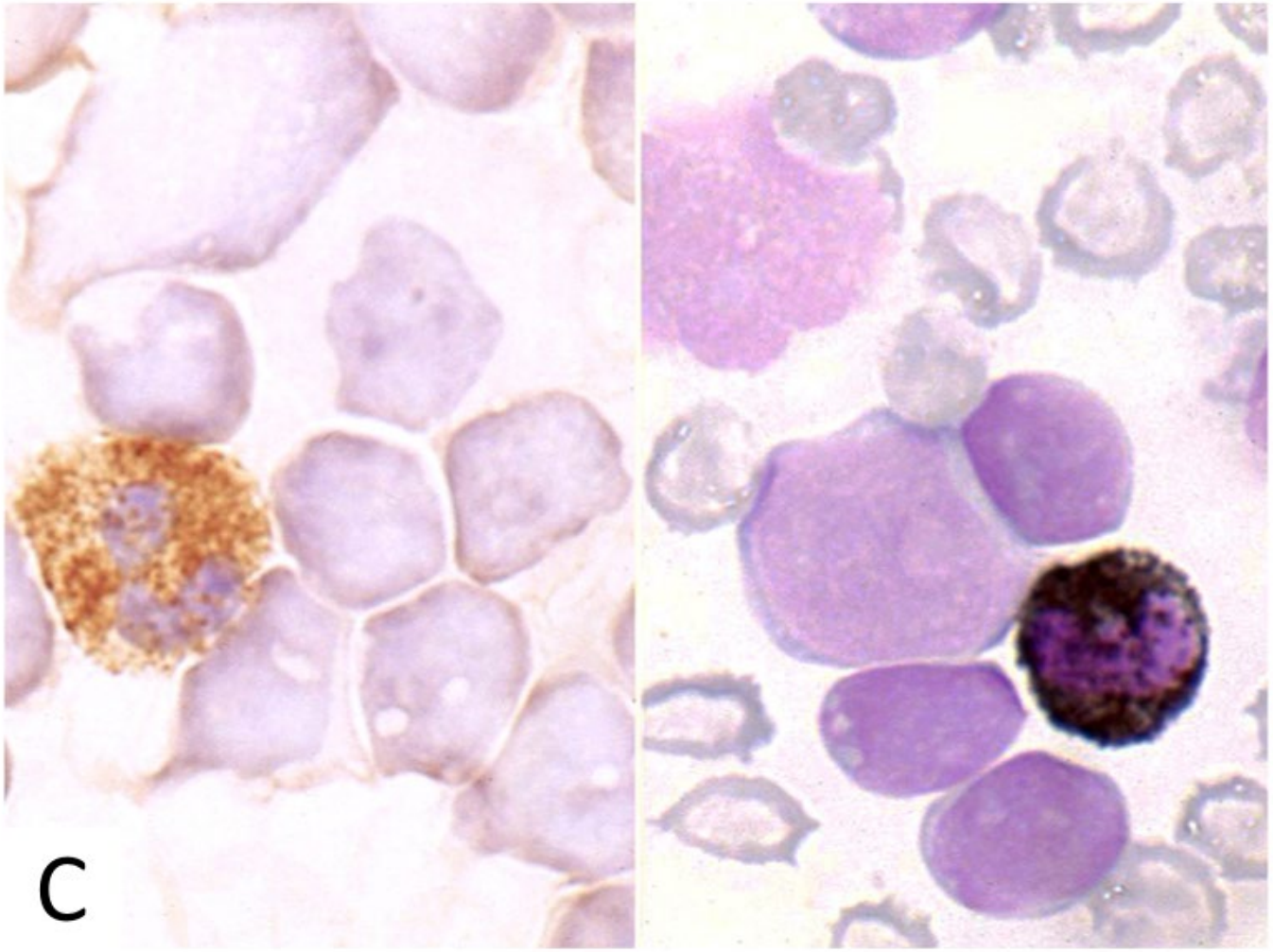


Kenmerken van blasten
-Auerse staaf
-Korreling
-Nucleolus
-Grote
-Cytoplasma
-Hand-mirror cel
-Chromatine

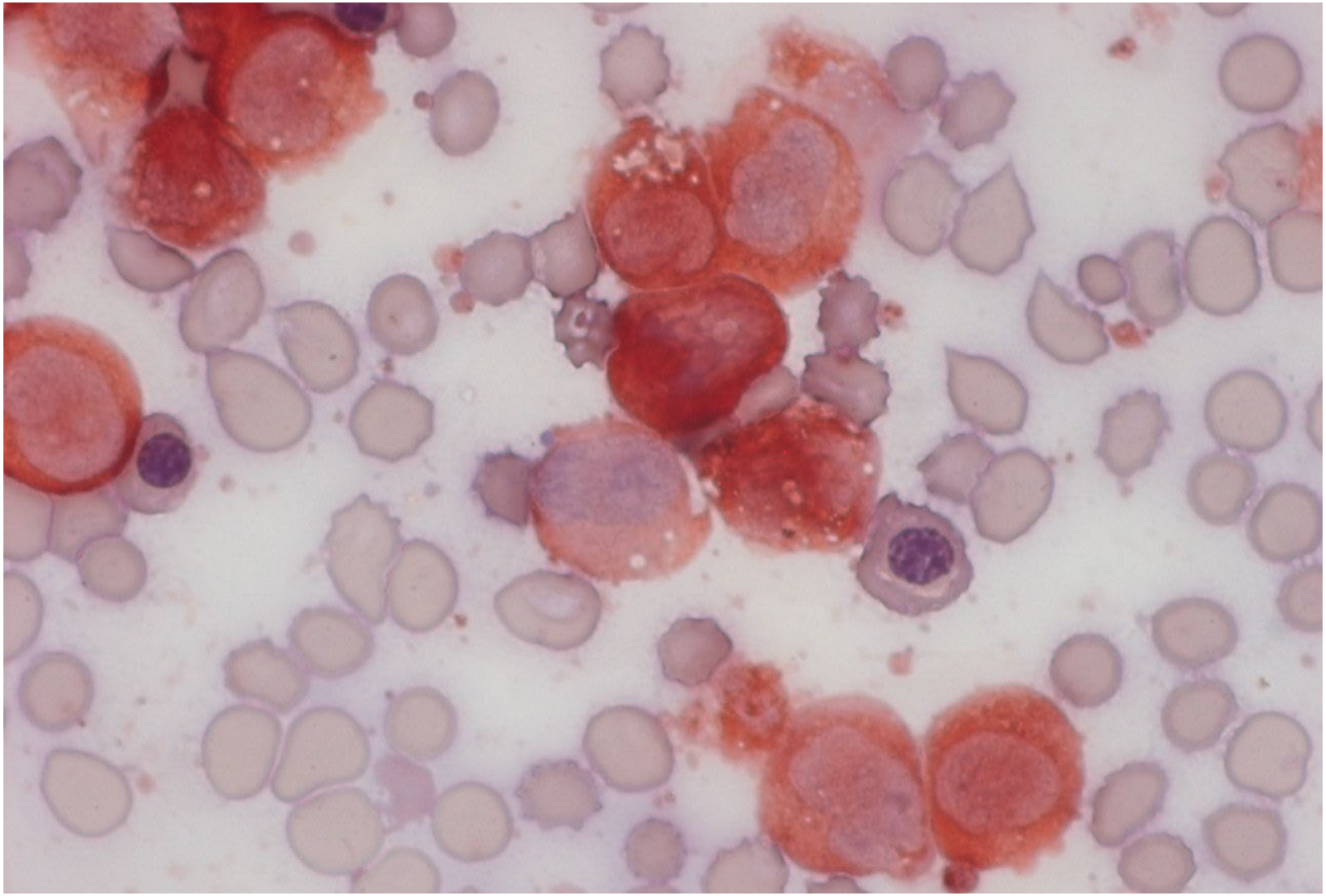
Cytochemische kleuringen

- Peroxidase
  - Kleuring van Myeloperoxidase
- Sudan-Black
  - Kleurstof voor fosfolipiden van primaire en secundaire granules myelo en monocytaire cellen
- Esterase m.b.v. alfa-naftylacetaat
  - Aantonen van esterase enzymen in met name monocyten, T-cellen en megakaryocyten

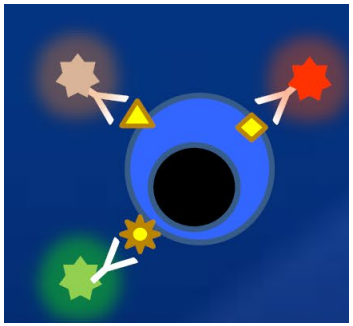
MPO en Sudan Black

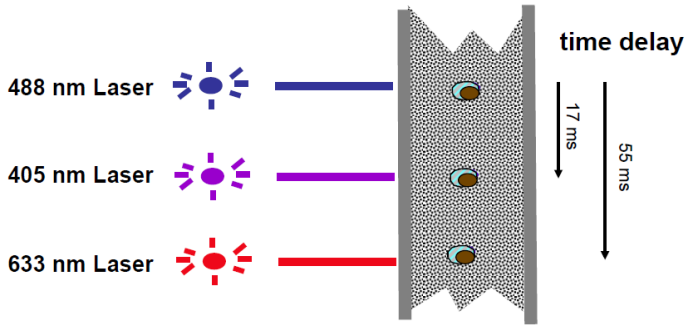


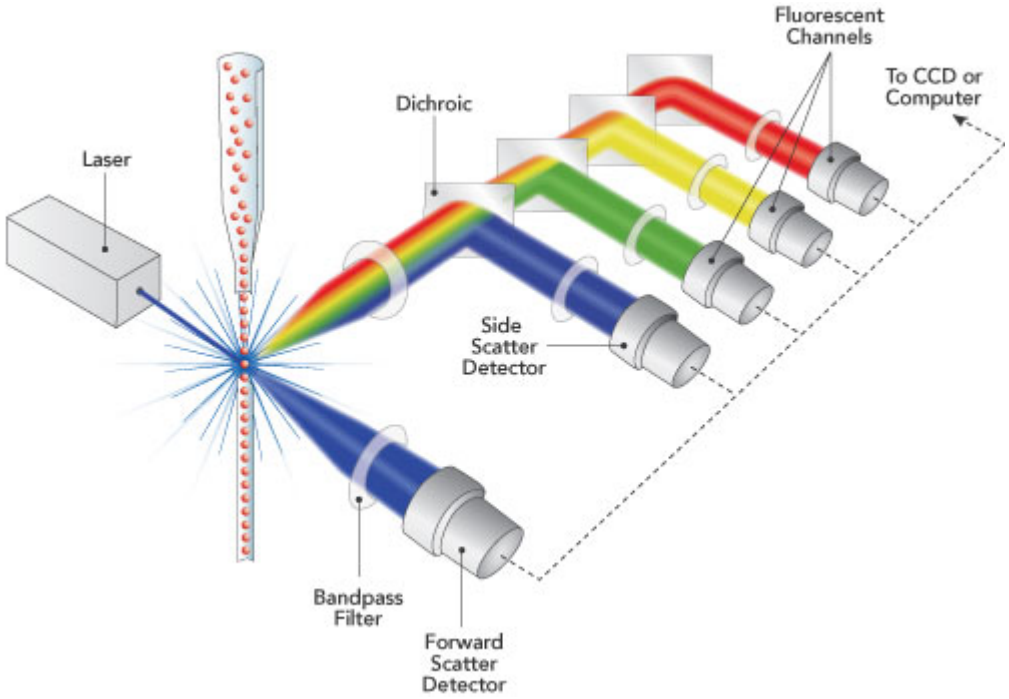
Esterase



Flow cytometrie





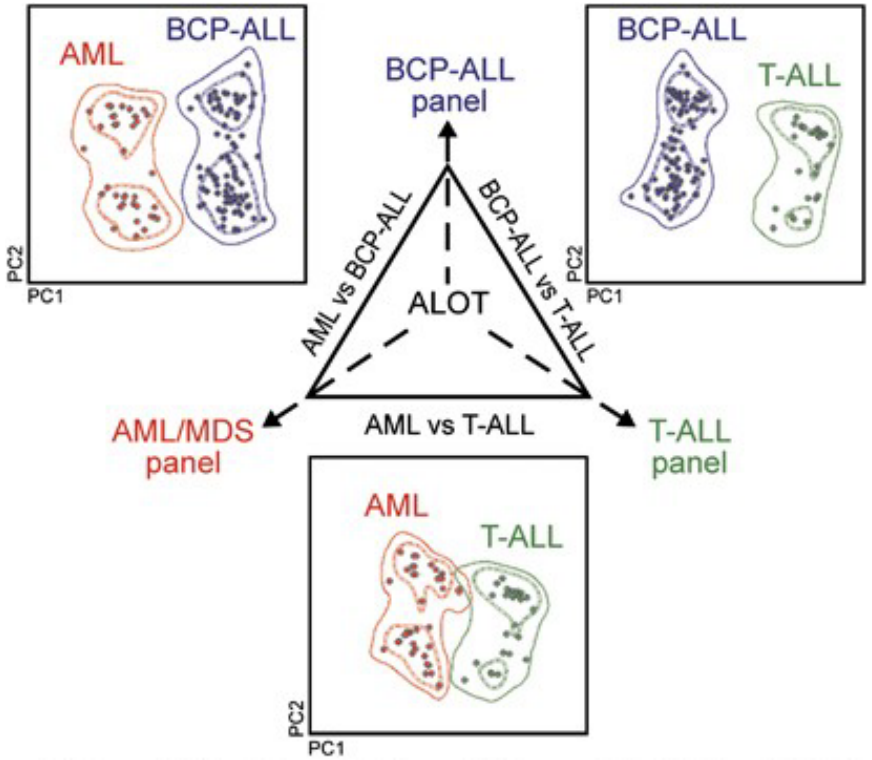


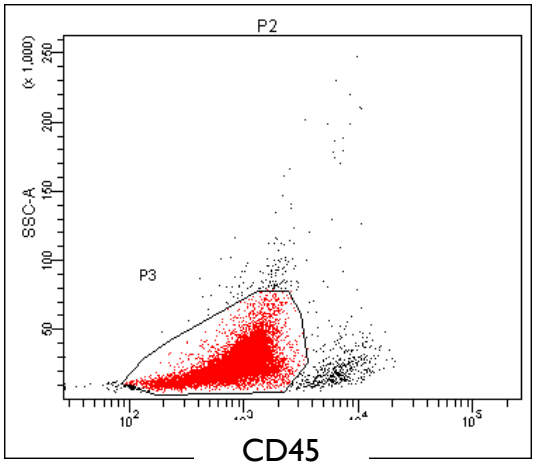
EuroFlow

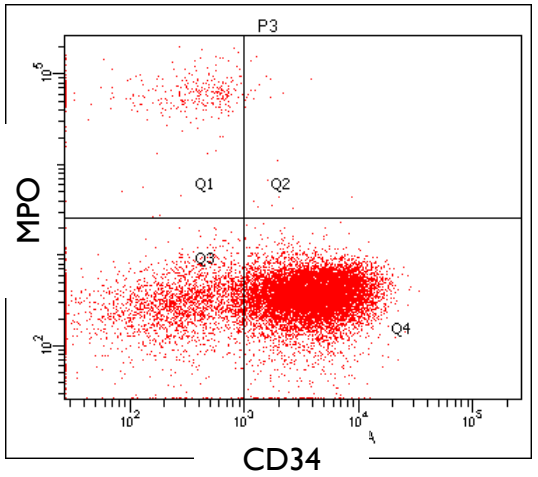
Acute Leukemia Orientation Tube (ALOT)

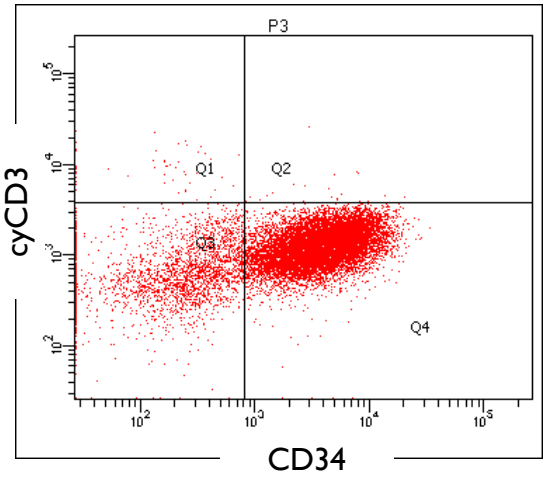
cyCD3	CD45	cyMPO	cyCD79a	CD34	CD19	CD7	CD3

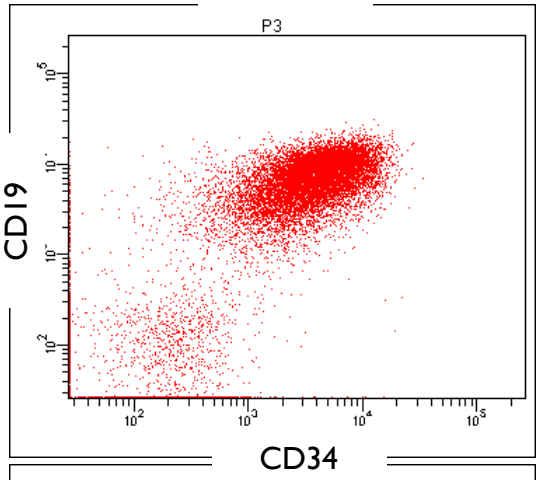
Lijn	Markers
Myeloid	MPO (CD117, CD13, CD33)
	2x (CD11c, CD14, CD64)
T lineage	Cytoplasmatisch CD3
	Oppervlakte CD3
B lineage	CD19 sterk + (CD79a, CD22 of CD10)
	CD19 zwak + 2x (CD79a, CD22 of CD10)

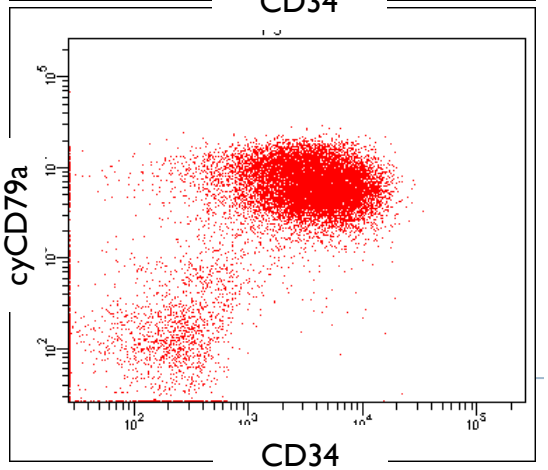












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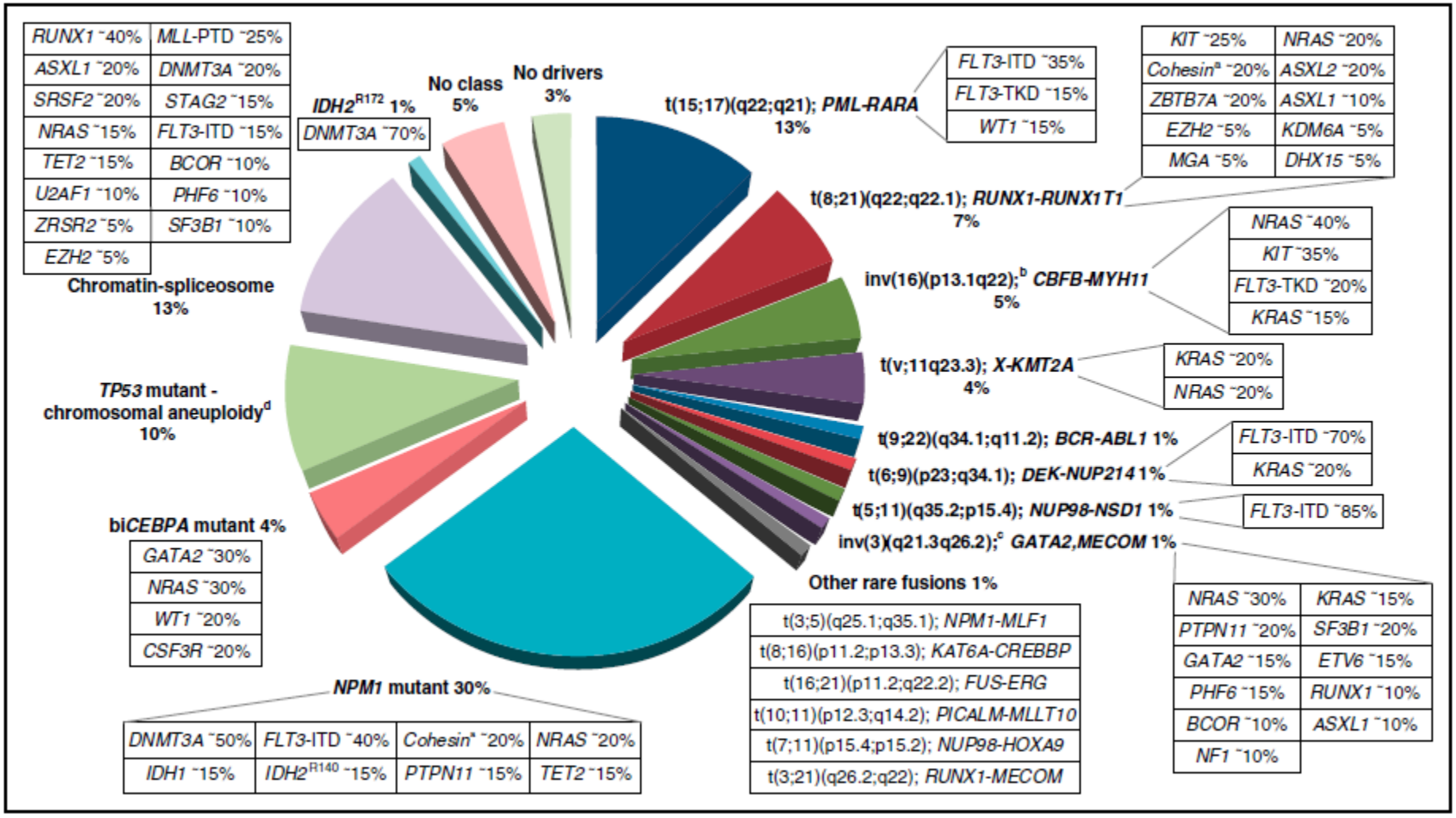
B lineage	CD19 sterk + (CD79a, CD22 of CD10)
	CD19 zwak + 2x (CD79a, CD22 of CD10)

Leukemie classificatie FAB → WHO

- FAB (French-American-British classification) 1976
  - Based on morphology and cytochemistry
- FAB 1982
  - Based on morphology and cytochemistry
- WHO 2001
  - Based on morphology, cytochemistry, (immunophenotyping), cytogenetics and clinical presentation
- WHO 2008
  - Based on morphology, cytochemistry, (immunophenotyping), cytogenetics, clinical presentation (and *molecular biology)*
- WHO 2016 revision/2017
  - Based on morphology, cytochemistry, immunophenotyping, cytogenetics, clinical presentation and more *molecular biology*

AML is complex (genetisch)



FOKKE & SUKKE
HADDEN EEN GESCHIL

Leukemia

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The 5th edition of the World Health Organization Classification of Haematolymphoid Tumours: Myeloid and Histiocytic/ Dendritic Neoplasms

blood®

Special Report

Check for updates

International Consensus Classification of Myeloid Neoplasms and Acute Leukemias: integrating morphologic, clinical, and genomic data

AML Classificatie ICC/WHO 2023

1. AML met genetische veranderingen
   1. % blasten maakt niet uit/ICC >10% blasten
   2. >20% blasten BCR:ABL1

2. AML met TP53 mutaties
   1. >20% blasten
   2. VAF >10% TP53 mutatie

3. AML met myelo dysplasie gerelateerde afwijkingen
   1. MDS gerelateerde genmutaties
   2. MDS gerelateerde cytogenetische afwijkingen

4. AML NOS (niet nader gespecificeerd)/op basis van differentatie

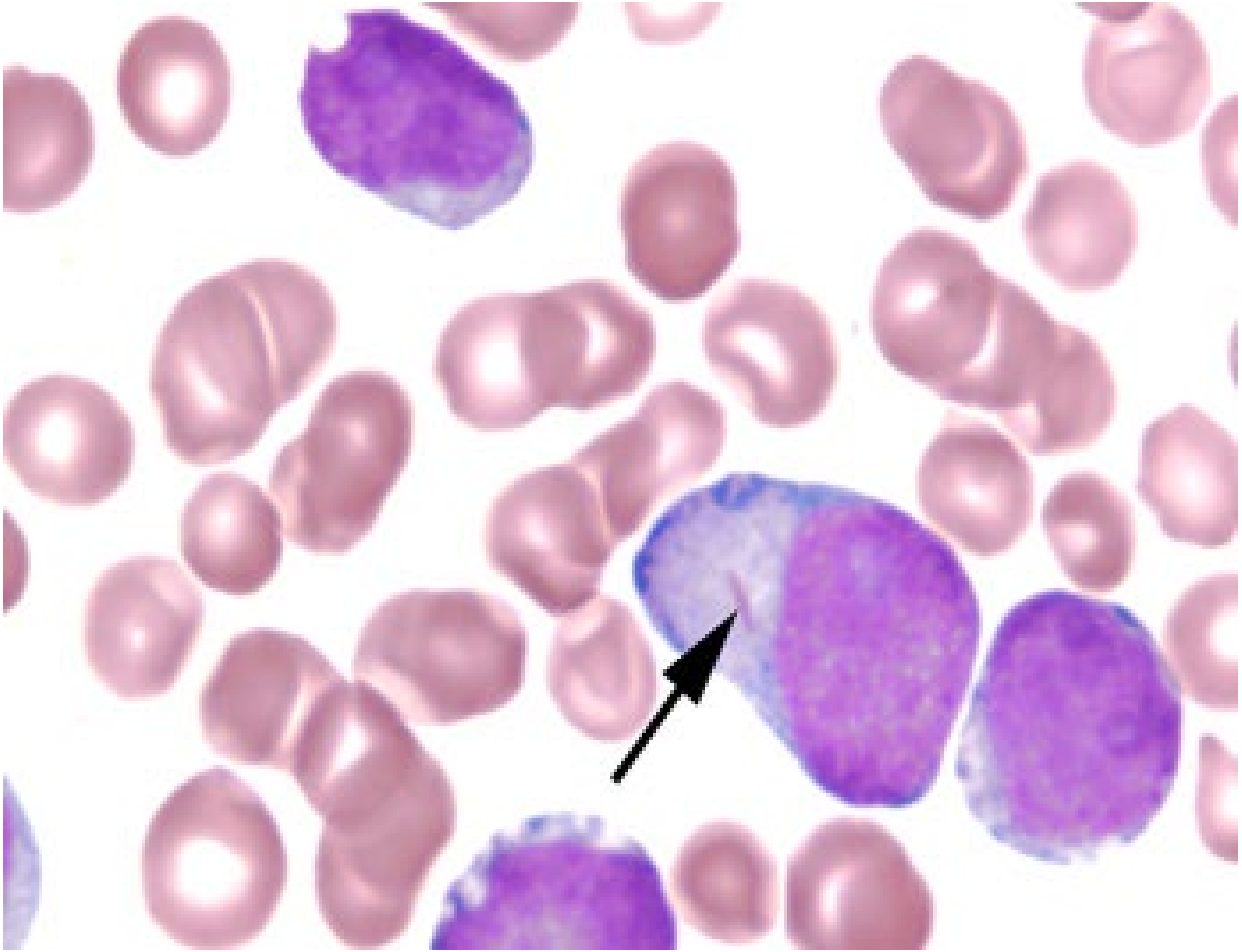
Diagnostiek AML

- Morfologie (200 cellen bloed/500 beenmerg)
- Flowcytometrie
- Moleculaire diagnostiek (PCR of NGS)
  - **FLT3-ITD, FLT3-TKD**, **IDH1, IDH2,** NPM1, CEBPA, DDX41, TP53, ASXL1, BCOR, EZH2, RUNX1, SFRB1, SRSF2, U2AF1 en ZRSR2.
  - Translocaties; PML::RARA, CBFB::MYH11, RUYNX1::RUNXT1, BCR::ABL1

  Aanbevolen wordt ook;
  - ANKRD26, BCORL1, BRAF, CBL, CSF3R, DMT3A, ETV6, GATA2, JAK2, KIT, KRS, NRAS, NF1, PHF6, PPM1D, PTPN11, RAD21, SETBP1, TET2 en WT1
- Cytogenetica
- Pathologie

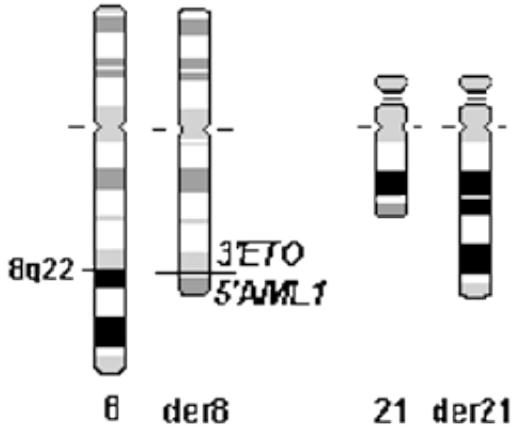
AML met genetische veranderingen

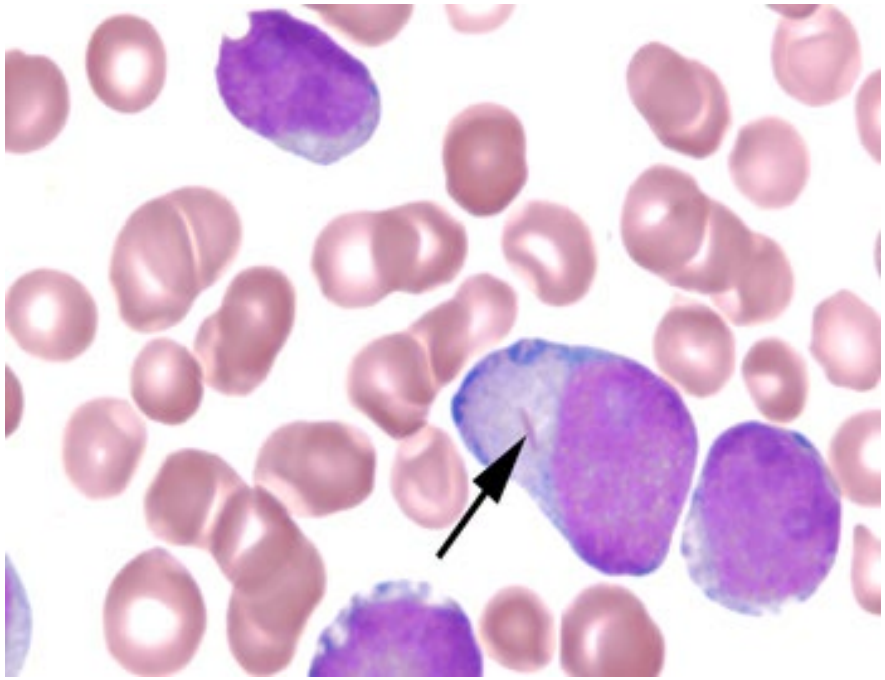
Acute myeloid leukaemia with defining genetic abnormalities
Acute promyelocytic leukaemia with *PML*::*RARA* fusion
Acute myeloid leukaemia with *RUNX1*::*RUNX1T1* fusion
Acute myeloid leukaemia with *CBFB*::*MYH11* fusion
Acute myeloid leukaemia with *DEK*::*NUP214* fusion
Acute myeloid leukaemia with *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

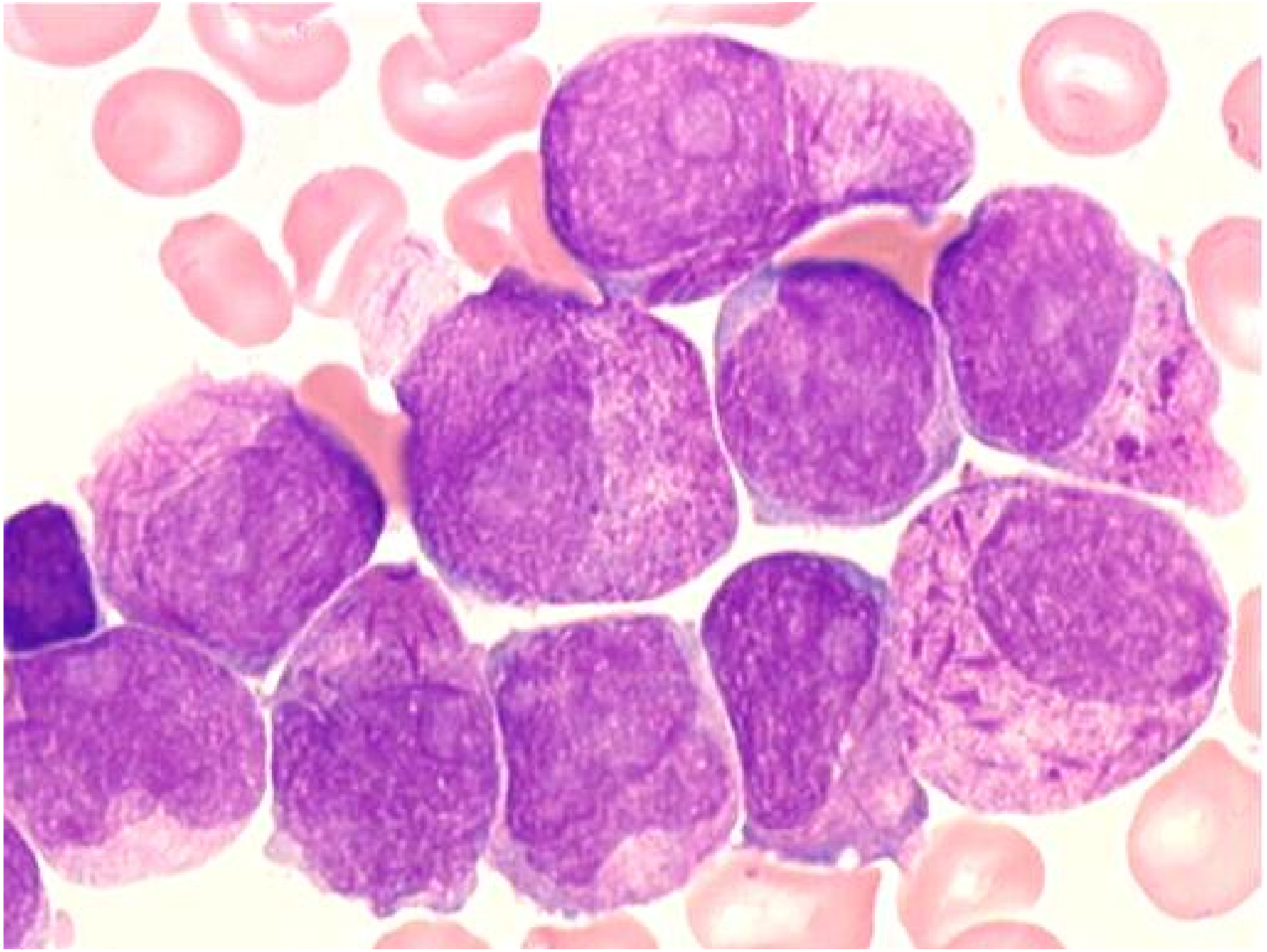


AML met t(8;21); RUNX1::RUNX1T1

- Bij <20% blasten AML
- Prevalentie c.a. 5% van de AML patiënten
- Vaak uitrijping tot neutrofielen
- Grote blasten met vaak met fijne auerse staaf.
- Flow cytometrie vaak positief voor CD19 en CD56

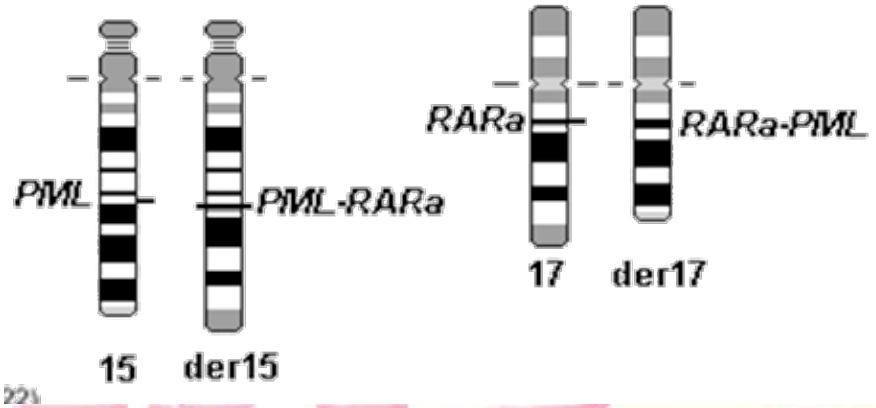


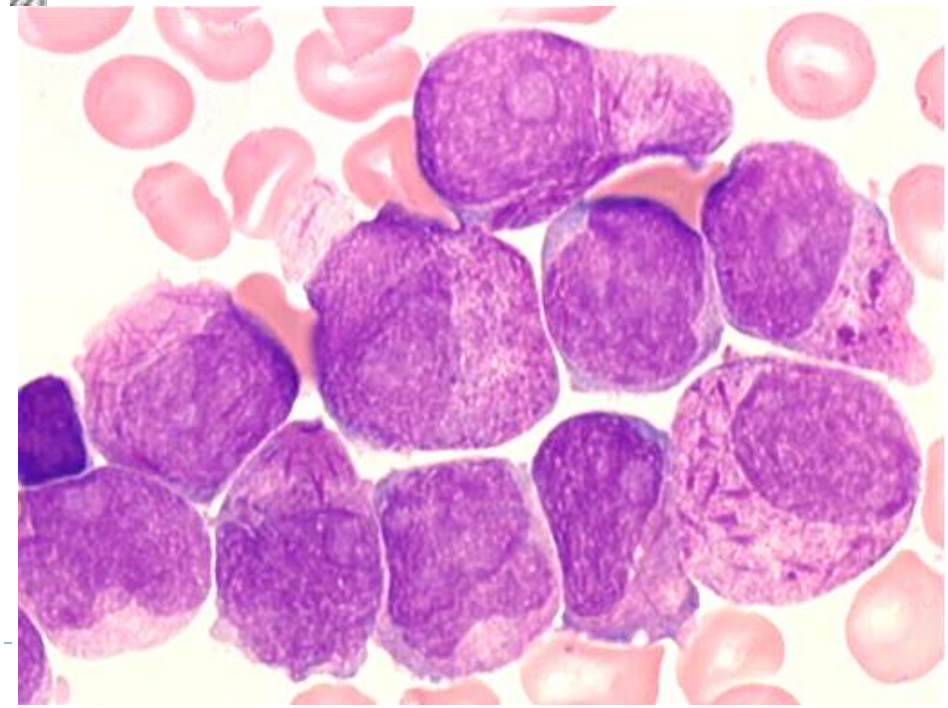


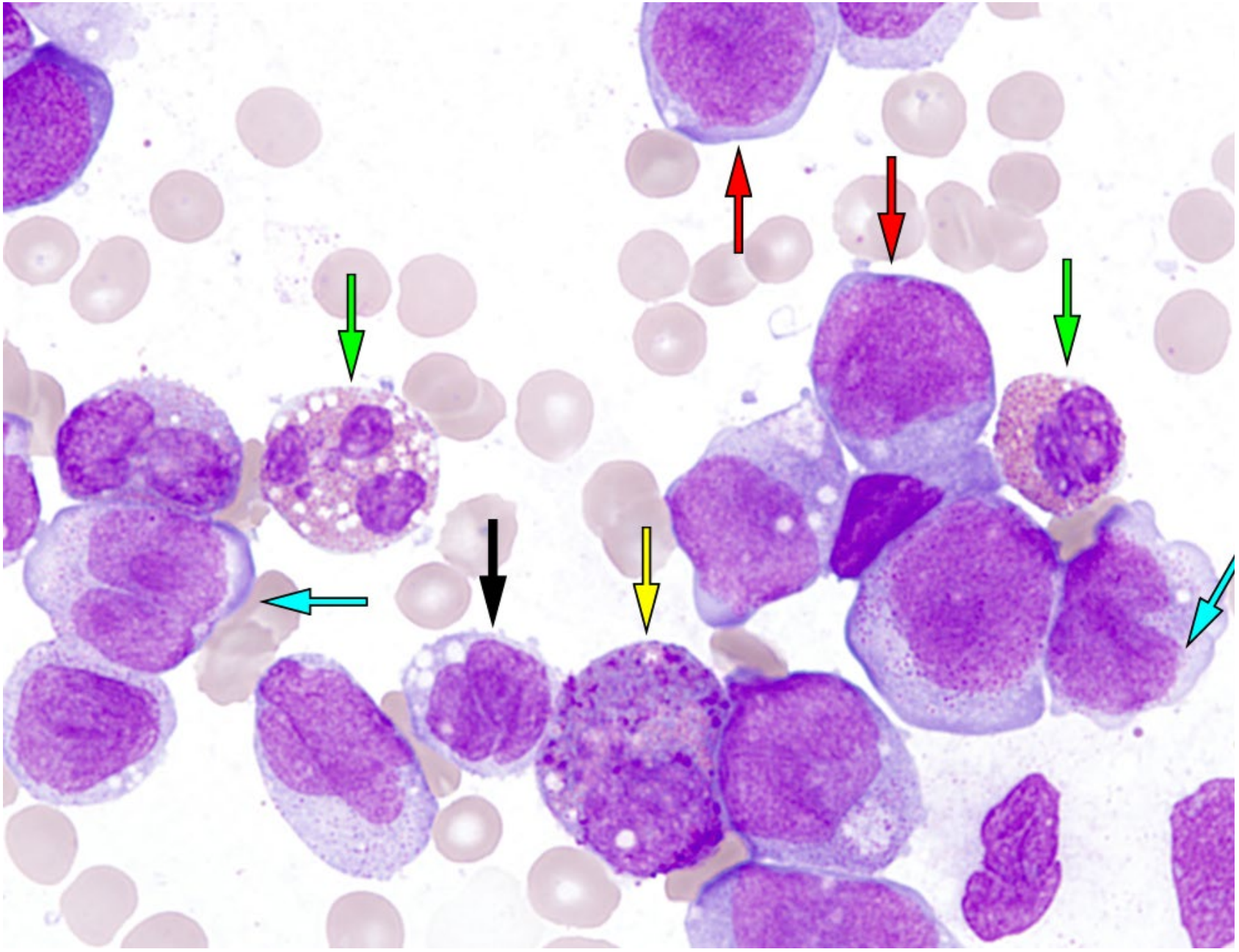


APL met t(15;17); PML::RARA

- Ook bij <20% blasten/promyelocyten nog steeds APL
- Mortaliteit hoog bij late diagnose
- DIS met hersenbloedingen
- Snel starten met ATRA
- Goede prognose

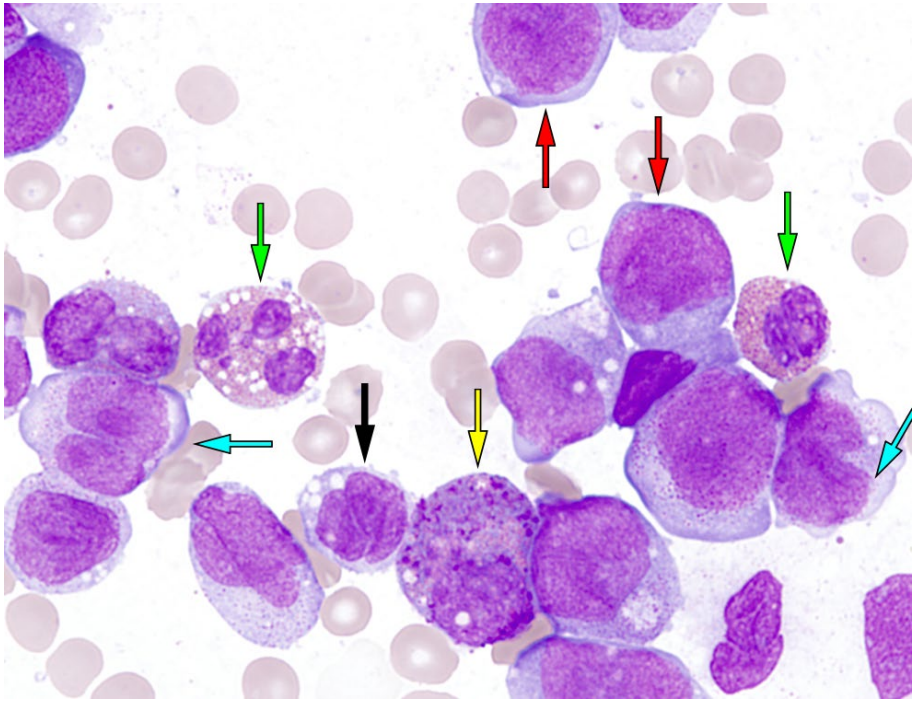


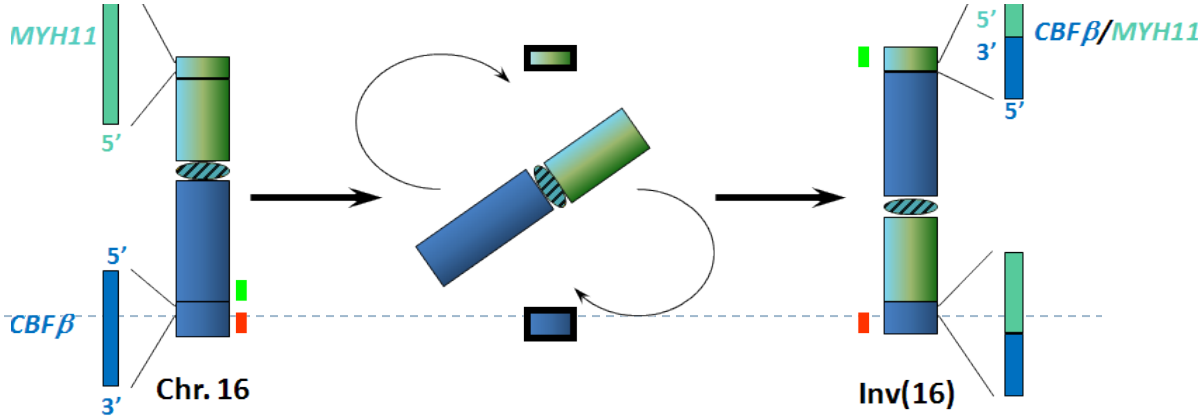


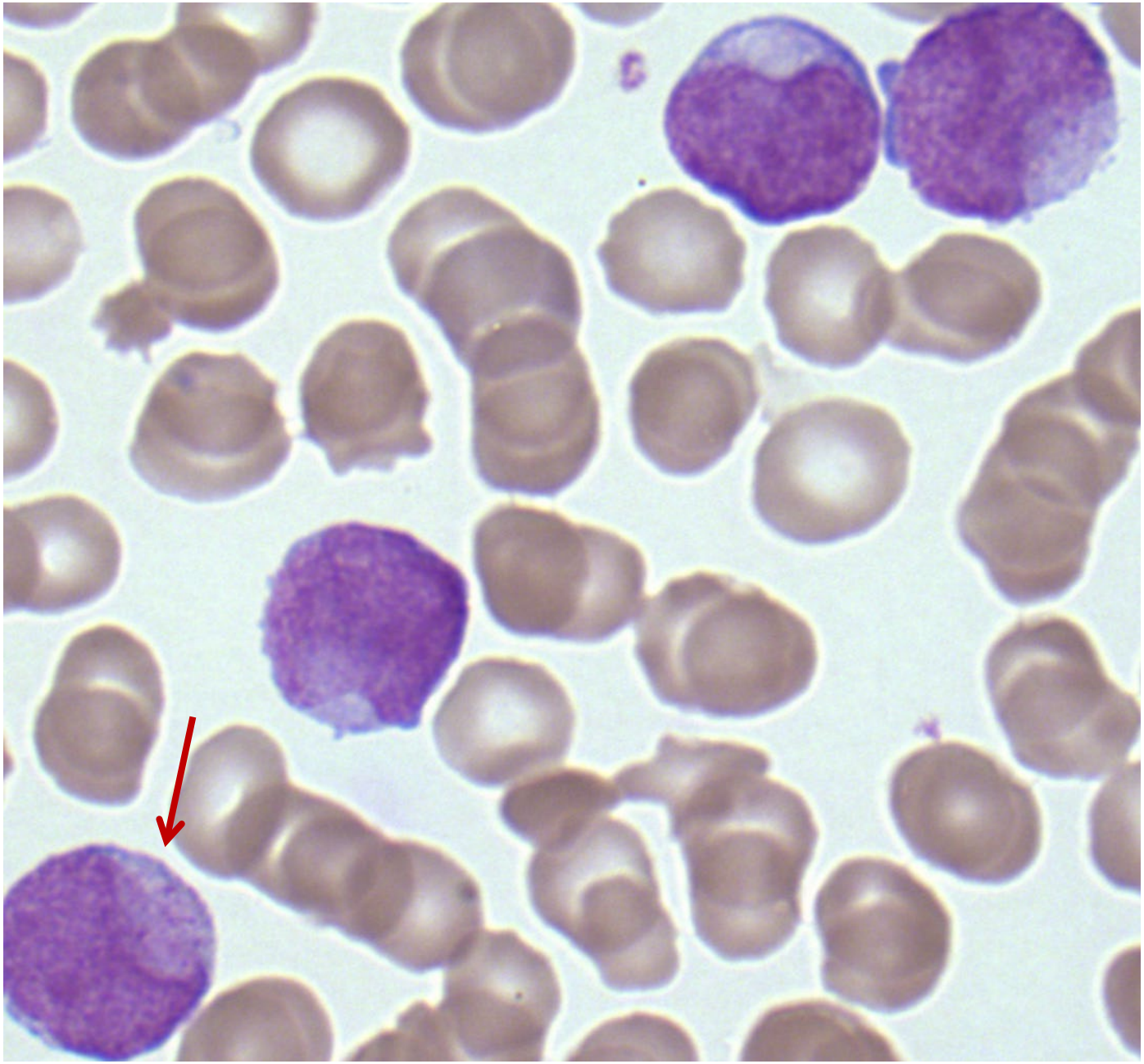


AML met inv(16) of t(16;16); CBFb::MYH11

- Bij <20% blasten AML
- Prevalentie c.a. 5% van de AML patiënten
- Monocytaire uitrijping met afwijkende eo's
- CD2 expressie met flow
- Goede prognose

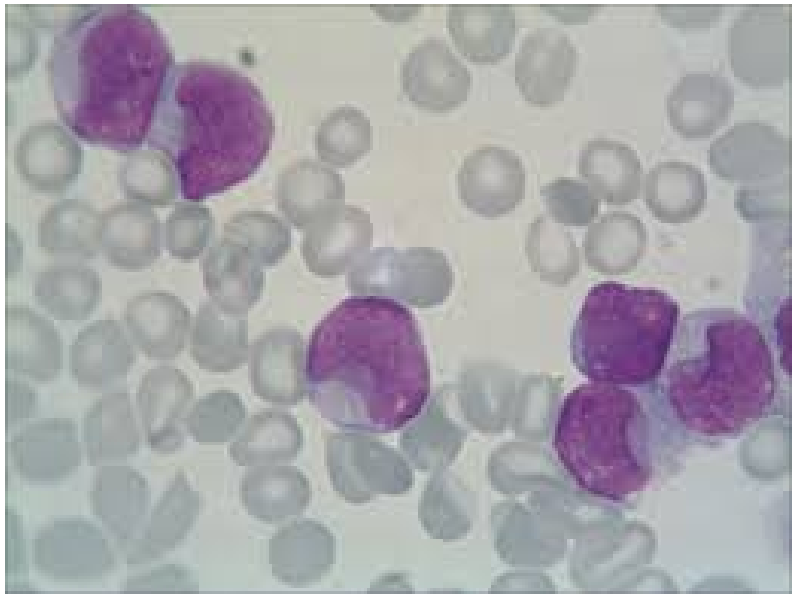






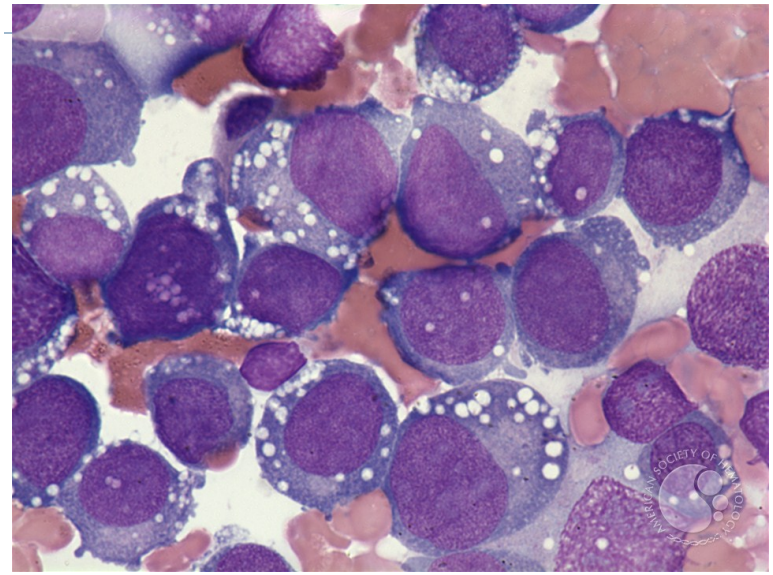
AML met NPM1 mutatie

- Bij <20% blasten AML
- Meest voorkomende mutatie bij AML (30%)
- Goede prognose, mits…
- Grote blasten met een deuk (cup-like cells).
- Flow cytometrie vaak negatief voor CD34 en HLA-DR



AML met TP53 mutatie

- Blasten >20%
- Variant allel fractie TP53 mutatie >10% zijn
- Puur erytroide leukemie
- Slechte prognose



AML met dysplasie gerelateerde veranderingen

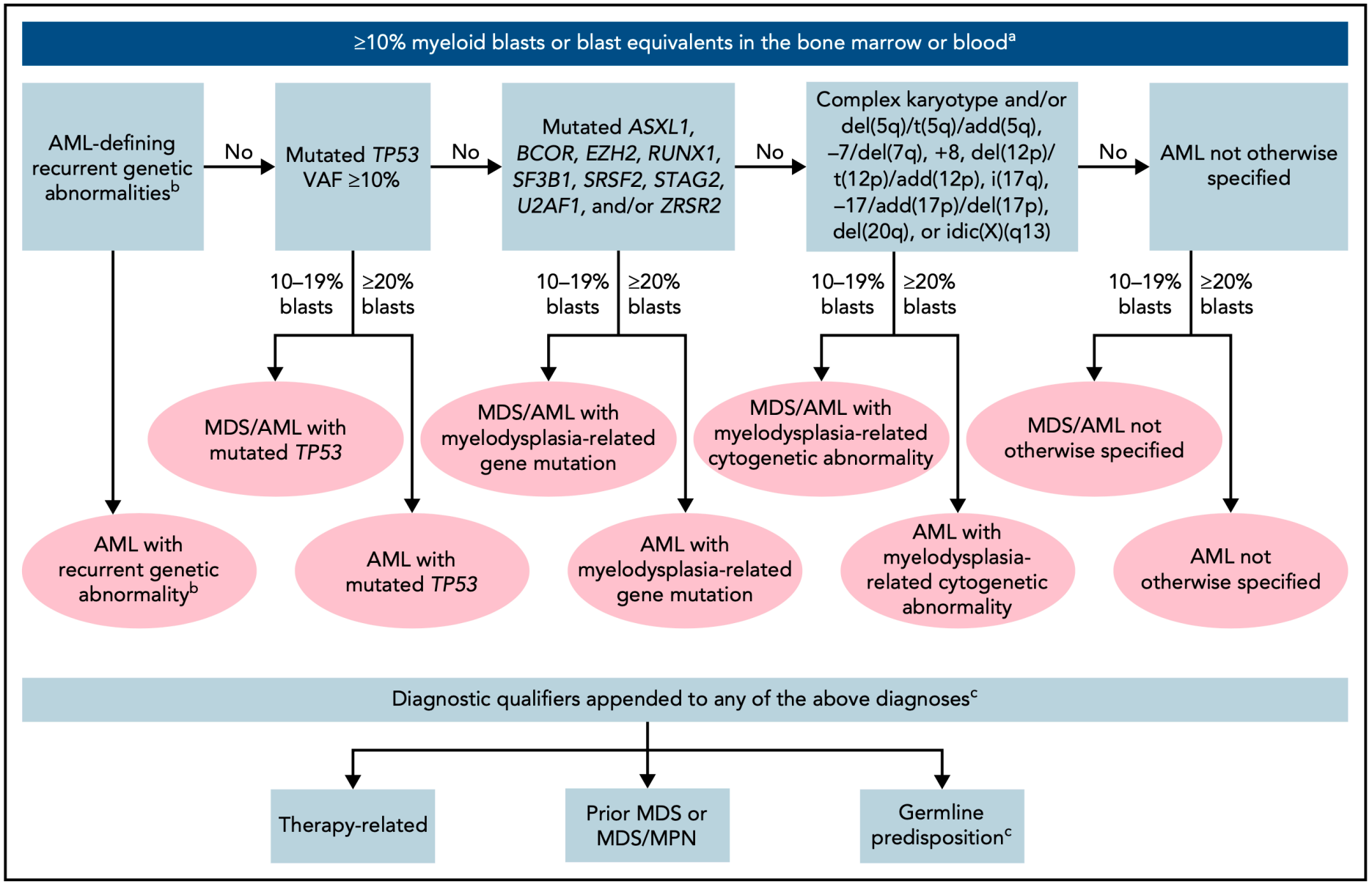
Criteria

- >20% blasten
- MDS gerelateerde genmutaties
- Of MDS gerelateerde cytogenetische afwijkingen
- Morfologie telt niet meer als criterium!!!

AML met dysplasie gerelateerde veranderingen

Unbalanced	balanced
-7 / del(7q)	t(11;16)
-5 / del(5q)	t(3;21)
inv(17q) / t(17p)	t(1;3)
-13 / del(13q)	t(2;11)
+8	t(5;12)
del(12p) / t(12p)	t(5;7)
Idic(X)(q13)	t(5;17)
Complex karyotype (> 3)	t(5;10)
	t(3;5)

- Moleculaire mutaties
  - ASLX1, BCOR, EZH2, RUNX1, SF3B1, SRSF2, STAG2, U2AF1 en ZRSR2

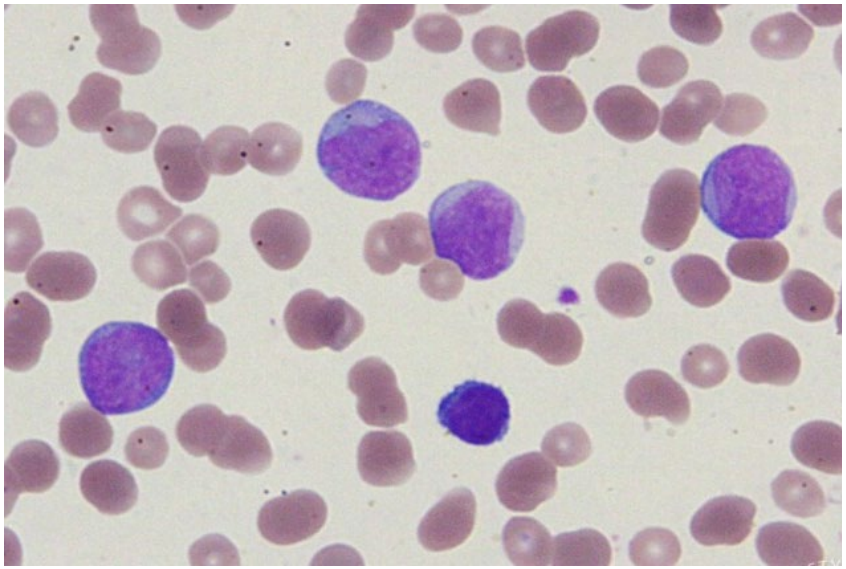

≥10% myeloid blasts or blast equivalents in the bone marrow or blood[a]
AML-defining recurrent genetic abnormalities[b]
No
Mutated TP53 VAF ≥10%
No
Mutated ASXL1, BCOR, EZH2, RUNX1, SF3B1, SRSF2, STAG2, U2AF1, and/or ZRSR2
No
Complex karyotype and/or del(5q)/t(5q)/add(5q), –7/del(7q), +8, del(12p)/t(12p)/add(12p), i(17q), –17/add(17p)/del(17p), del(20q), or idic(X)(q13)
No
AML not otherwise specified
10–19% blasts
≥20% blasts
MDS/AML with mutated TP53
MDS/AML with myelodysplasia-related gene mutation
MDS/AML with myelodysplasia-related cytogenetic abnormality
MDS/AML not otherwise specified
AML with recurrent genetic abnormality[b]
AML with mutated TP53
AML with myelodysplasia-related gene mutation
AML with myelodysplasia-related cytogenetic abnormality
AML not otherwise specified
Diagnostic qualifiers appended to any of the above diagnoses[c]
Therapy-related
Prior MDS or MDS/MPN
Germline predisposition[c]

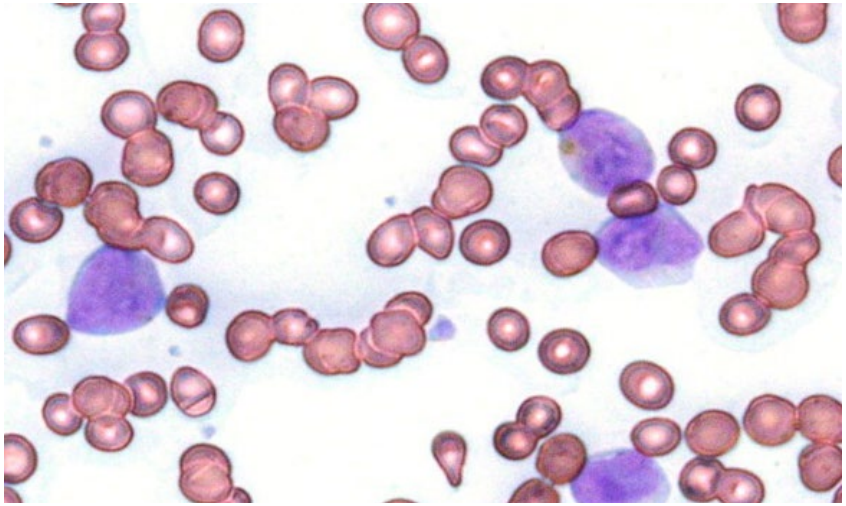
AML, Not Otherwise Specified (NOS)

1. AML met minimale differentiatie
2. AML zonder uitrijping
3. AML met uitrijping
4. Acute myelomonocytaire leukemie
5. Acute monoblasten/monocytaire leukemie
6. Pure erytroïde leukemie
7. Acute megakarycytaire leukemie

AML met minimale differentiatie

- >20% blasten
- Morfologisch
  - Geen myeloïde differentiatie
  - <3% Peroxidase kleuring
- Flowcytometrie
  - onderscheid met ALL

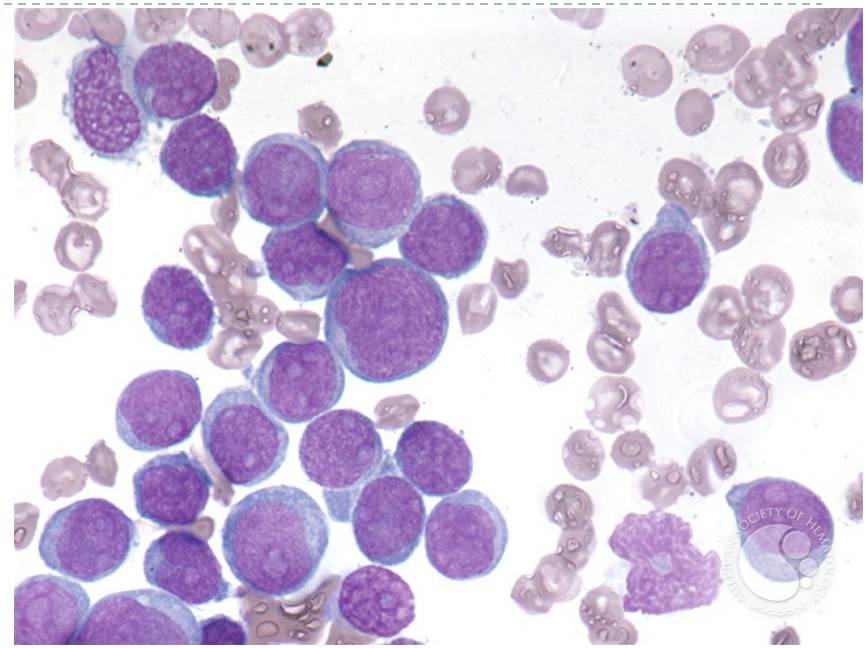


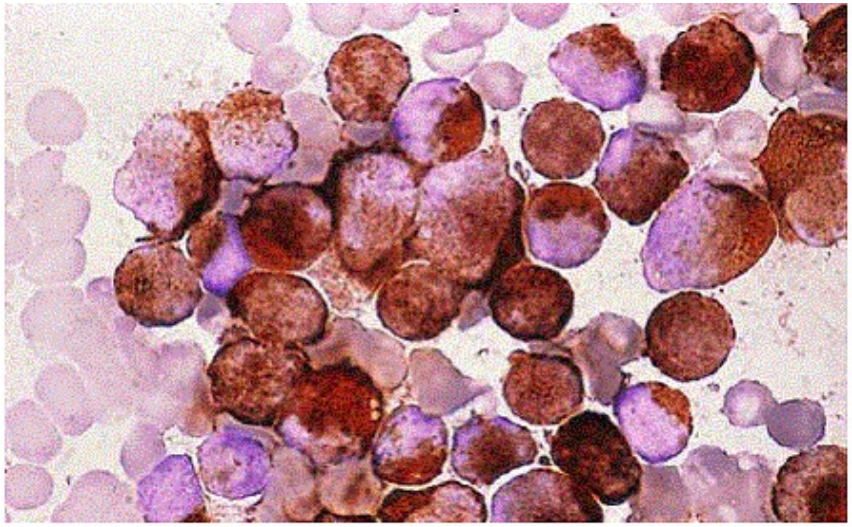


AML zonder uitrijping

- >20% blasten
- <10% granulocytaire uitrijping

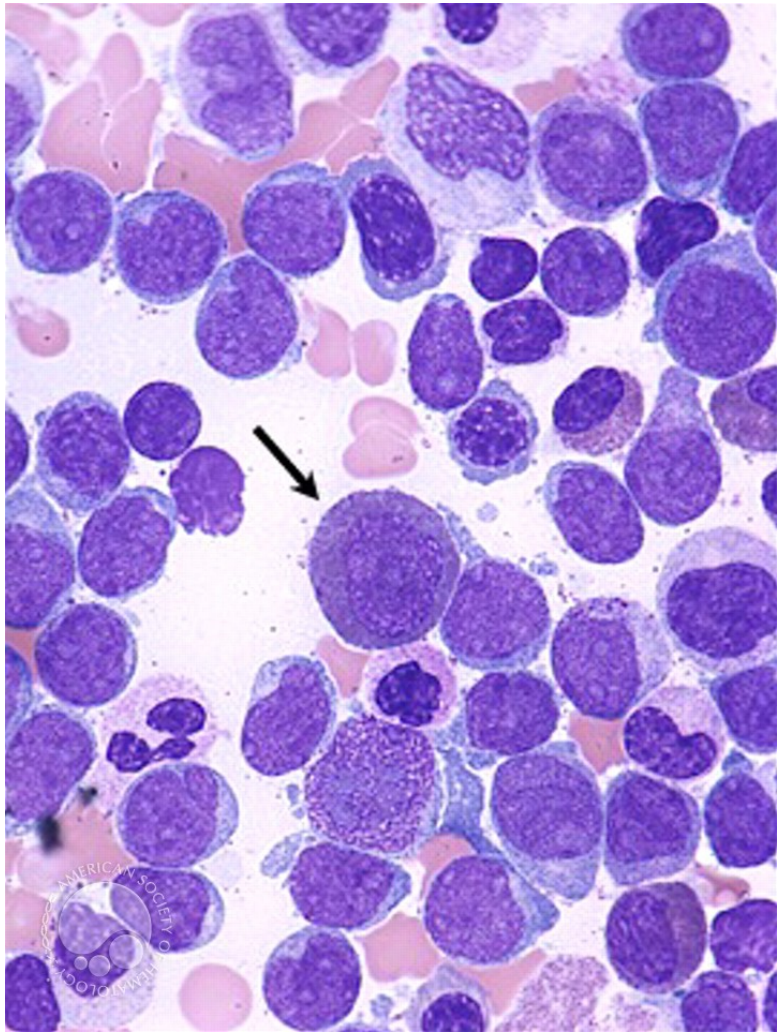
- Minimale differentiatie vs zonder uitrijping wel myeloïde differentiatie (MPO)
  - >3% Peroxidase kleuring





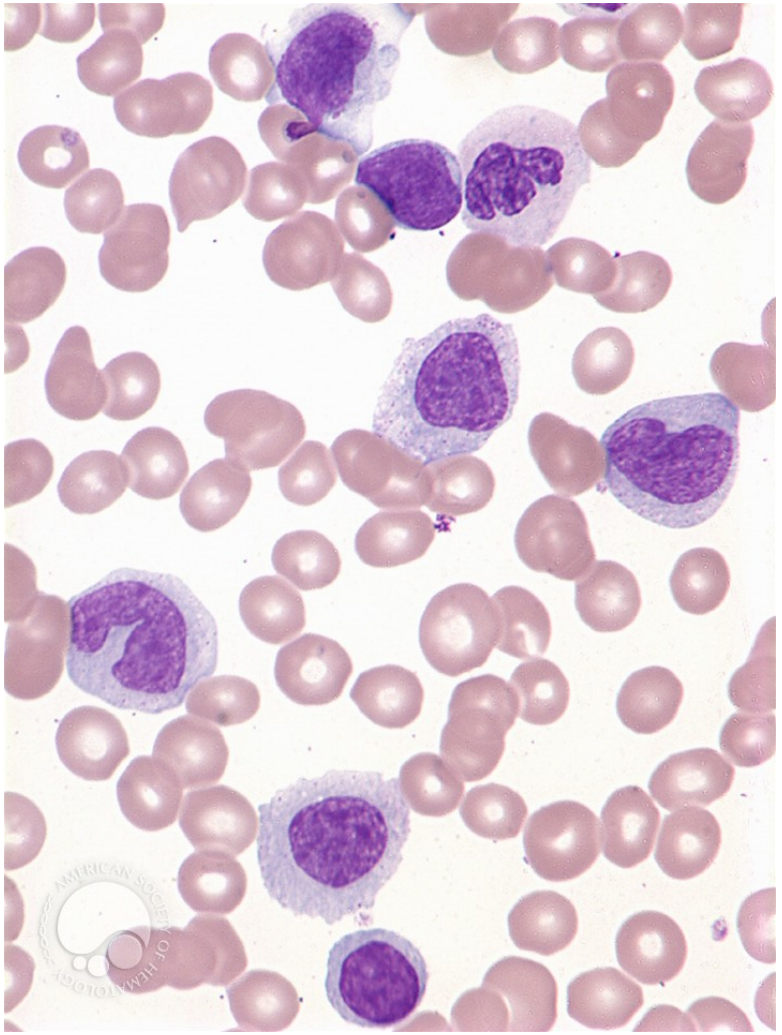
AML met uitrijping

- >20% blasten
- >10% granulocytaire uitrijping
- <20% monocytaire cellen



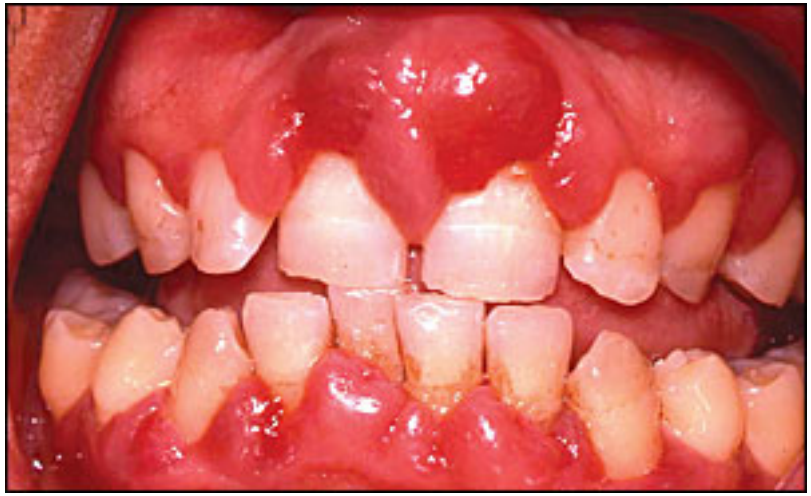
Acute myelomonocytaire leukemie

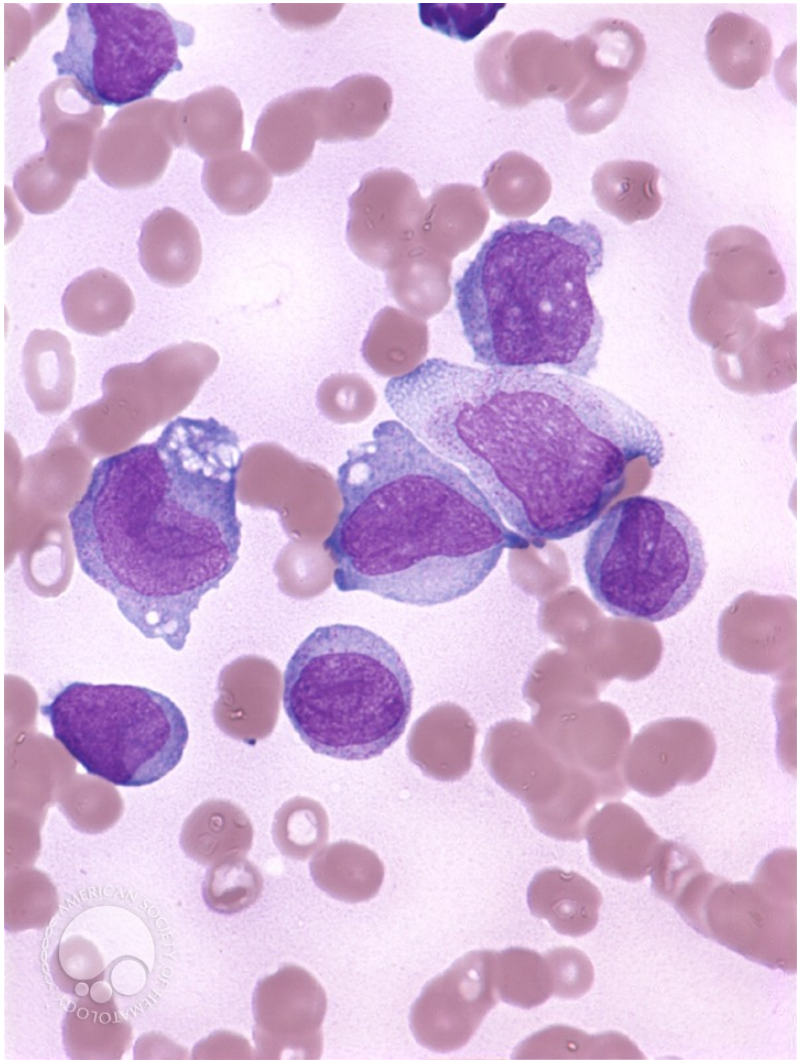
- >20% blasten inclusief promonocyten
  - >20% granulocytaire uitrijping
  - >20% monocytaire cellen
- Bloed kan lijken op CMMML
- Flow cytometrie ook monocytaire markers (CD4, CD64 en CD14)



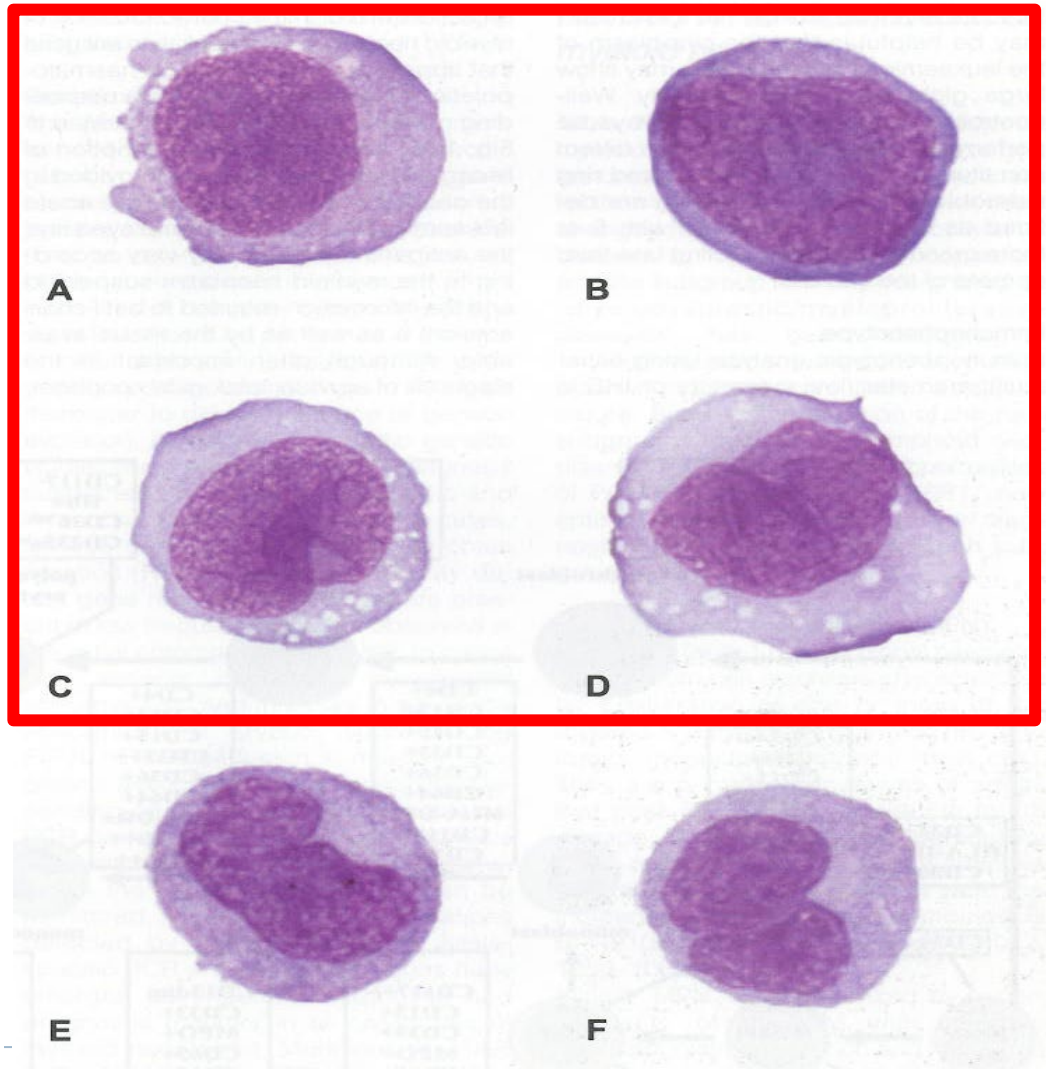
Acute monoblasten/monocytaire leukemie

- >20% blasten
  - >80% monoblasten/promonocyten
  - <20% granulocytaire uitrijping
- Tandvlees hypertrofie

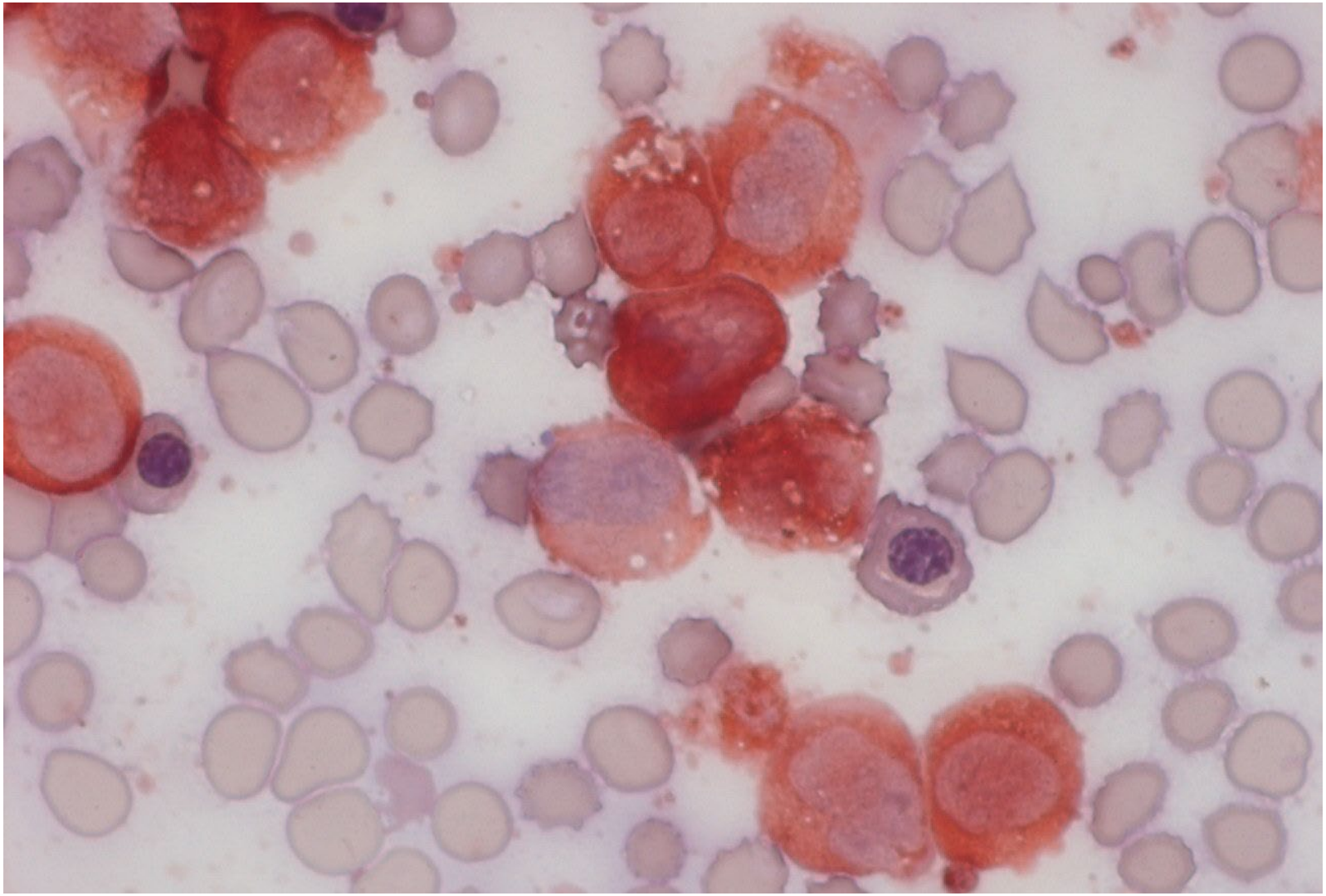


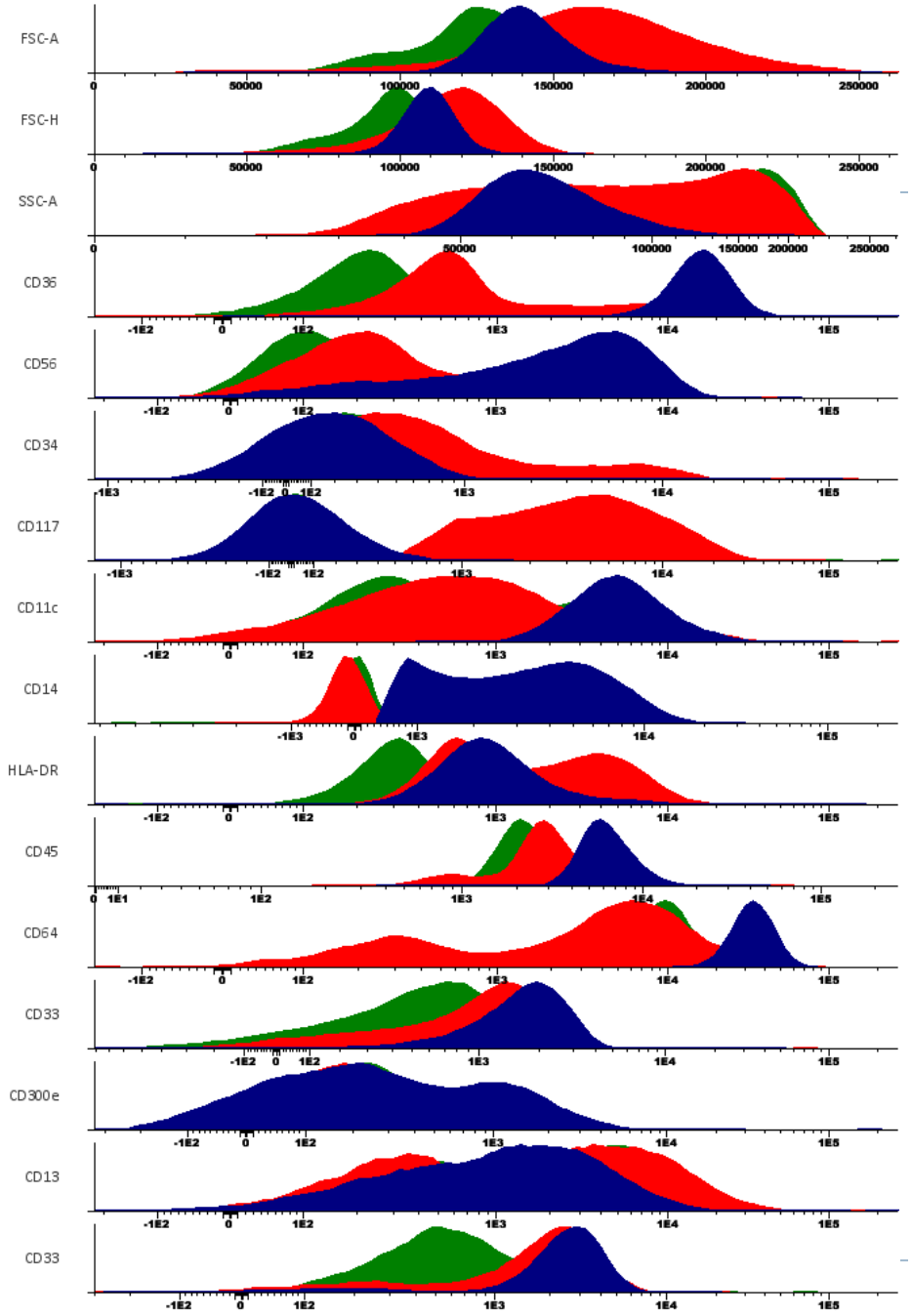


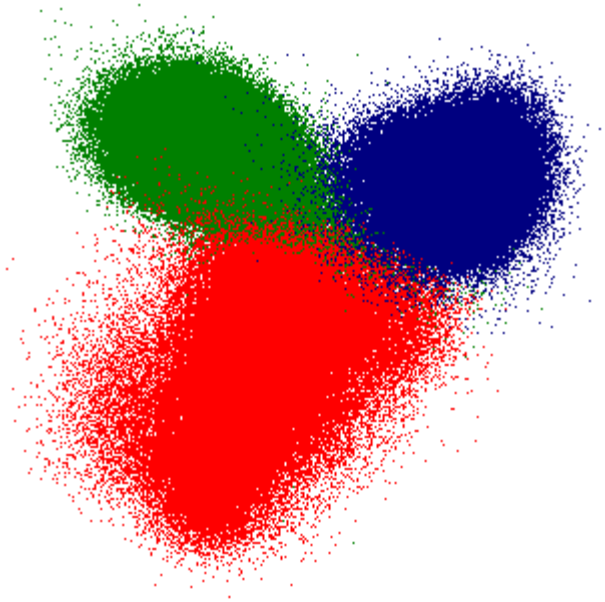
Monoblast/promonocyt/monocyt



Esterase kleuring







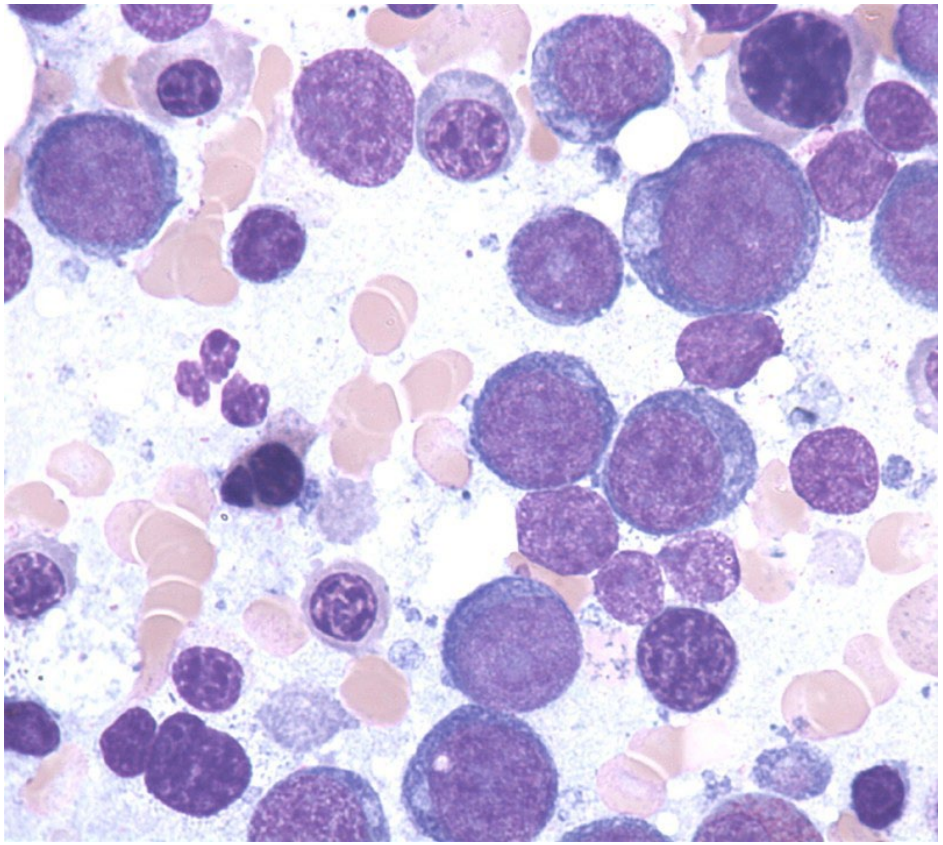
Monocytaire cellen: blauw

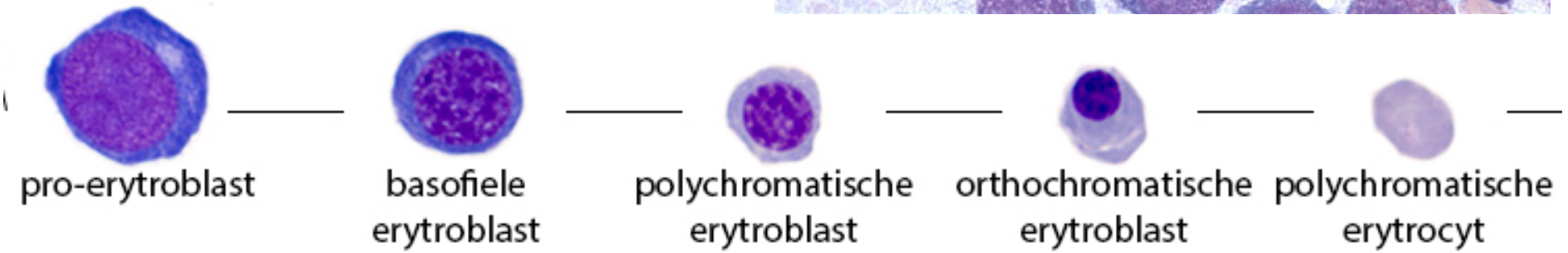
- CD64 sterk
- Monocyten: CD300e positief
- Onrijp: CD300e negatief

- Soms verlies of verzwakte:
  - CD36
  - HLA-DR
  - CD11c

Pure erytroïde leukemie

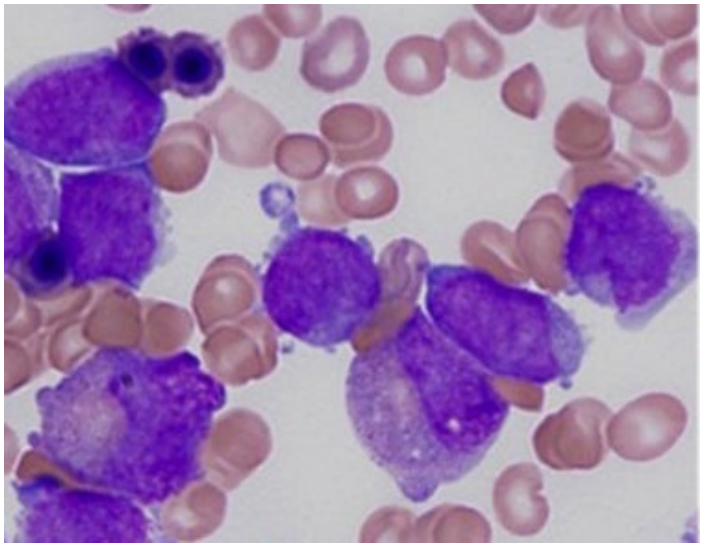
- WHO 2016
  - 80% erytroblasten met
  - >30% pro-erytroblasten

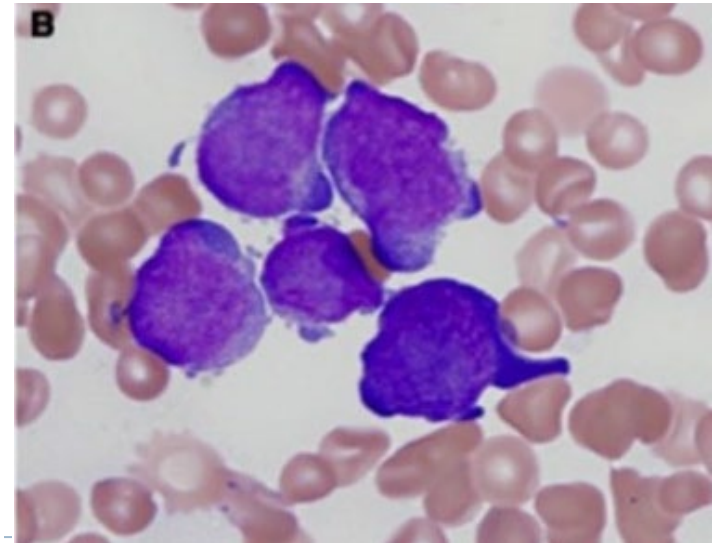




Acute megakaryocytaire leukemie

- >20% blasten
  - 50% megakaryoblast
- Cytoplasmatische blebs
- Fibrose
- Megakaryocytaire merkers(CD41, CD61 of CD42b)





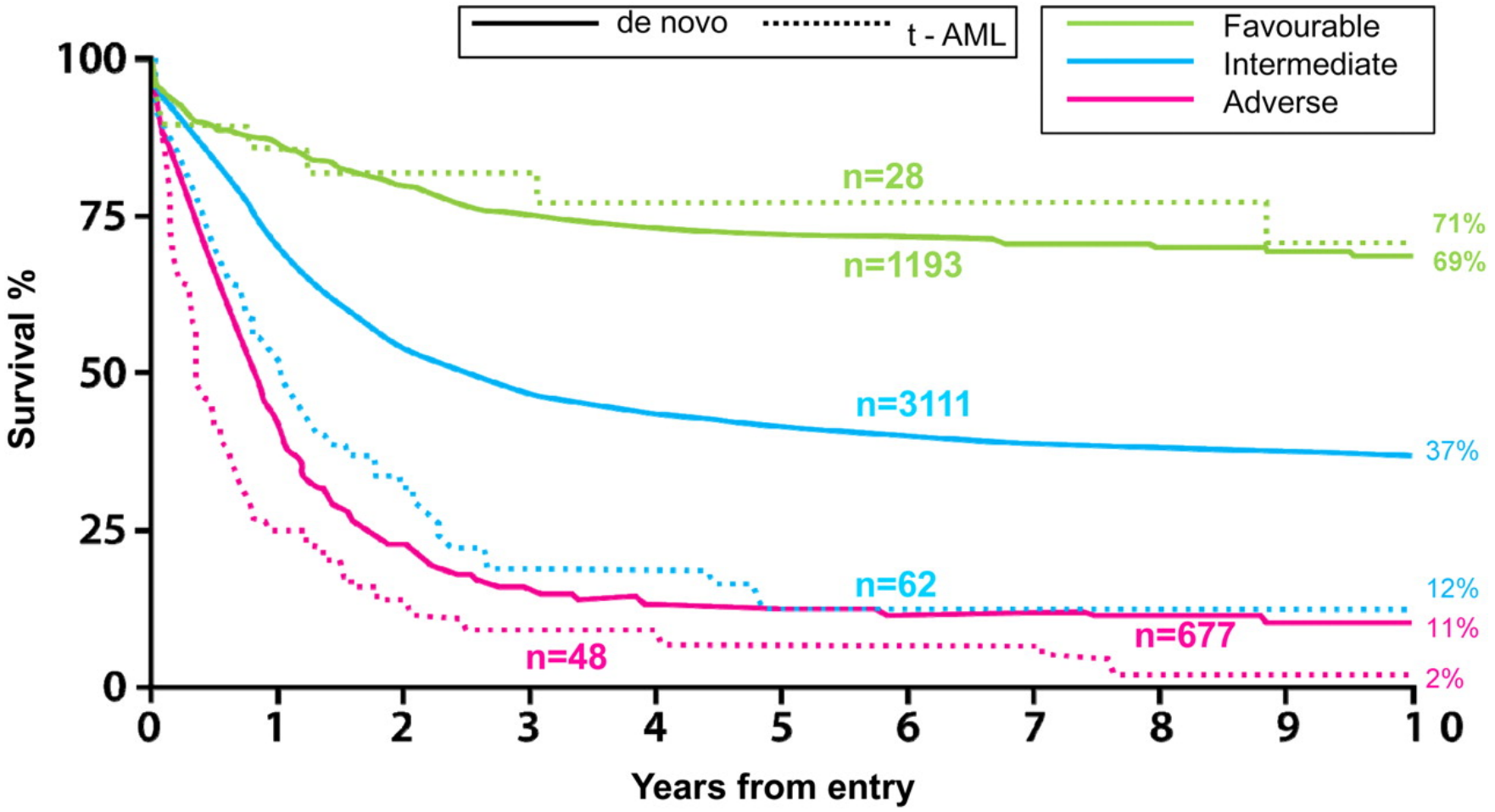
AML Prognose

Diagnosis and management of AML in adults: 2022 recommendations from an international expert panel on behalf of the ELN

Table 6. 2022 ELN risk classification by genetics at initial diagnosis*

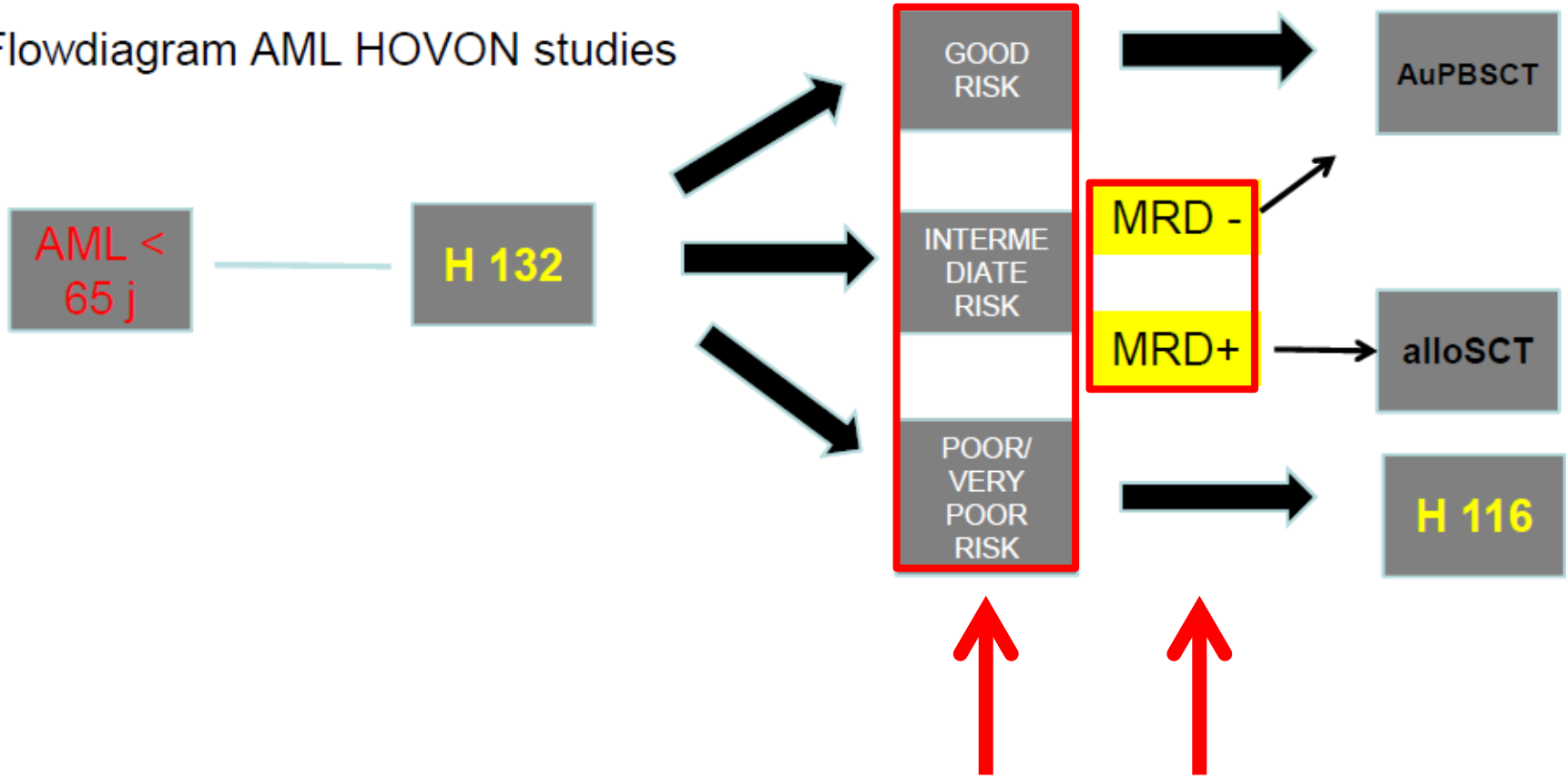
Risk category†	Genetic abnormality
Favorable	• t(8;21)(q22;q22.1)/*RUNX1*::*RUNX1T1*†,‡ • inv(16)(p13.1q22) or t(16;16)(p13.1;q22)/*CBFB*::*MYH11*†,‡ • Mutated *NPM1*†,§ without *FLT3*-ITD • bZIP in-frame mutated *CEBPA*‖
Intermediate	• Mutated *NPM1*†,§ with *FLT3*-ITD • Wild-type *NPM1* with *FLT3*-ITD (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
Adverse	• 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 *TP53*[a]

AML prognose

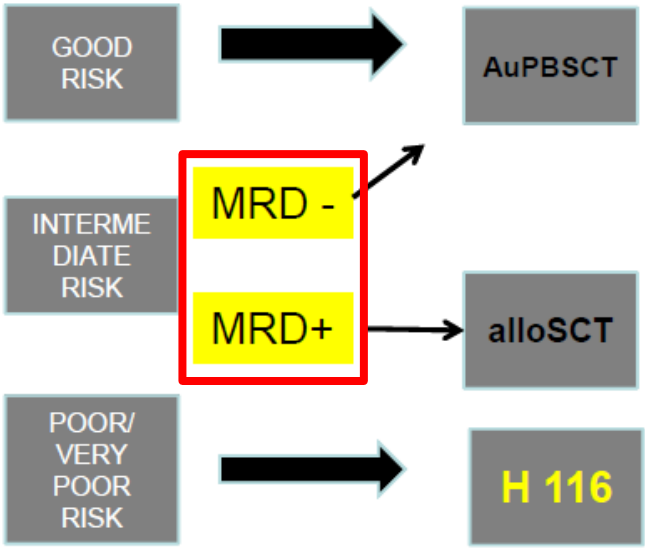


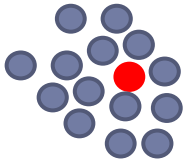
AML behandeling

Fig 1 Flowdiagram AML HOVON studies

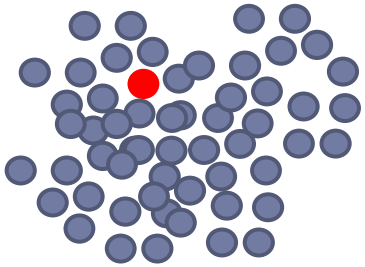


Minimale restziekte (MRD)

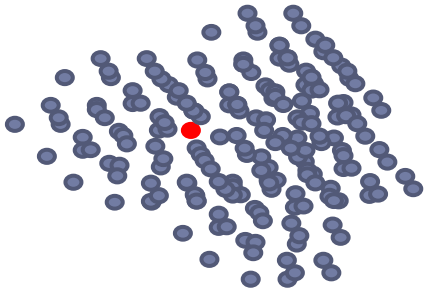




Morfologie
- 1 op 100 cellen

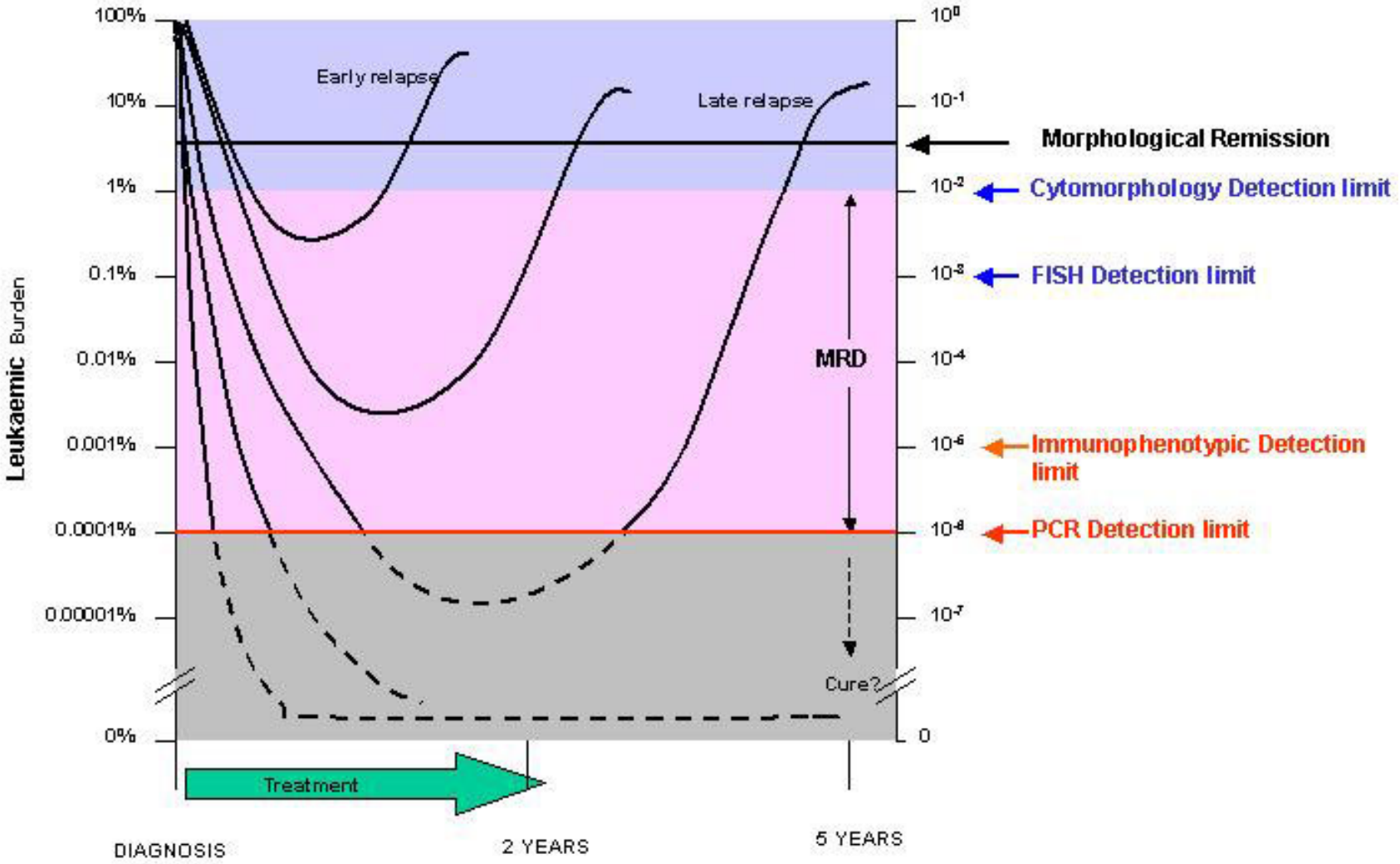


Flowcytometrie
- 1 op 10.000 cellen

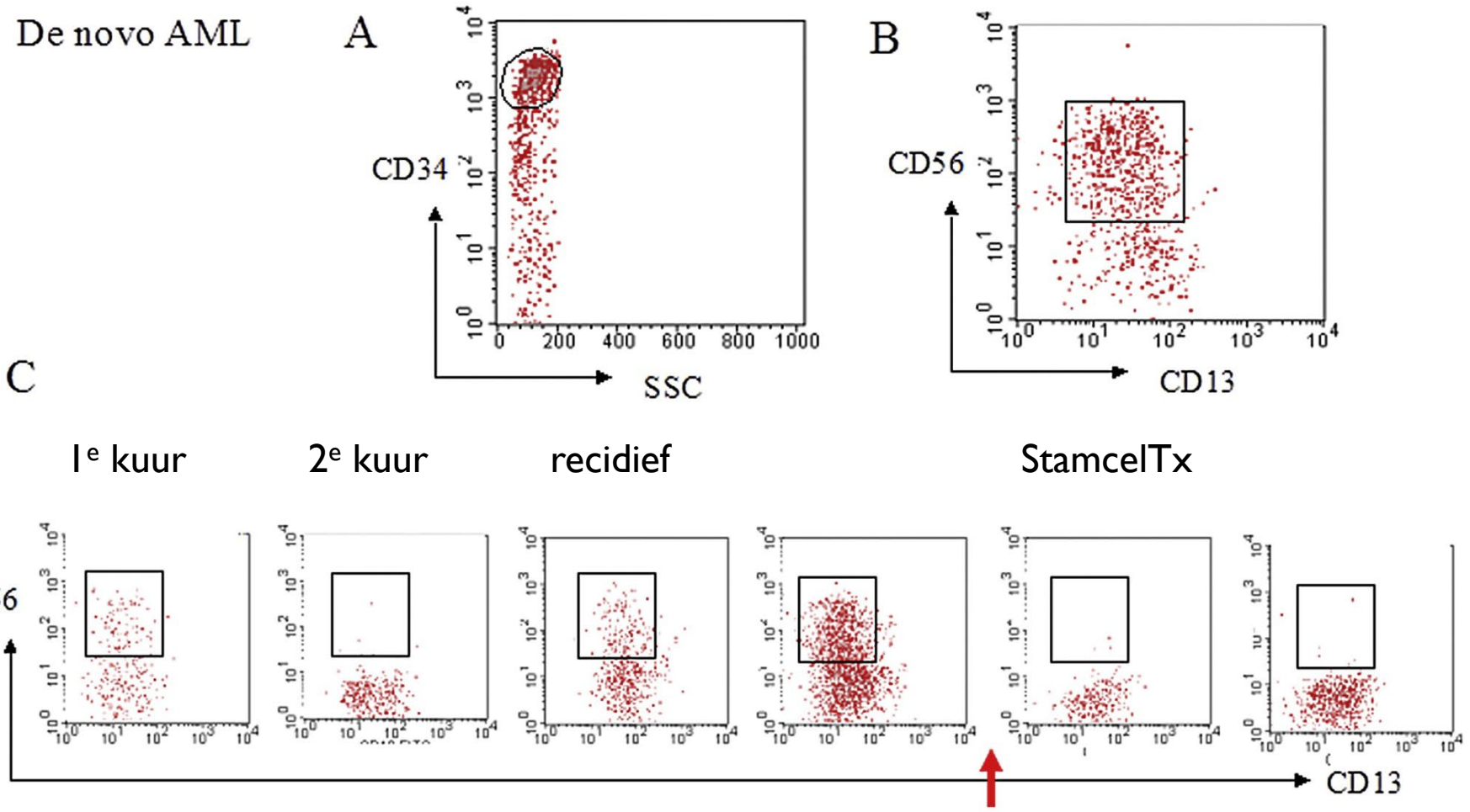


Moleculaire technieken
- 1 op 100.000 cellen

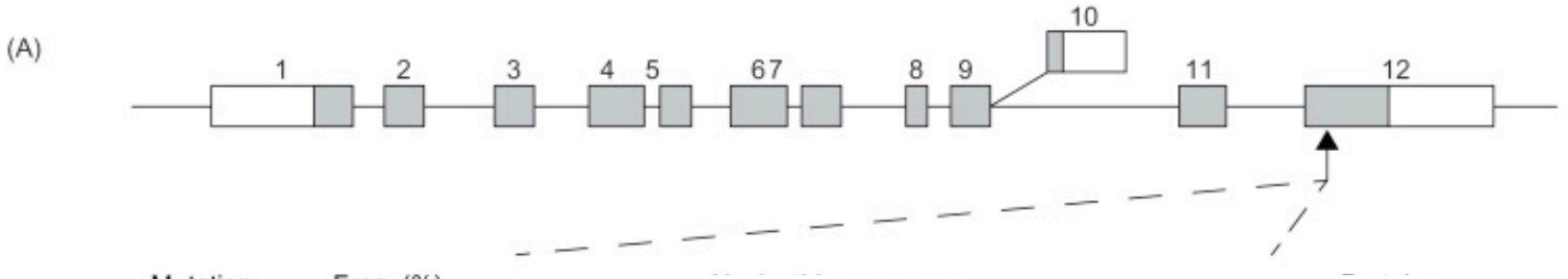
Minimale restziekte (MRD)



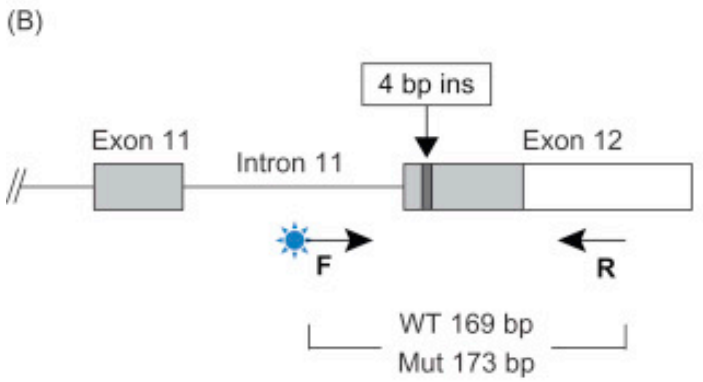
Flowcytometrie

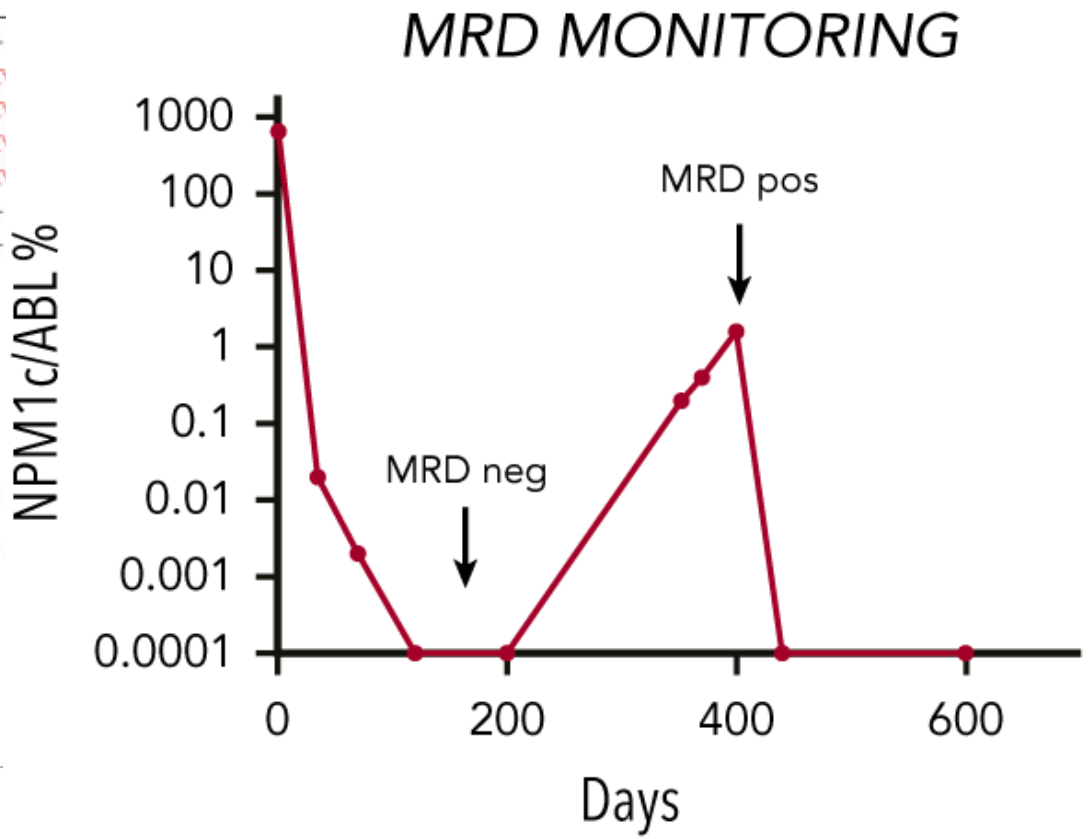


Moleculair

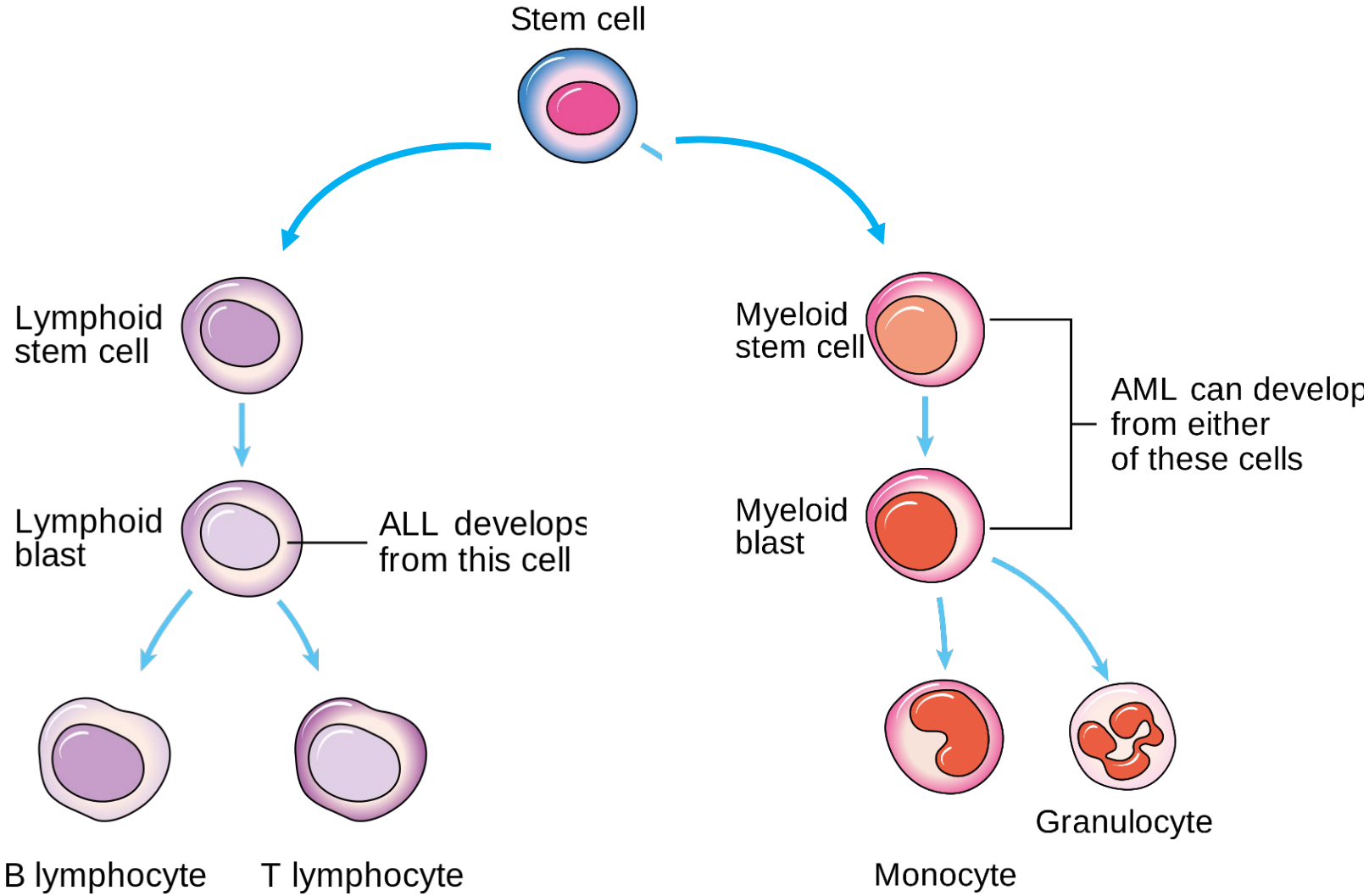


Mutation	Freq. (%)	
Wild Type		GATCTCTG---
A	80	GATCTCTGTCT
B	6	GATCTCTGCAT
C	<1	GATCTCTGCGT
D	6	GATCTCTGCCT
E	<1	GATCTCTG---
F	<1	GATCTCTG---
~45 more	7	





Acute lymfatische leukemie Acute myeloïde leukemie



British Journal of Haematology, 1976, **33**, 451.

Proposals for the Classification of the Acute Leukaemias

FRENCH–AMERICAN–BRITISH (FAB) CO-OPERATIVE GROUP

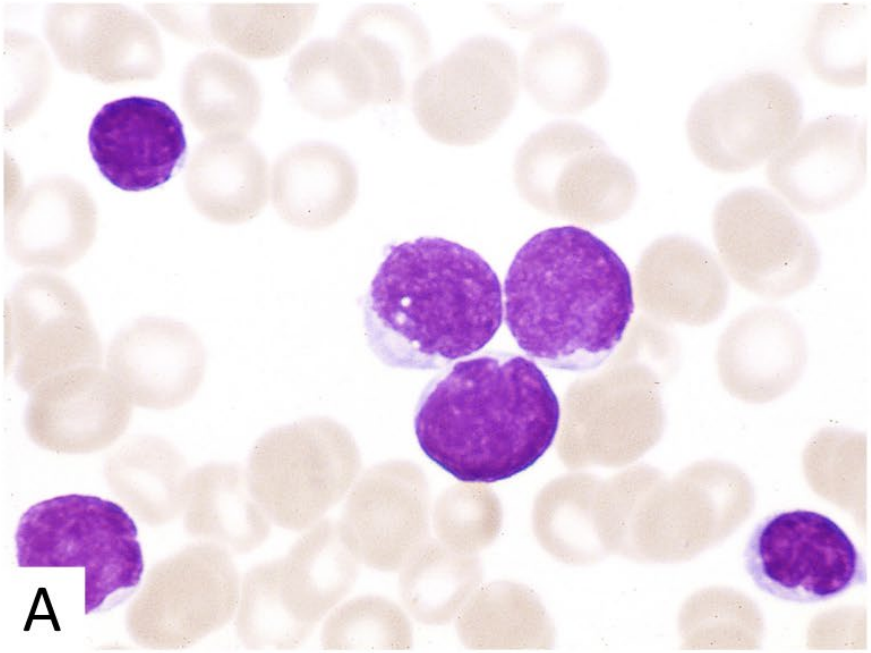
J. M. BENNETT,* D. CATOVSKY,† MARIE-THERÈSE DANIEL,‡ G. FLANDRIN,‡
D. A. G. GALTON,† H. R. GRALNICK§ AND C. SULTAN¶

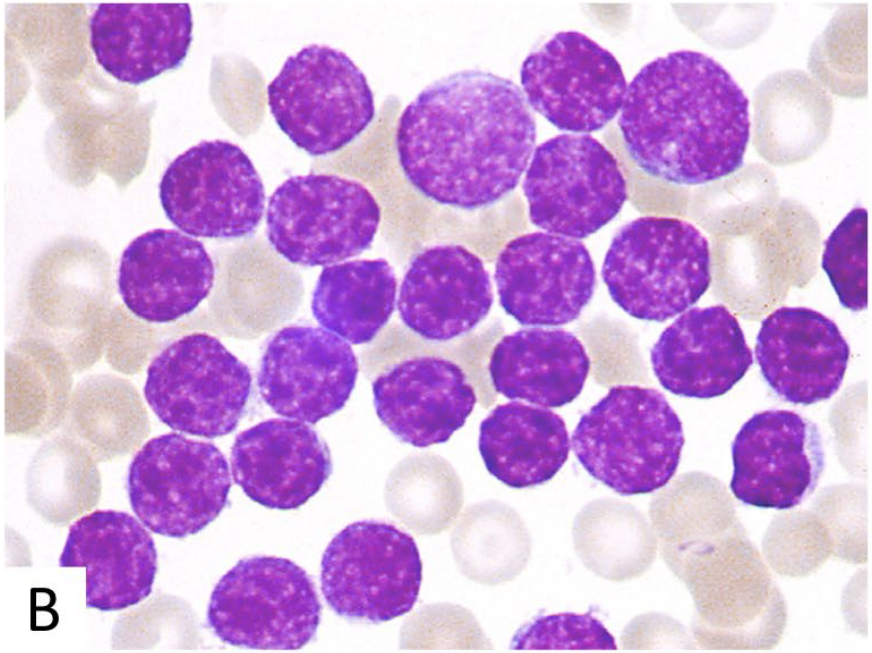
FAB classification of acute lymphoblastic leukaemia.

Criterion	Classification
High nucleus:cytoplasm ratio > 75% of cells	+
Low nucleus:cytoplasm ratio > 25% of cells	–
Nucleoli: 0–1 (small) > 75% of cells	+
Nucleoli: 1 or more (prominent) > 25% of cells	–
Irregular nuclear membrane > 25% of cells	–
Megaloblasts > 50% of cells	–

Obtain 1 classification in each category, sum total of signs.

Total classification (–4 to +2): 0 to +2 L1 acute lymphoblastic leukaemia; –1 to –4 L2 acute lymphoblastic leukaemia.





L1 Lymphoblastic leukaemia with homogeneous structure

Frequency:

Between 25% and 30% of cases in adults, and 85% of cases in children.

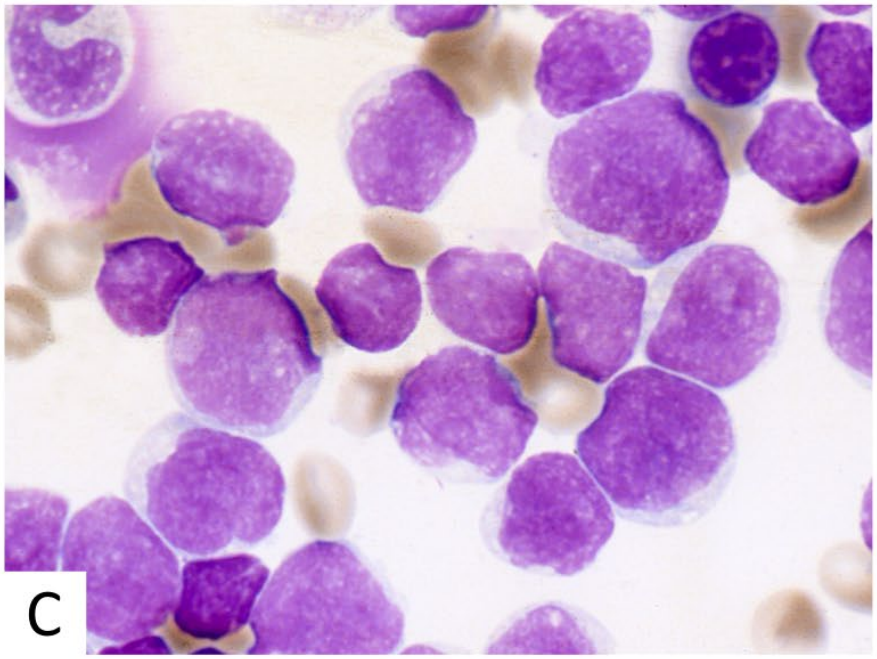
Morphology:

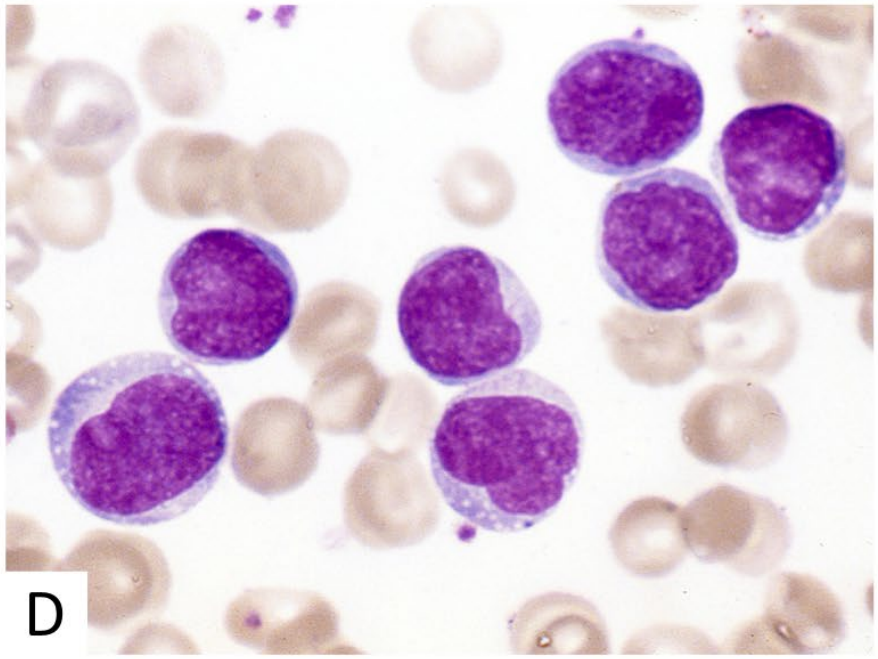
Blasts are homogeneous, nucleus is regular, chromatin is homogeneous, small or no nucleoli, scanty cytoplasm, and mild to moderate basophilia.

Immunophenotype

B:	T:
• CD19	•CD3
•CD22	•CD7
•CD79a	•CD5
•CD10	•CD2
•CD20	•CD4

•Cytoplasmic or superficial immunoglobulir





L2 Lymphoblastic leukaemia with varied structure

Frequency:

Accounts for 70% of cases in adults, and 14% in children.

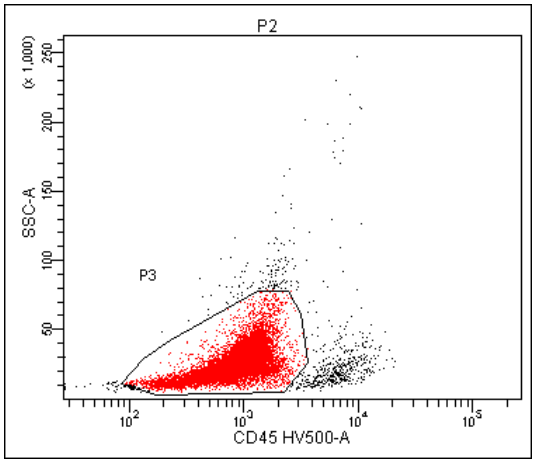
Morphology:

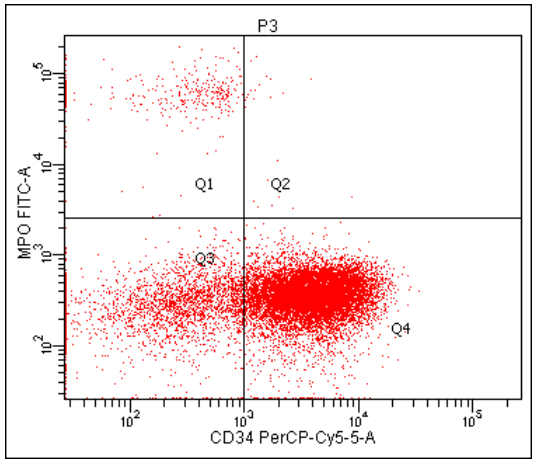
Nucleus is irregular, heterogeneous chromatin structure, large nucleoli.

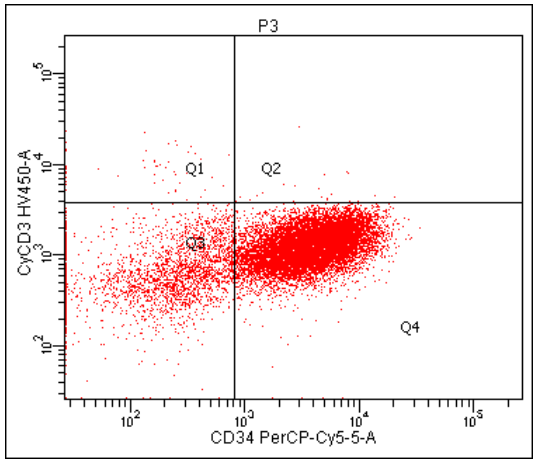
Immunophenotype

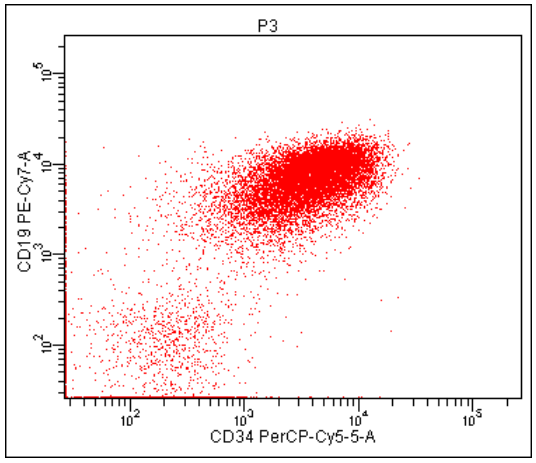
B:	T:
•CD19	•CD3
•CD22	•CD7
•CD79a	•CD5
•CD10	•CD2
•CD20	•CD4

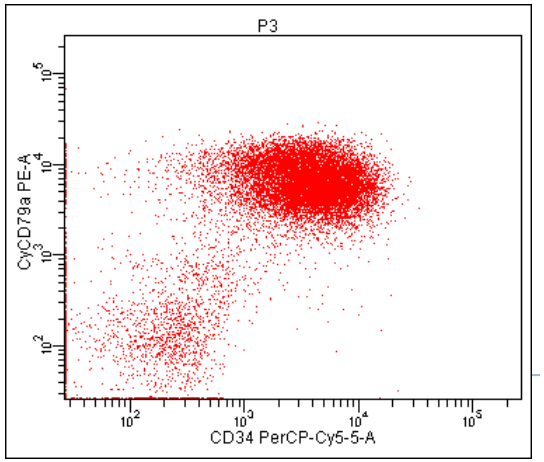
•Cytoplasmic or superficial immunoglobulin



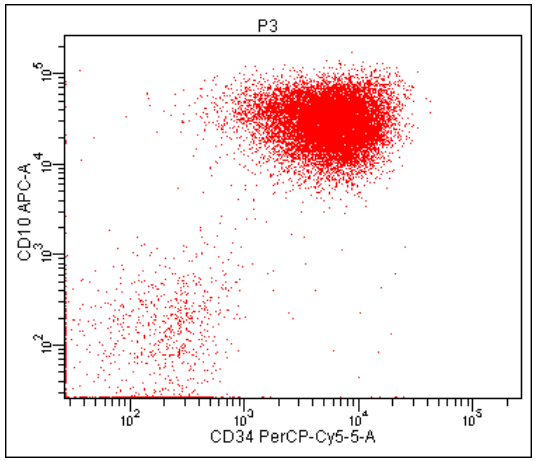


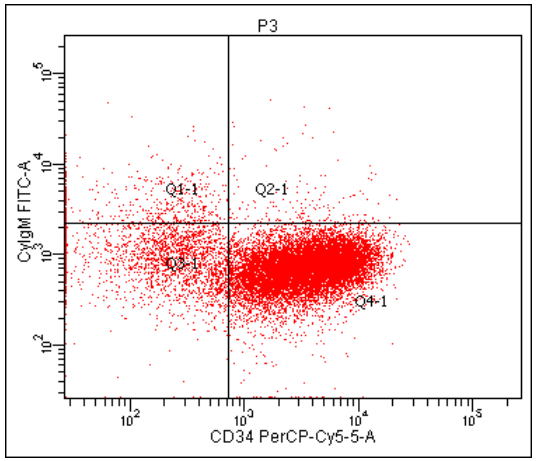


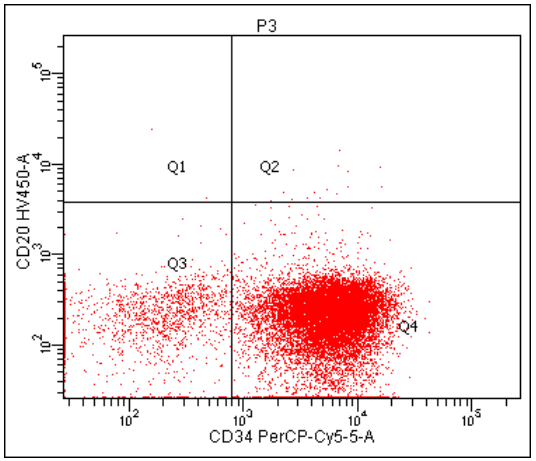


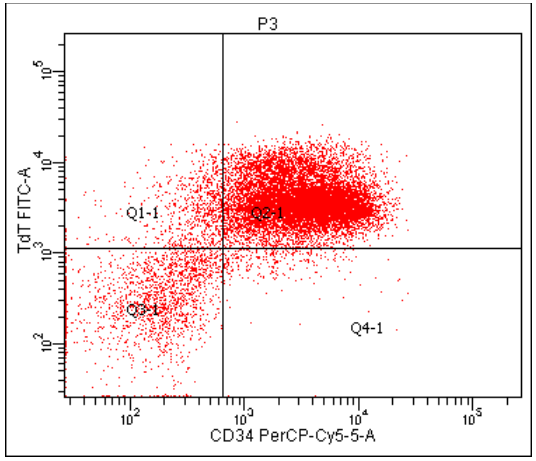


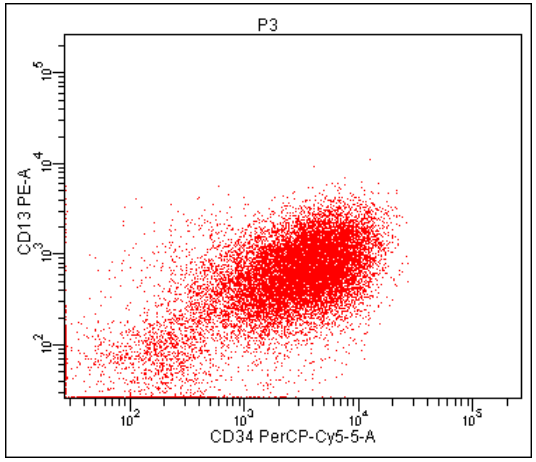
Lijn	Markers
Myeloid	MPO
	2x (CD11c, CD14, CD64)
T lineage	Cytoplasmatisch CD3
	Oppervlakte CD3
B lineage	CD19 sterk + (CD79a, CD22 of CD10)
	CD19 zwak + 2x (CD79a, CD22 of CD10)

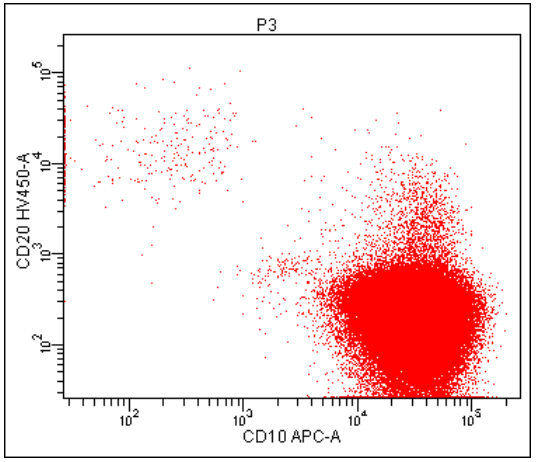

P3
CD10 APC-A
CD34 PerCP-Cy5-5-A


P3
CyIgM FITC-A
Q1-1
Q2-1
Q3-1
Q4-1
CD34 PerCP-Cy5-5-A

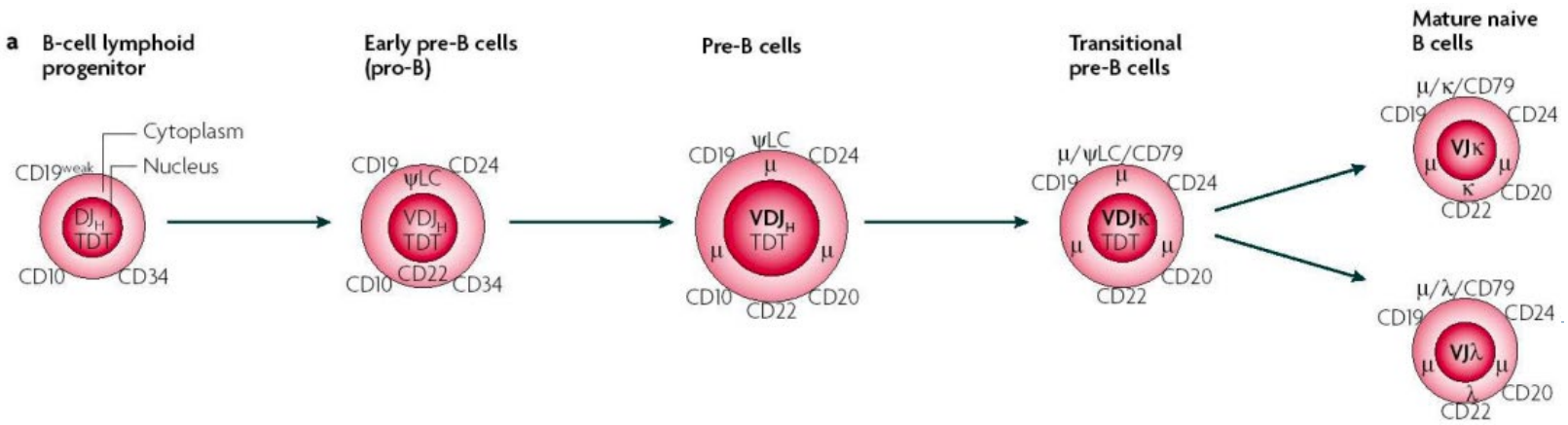

P3
CD20 HV450-A
Q1
Q2
Q3
Q4
CD34 PerCP-Cy5-5-A


P3
TdT FITC-A
Q1-1
Q2-1
Q3-1
Q4-1
CD34 PerCP-Cy5-5-A


P3
CD13 PE-A
CD34 PerCP-Cy5-5-A


P3
CD20 HV450-A
CD10 APC-A

Markers	pro-B-ALL (EGIL B-I)	common ALL (EGIL B-II)	pre-B-ALL (EGIL B-III)	transitional pre-B-ALL* (geen EGIL-code)
TdT	++**	++	++	++
CD10	–	++	++	++
CD19	++	++	++	++
CD20	–	+	+	+
CD22	++	++	++	++
CyCD79	++	++	++	++
CyIgμ	–	–	++***	++***
SmVpre-B/λ5	–	–	–	++
SmIg-CD79	–	–	–	++
CD34	+	+	+	+

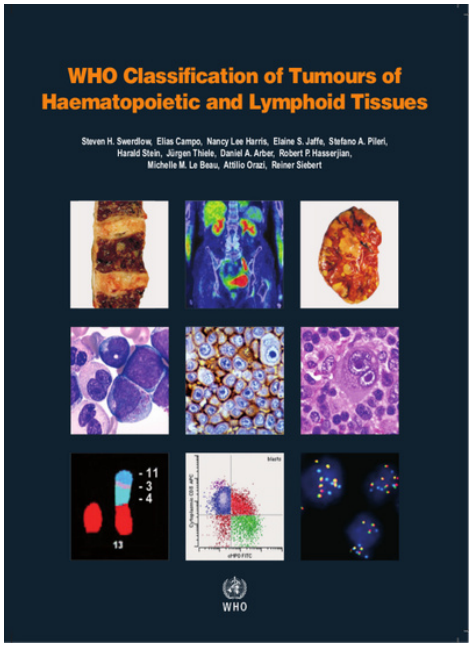


WHO 2022 ALL Classificatie

Precursor B-cell neoplasms	
B-cell lymphoblastic leukaemias/lymphomas	
B-lymphoblastic leukaemia/lymphoma, NOS	(Same)
B-lymphoblastic leukaemia/lymphoma with high hyperdiploidy	B-lymphoblastic leukaemia/lymphoma with hyperdiploidy
B-lymphoblastic leukaemia/lymphoma with hypodiploidy	(Same)
B-lymphoblastic leukaemia/lymphoma with iAMP21	(Same)
B-lymphoblastic leukaemia/lymphoma with *BCR::ABL1* fusion	B-lymphoblastic leukaemia/lymphoma with t(9;22)(q34;q11.2); *BCR-ABL1*
B-lymphoblastic leukaemia/lymphoma with *BCR::ABL1*-like features	B-lymphoblastic leukaemia/lymphoma, *BCR-ABL1*-like
B-lymphoblastic leukaemia/lymphoma with *KMT2A* rearrangement	B-lymphoblastic leukaemia/lymphoma with t(v;11q23.3); *KMT2A*-rearranged
B-lymphoblastic leukaemia/lymphoma with *ETV6::RUNX1* fusion	B-lymphoblastic leukaemia/lymphoma with t(12;21)(p13.2;q22.1); *ETV6-RUNX1*
B-lymphoblastic leukaemia/lymphoma with *ETV6::RUNX1*-like features	*Not previously included*
B-lymphoblastic leukaemia/lymphoma with *TCF3::PBX1* fusion	B-lymphoblastic leukaemia/lymphoma with t(1;19)(q23;p13.3); *TCF3-PBX1*
B-lymphoblastic leukaemia/lymphoma with *IGH::IL3 fusion*	B-lymphoblastic leukaemia/lymphoma with t(5;14)(q31.1;q32.1); *IGH/IL3*
B-lymphoblastic leukaemia/lymphoma with *TCF3::HLF* fusion	*Not previously included*
B-lymphoblastic leukaemia/lymphoma with other defined genetic abnormalities	(Same)

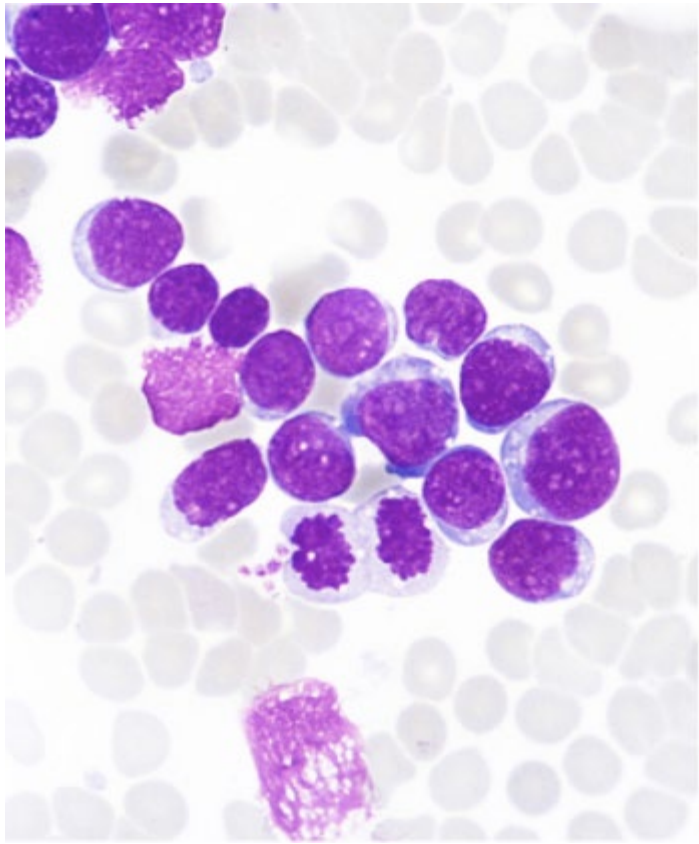
B-ALL Not further classified

- Definitie B-ALL/LBL
  - Neoplasm van precursor B-cellen
  - B-LBL
    - Nodale massa
    - Minimaal bloed en beenmerg
  - B-ALL
    - Extensieve bloed en beenmerg betrokkenheid
    - WHO geen vast grens % blasten (>20%)



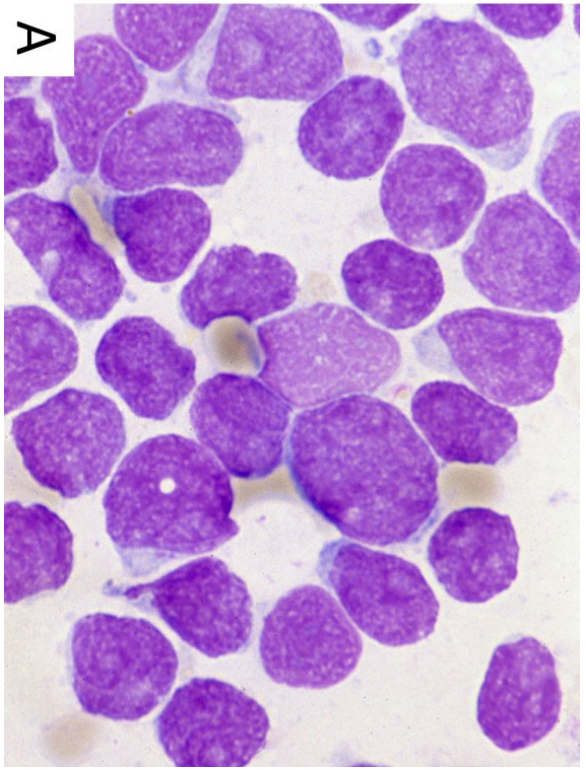
T-ALL Not Otherwise Specified

- Prevalentie
  - c.a. 15% van de volwassenen met ALL
  - c.a. 80-90 LBL is T-LBL
- Presentatie
  - Leukocytose, mediastinale massa
  - Rest hematopoëse relatief gespaard
- Morfologie
  - Geen goed onderscheid met B-ALL
- Flowcytometrie
  - cyCD3, meestal TdT en CD7
  - Soms cyCD79a (10%), CD13 en CD33 (30%)

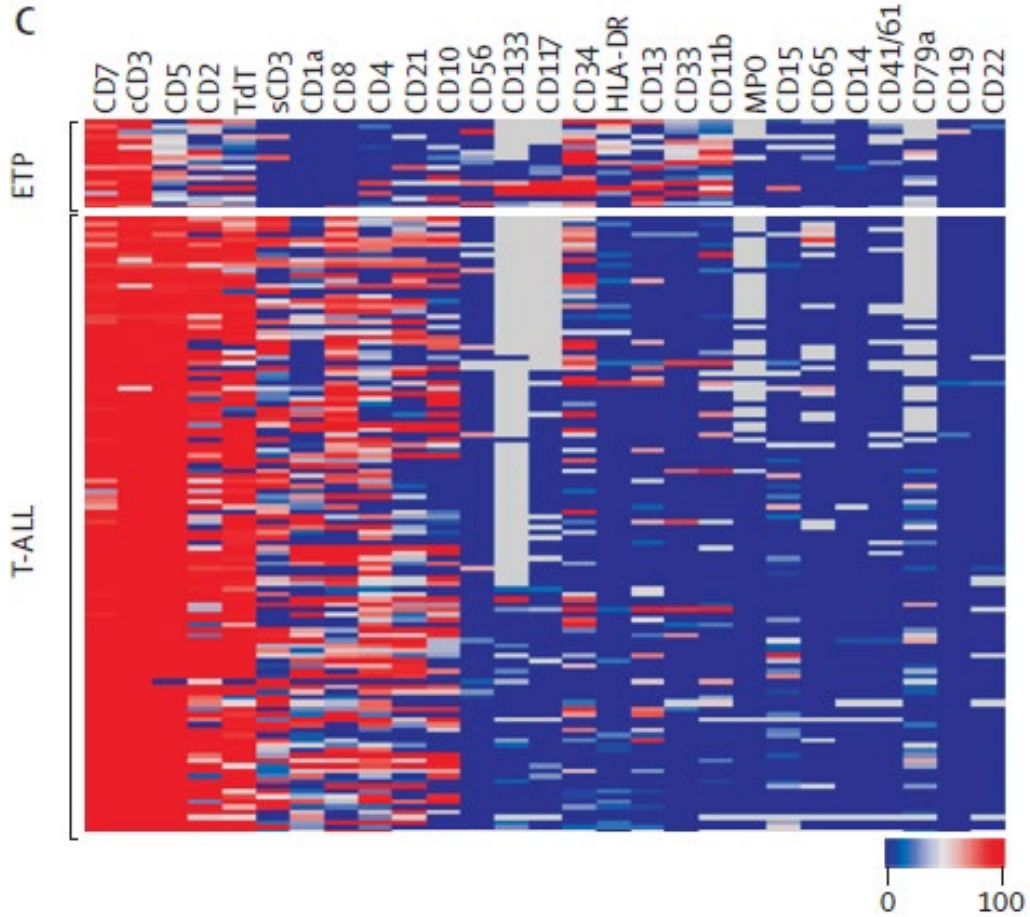


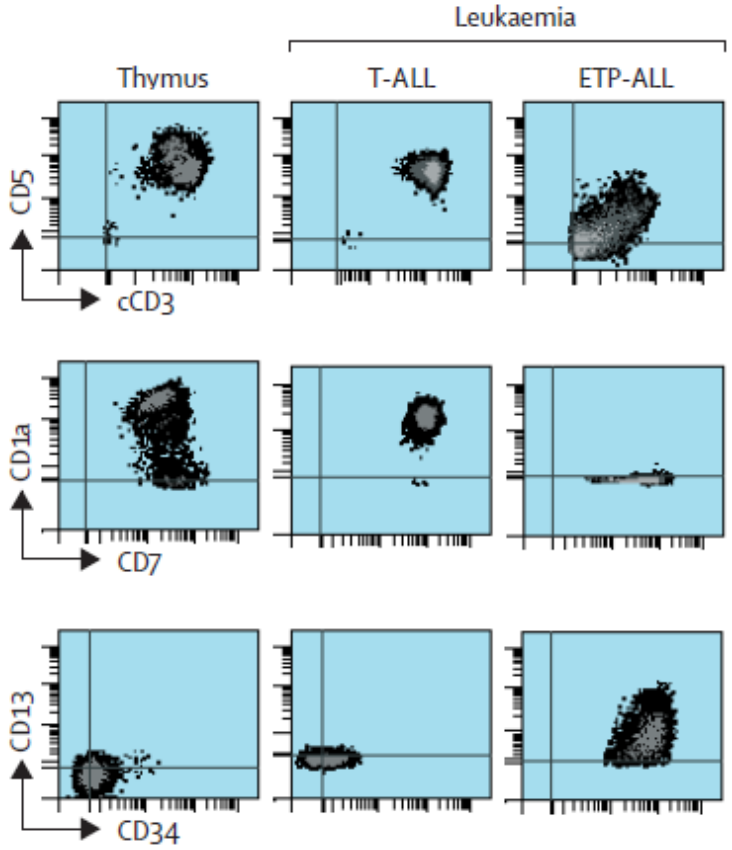
Early T-cel precursor ALL

- Prevalentie
  - c.a. 5-10% van de volwassenen
- Blast met minimale T cel differentiatie met myeloïde/stamcel kenmerken
- Immunofenotype
  - Positief CD7 en cyCD3
  - Negatief CD8 en CD1a
  - Positief voor stamcel/myeloide markers CD34, CD117, CD13, CD33
- Genetisch myeloïde mutaties
  - FLT3, NRAS/KRAS, DNMT3A, IDH1 en IDH2



ETP-ALL





E Coustan, CG Mullighan *Lancet Oncology* 2009