

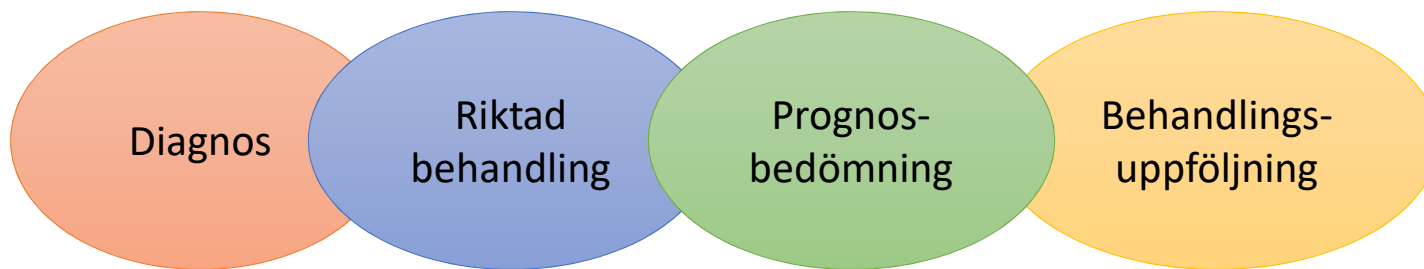
Hematopatologisk diagnostik inkl flödescytometri SK-kurs i AML 2021

Linda Fogelstrand

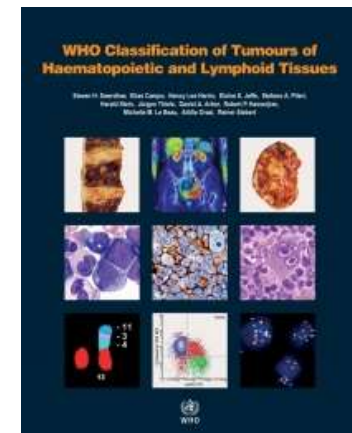
Klinisk kemi & Centrum för medicinsk genomik

Sahlgrenska Universitetssjukhuset, Göteborg

Diagnostik → underlag för att kunna ge rätt behandling



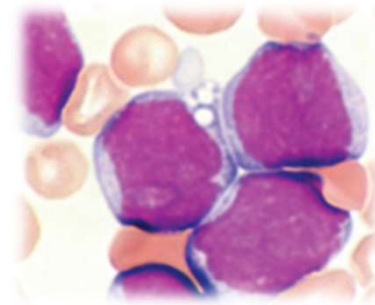
Morfologisk undersökning (aspirat +/- biopsi)
Immunofenotypning (flödescytometri)
Genetiska analyser (cyto- och molekulärgenetik)





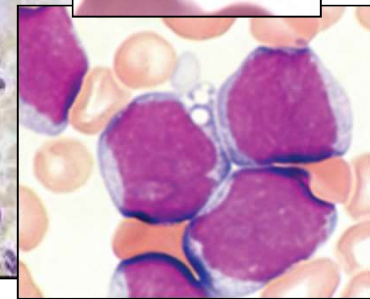
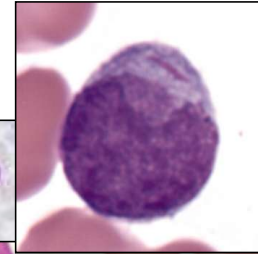
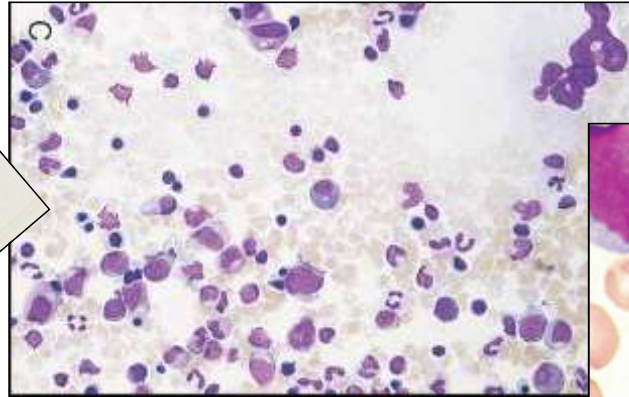
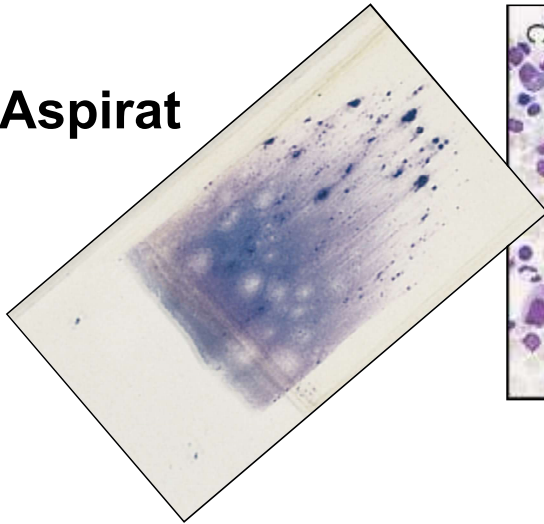
Morfologi

Akuteleukemi?



Benmärgsmorfologi

Aspirat



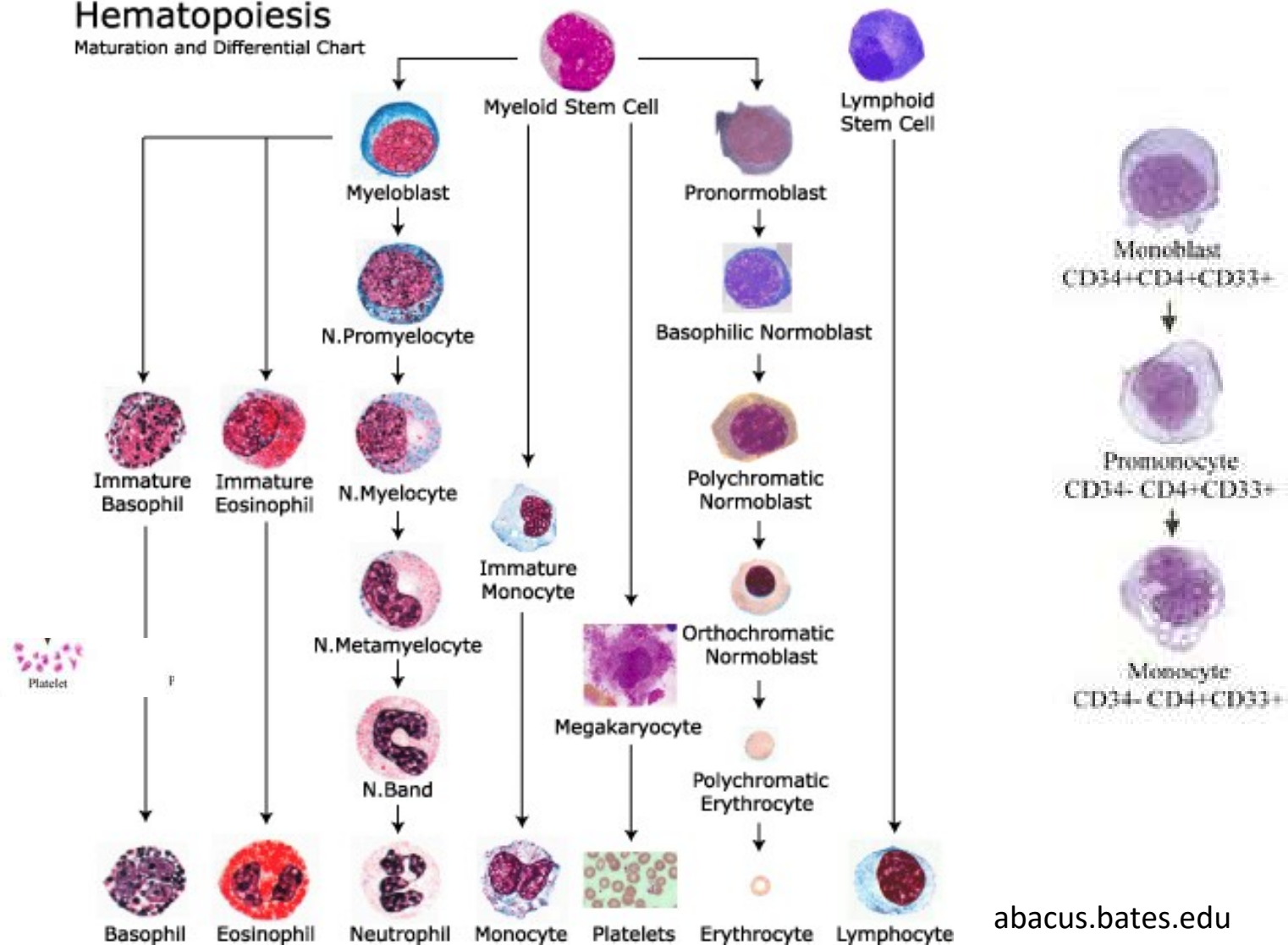
>20% blaster
i benmärg/blod
→ akut leukemi

Blastekvivalenter:

- Promonocyter
- Vid APL – promyelocyter

Hematopoiesis

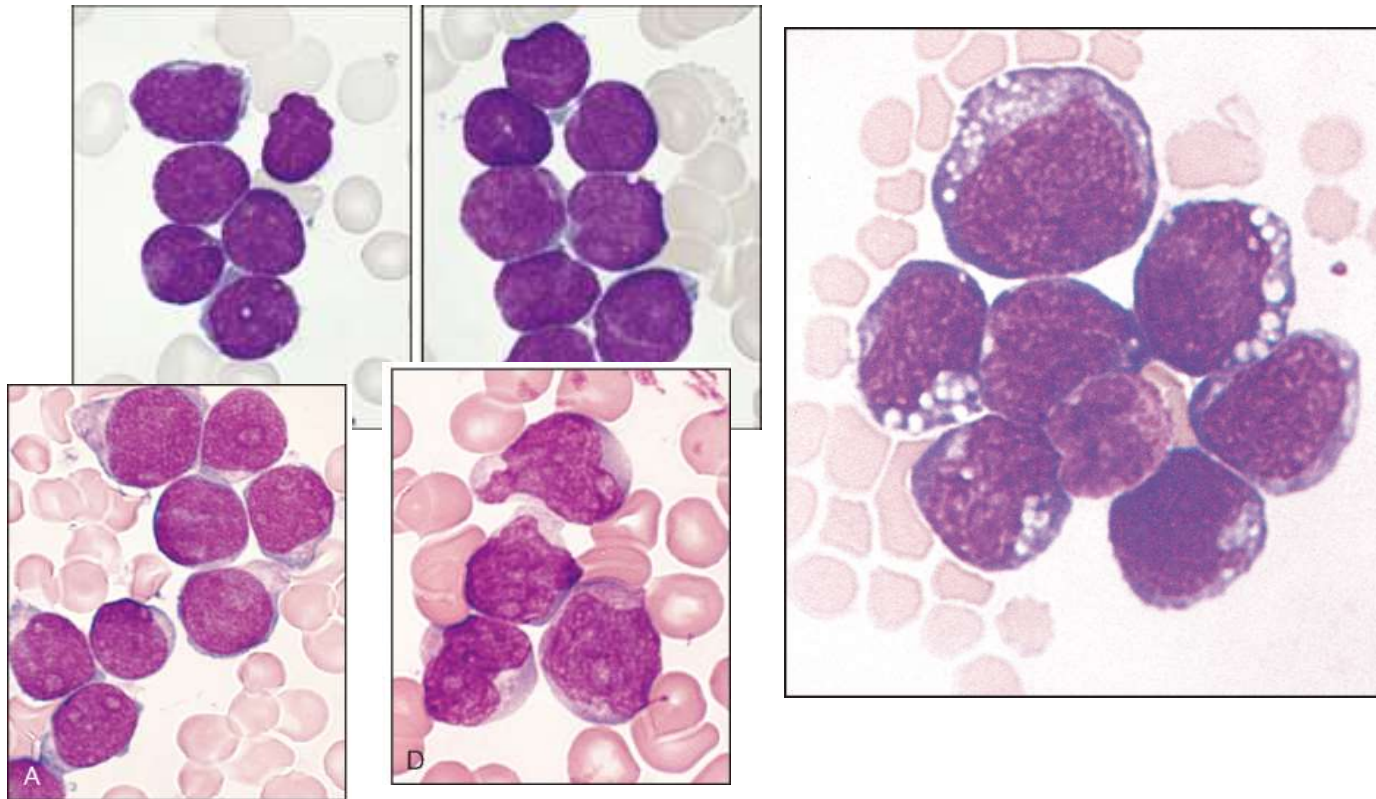
Maturation and Differential Chart



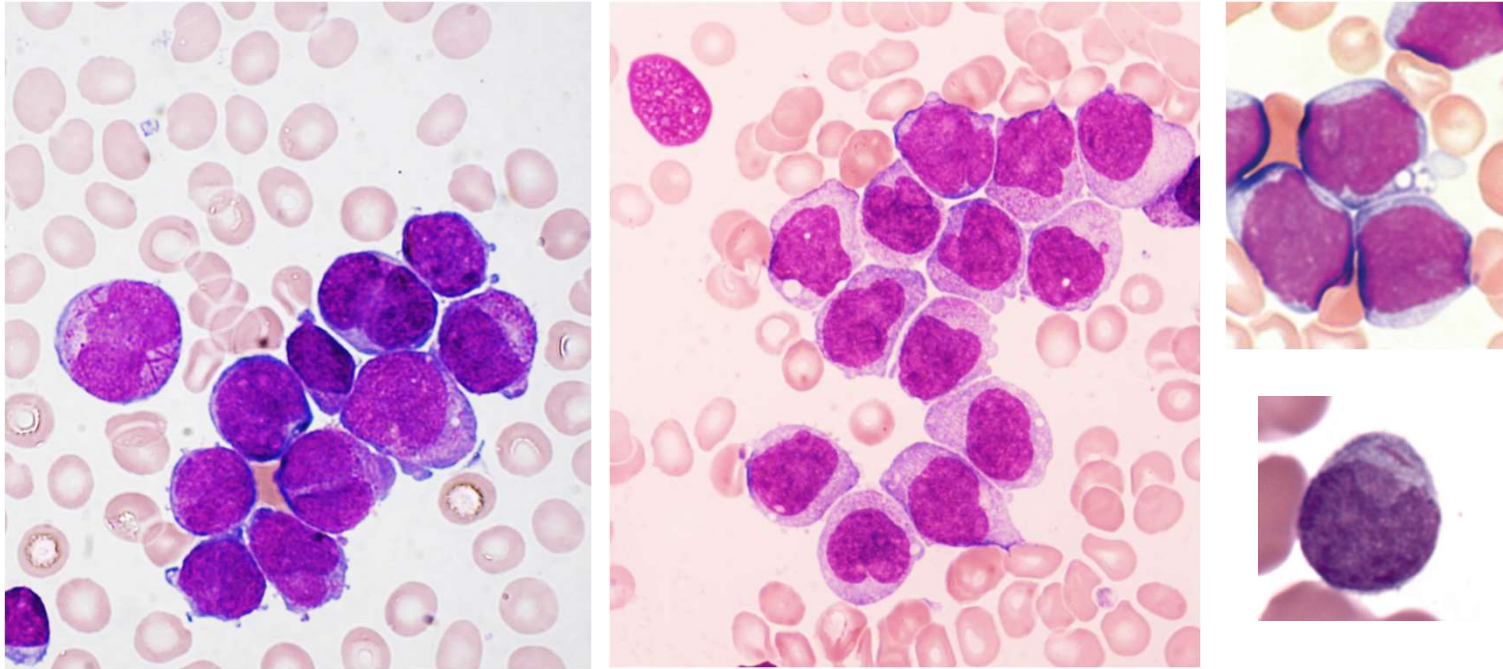
abacus.bates.edu

Attar, Global J Hematol Blood Transf 2014

Akut lymfatisk leukemi (ALL)



Akut myeloisk leukemi (AML)



Ledtrådar:

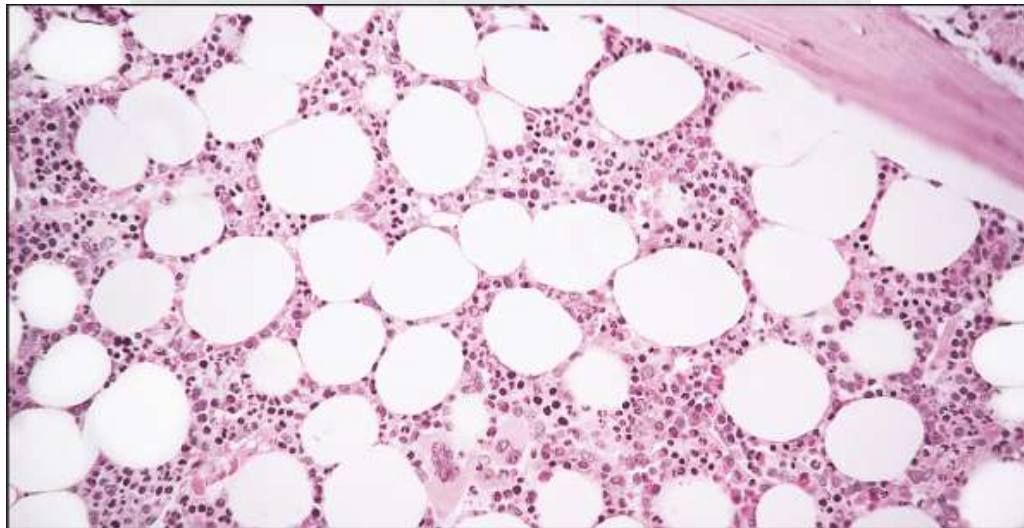
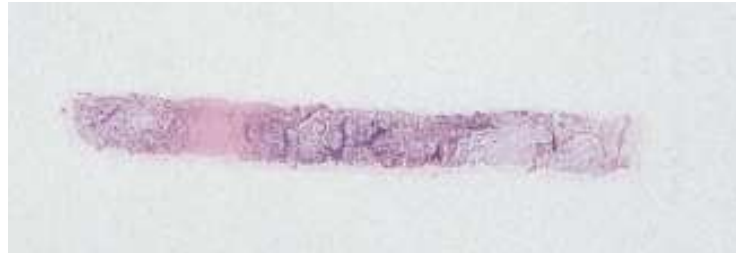
Morfologiska särdrag (Auerstavar)

Cytokemisk färgning: myeloperoxidas, monocytärt/myeloiskt esteras

När biopsi?

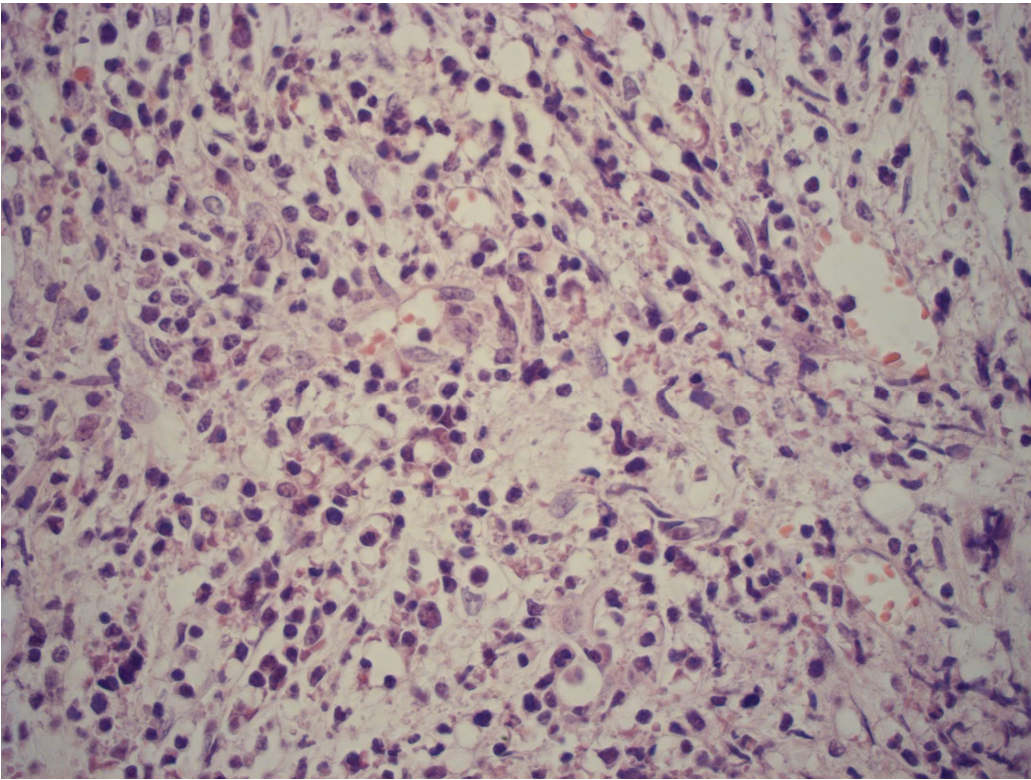
- Vid "dry tap"
- Om tidigare undersökning givit misstanke (t.ex. sparsam cellhalt/icke-representativt med blastökning)

Benmärgsbiopsi

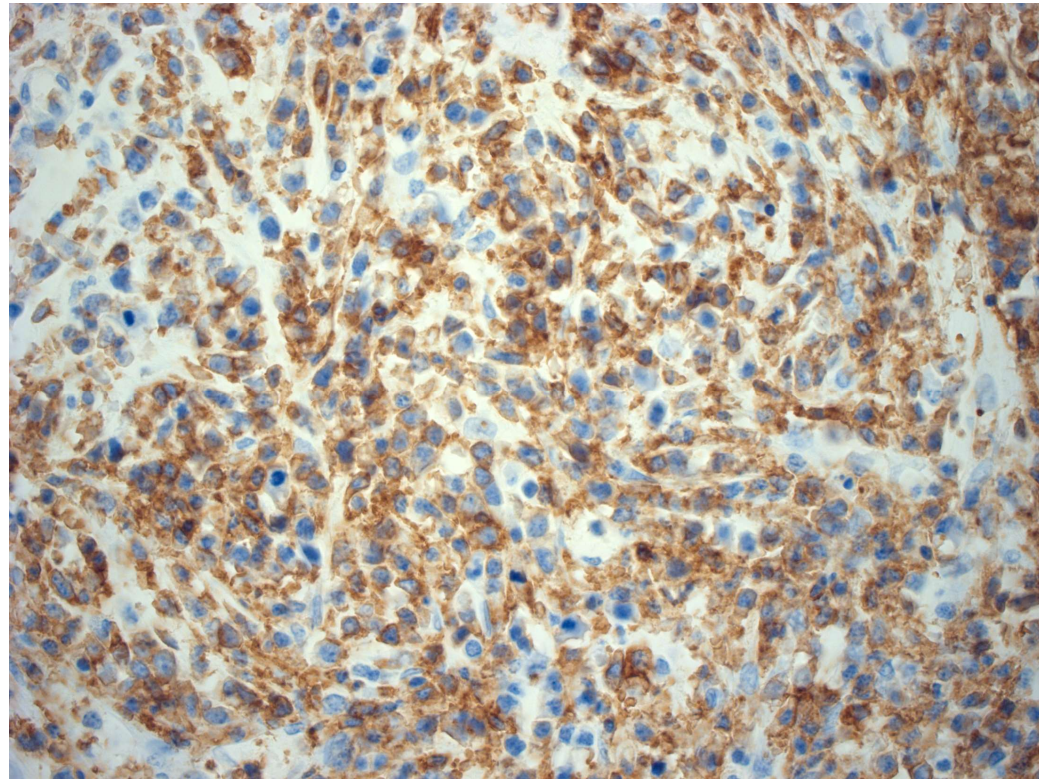


Kan visa akut leukemi även vid blastandel $<20\%$, speciellt vid fibros samt vid akut megakaryoblastleukemi

Benmärgsbiopsi: Akut megakaryoblastleukemi



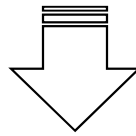
H&E stain



CD61 stain

Morfologi

Akutleukemi?

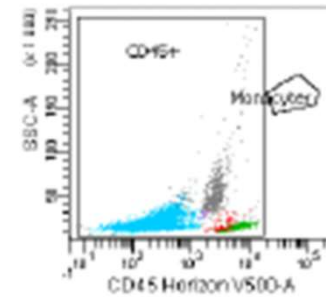
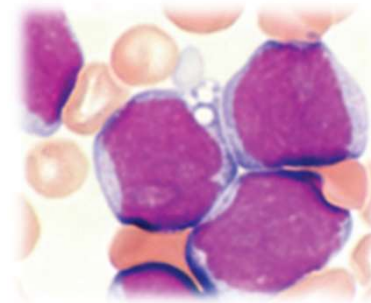


Pre-B-ALL

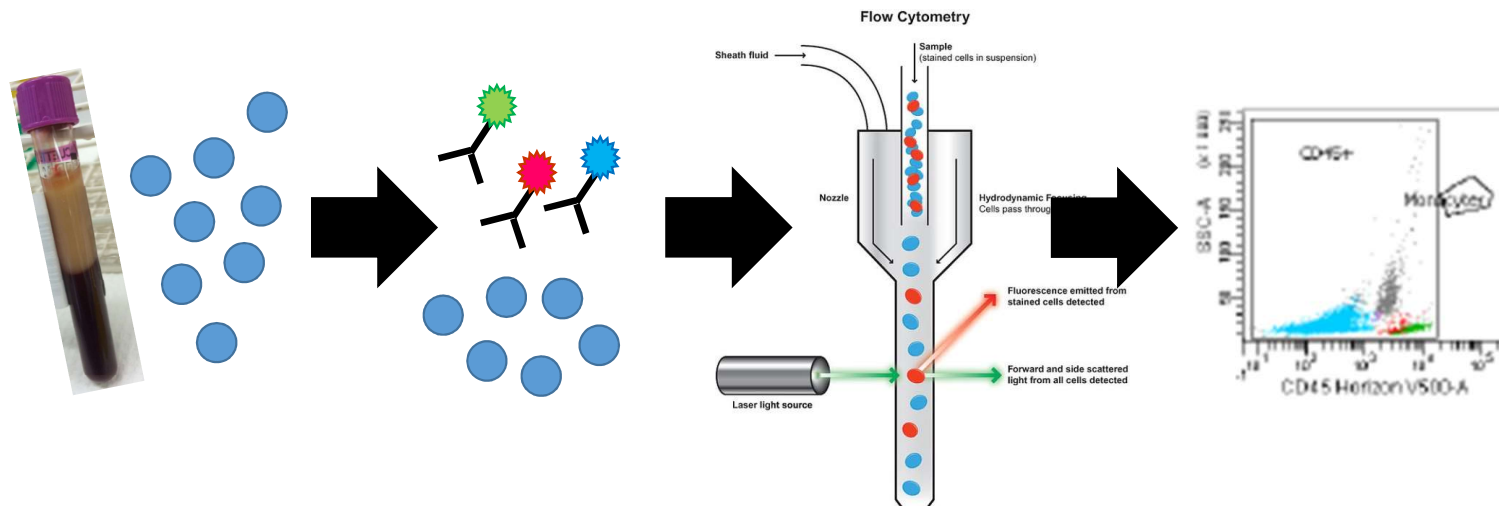
T-ALL

AML

Immunofenotyping

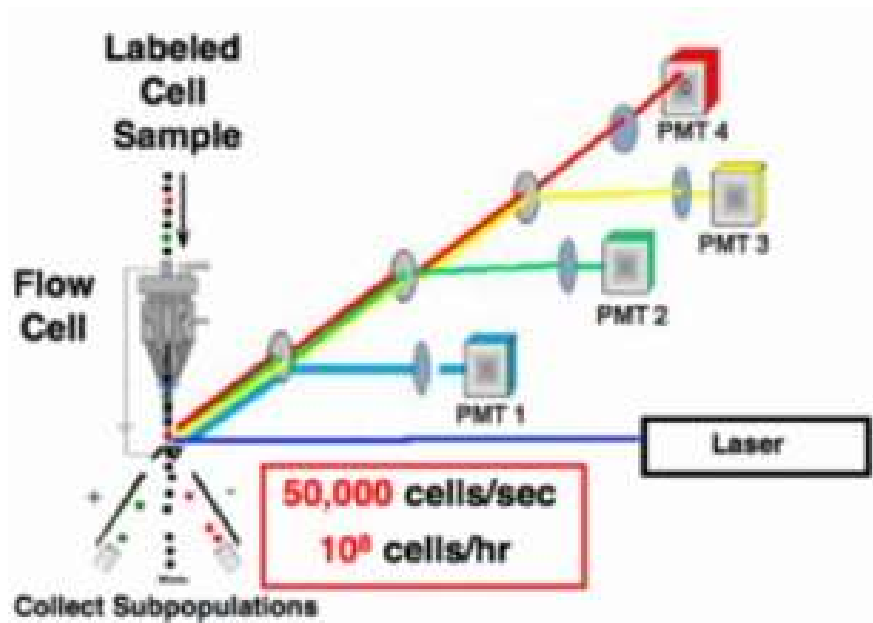


Immunofenotypning vid AL



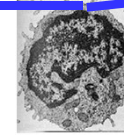
- avgör vilken typ av akutleukemi det är: B/T-ALL / AML / AL med oviss linjetillhörighet
- karakteriserar leukemicellerna för framtida MRD-analys

Immunofenotypning

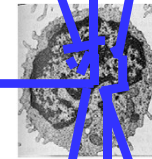


FSC

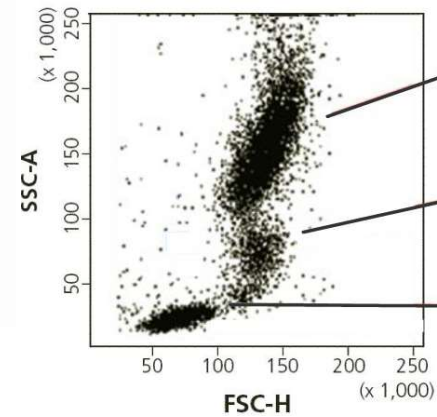
Forward scatter → storlek



SSC

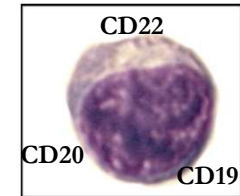
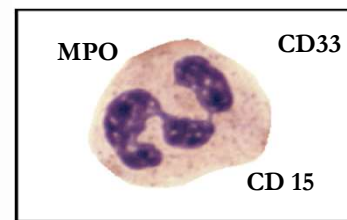
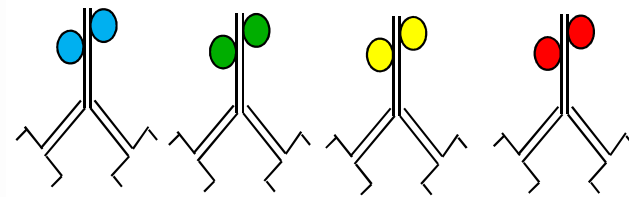
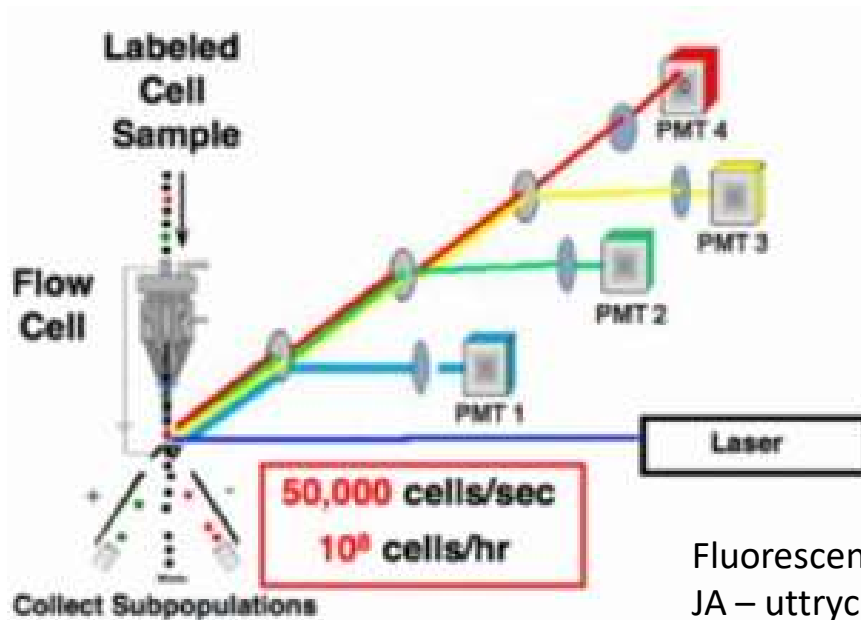


Side scatter → granulering/
Kärn-
komplexitet



Immunofenotypning

3 lasrar → 8 fluorescenskanaler



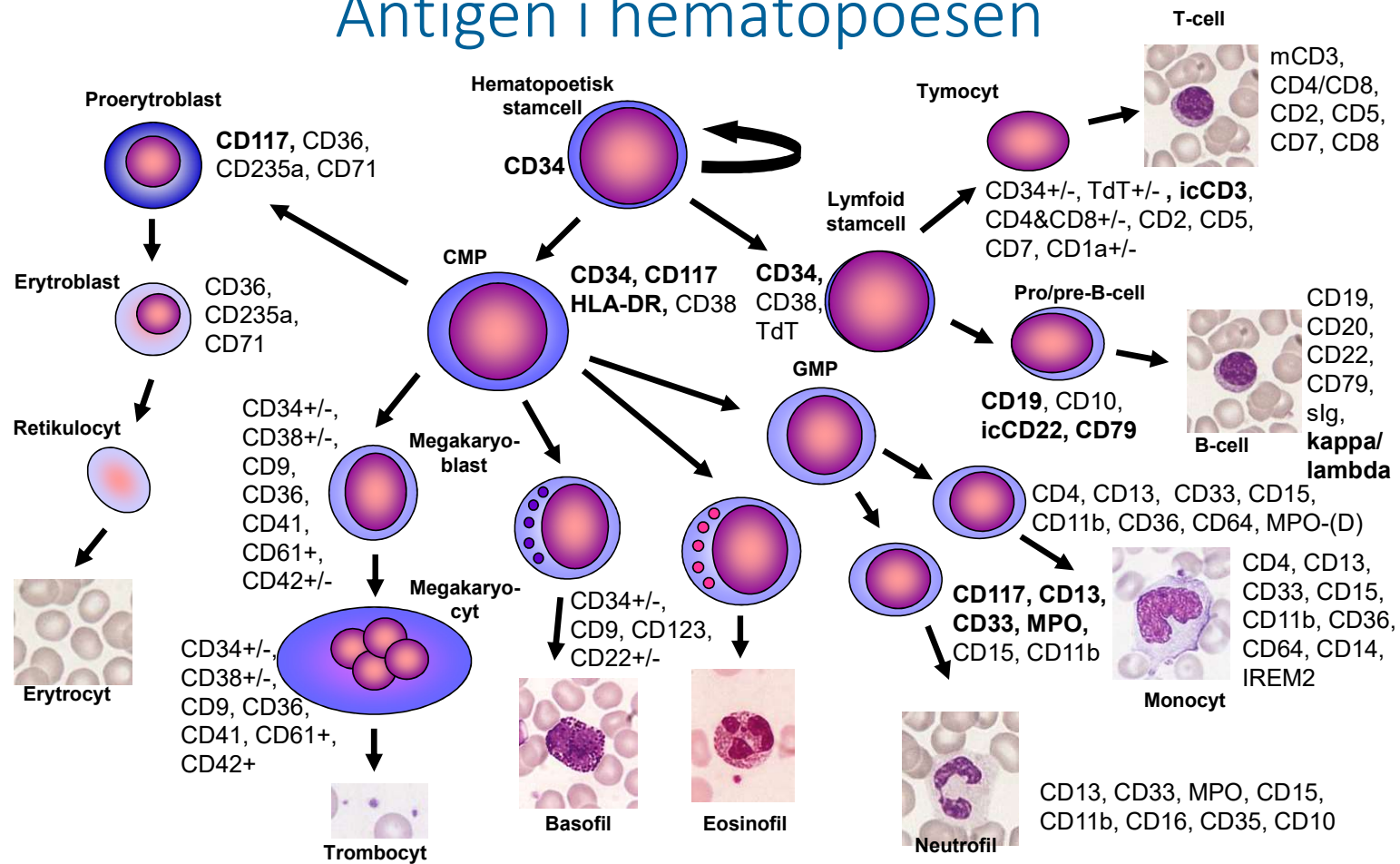
Fluorescenskanalerna → har cellen bundit antikroppen?

JA – uttrycker antigenet/proteinet

NEJ – uttrycker inte antigenet/proteinet

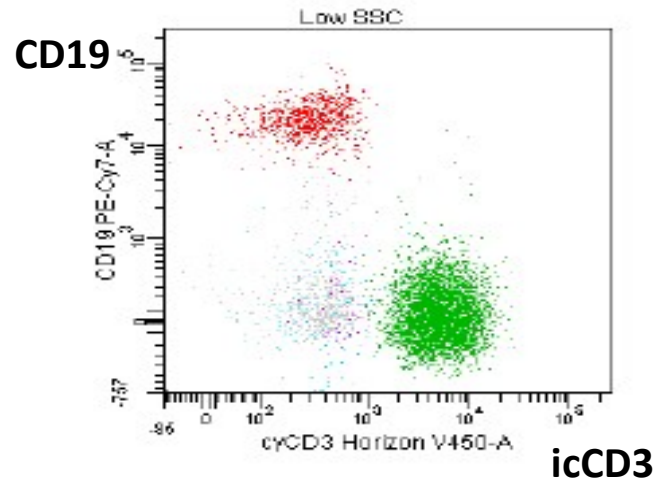
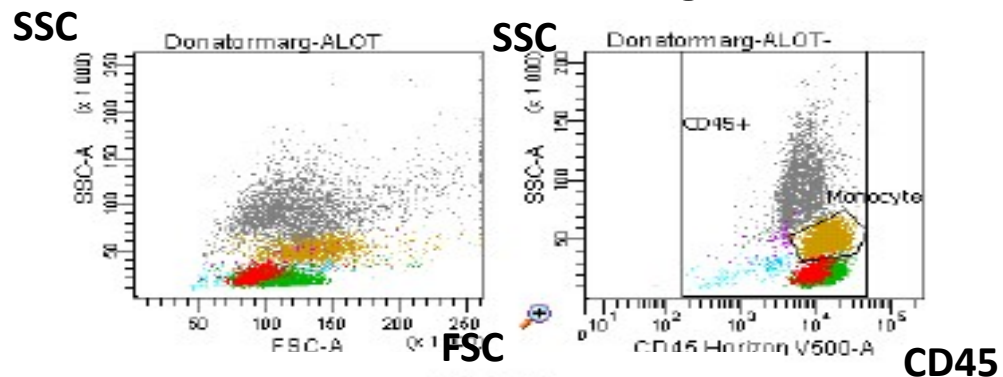
Kombination av antigenuttryck (8 kanaler) → celltyp /
cellinjetillhörighet av "blaster"

Antigen i hematopoesen

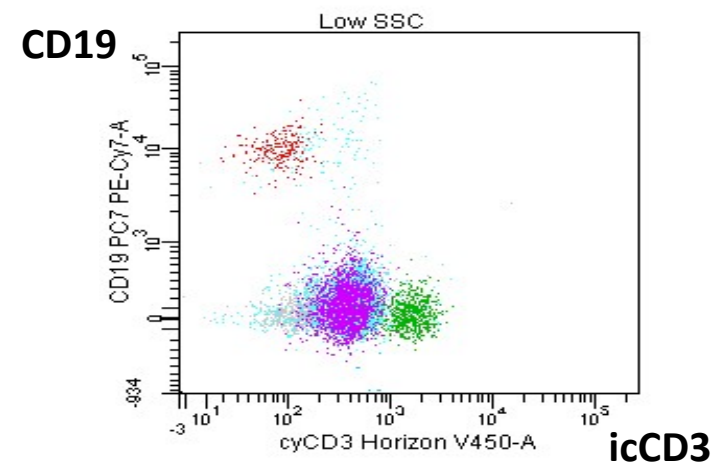
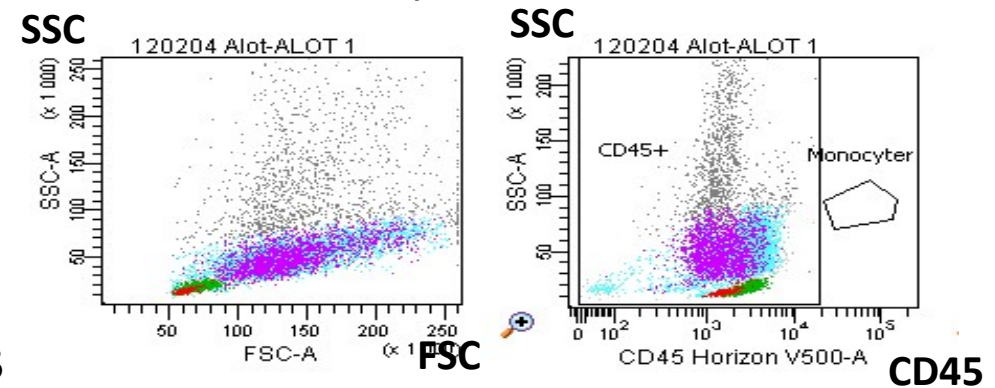


Immunofenotypning vid akut leukemi

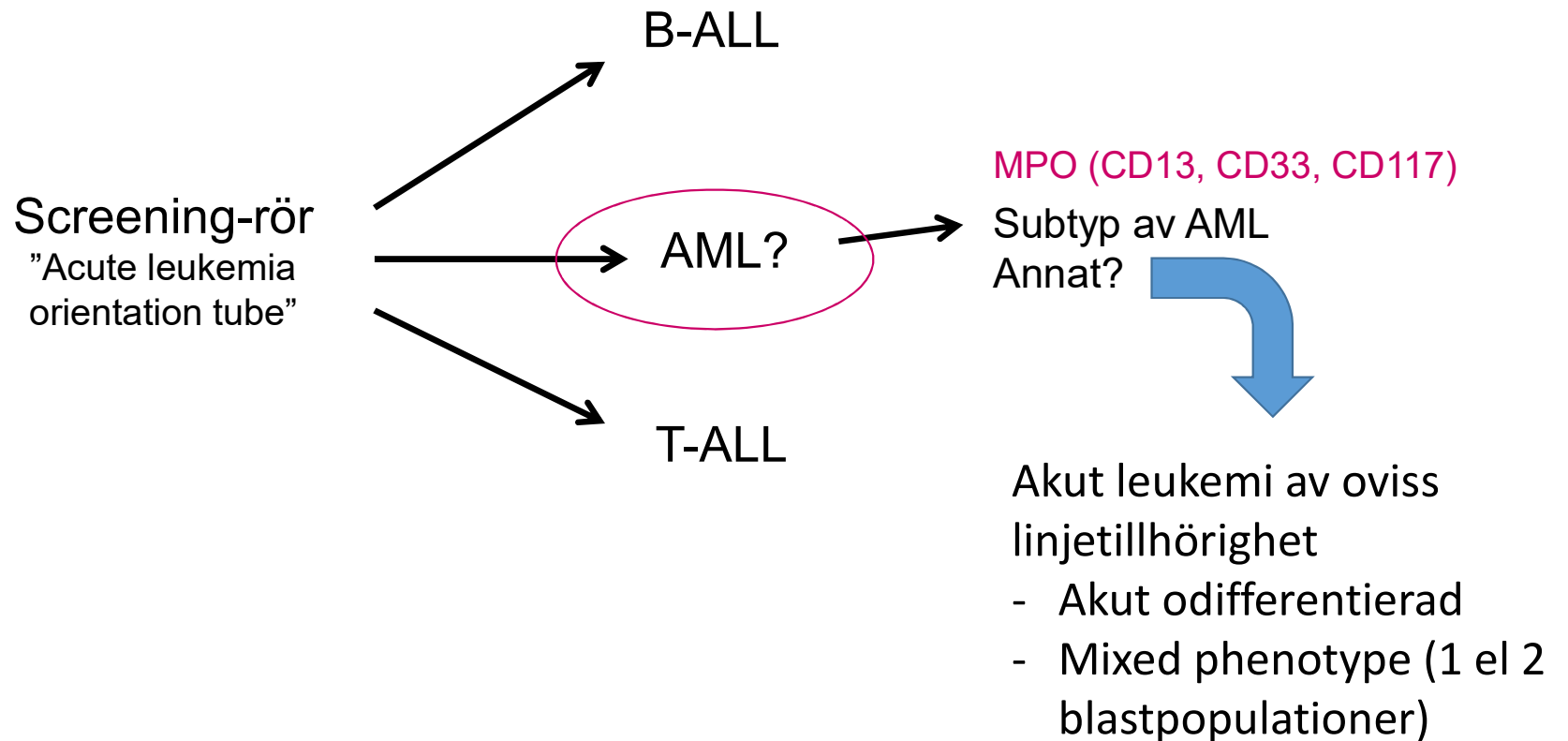
Normal benmärg



Akut myeloisk leukemi



Immunofenotypning vid akut leukemi



8-färgspanel vid AML

Tube	V450	V500	FITC	PE	PerCP-CY5,5	PE-CY7	APC	APC-H7
1.	HLA-DR 2 µl	CD45 2 µl	CD16 5 µl	CD13 5 µl	CD34 5 µl	CD117 5 µl	CD11b 2 µl	CD10 2 µl
2.	HLA-DR 2 µl	CD45 2 µl	CD35 5 µl	CD64 5 µl	CD34 5 µl	CD117 5 µl	IREM2 5 µl	CD14 2 µl
3	HLA-DR 2 µl	CD45 2 µl	CD36 5 µl	CD105 5 µl	CD34 5 µl	CD117 5 µl	CD33 2 µl	CD71 2 µl
4	HLA-DR 2 µl	CD45 2 µl	nuTdT 5 µl	CD56 5 µl	CD34 5 µl	CD117 5 µl	CD7 5 µl	CD19 5 µl
5	HLA-DR 2 µl	CD45 2 µl	CD15 5 µl	NG2 5 µl	CD34 5 µl	CD117 5 µl	CD22 2 µl	CD38 2 µl
6	HLA-DR 2 µl	CD45 2 µl	CD42a-5µl CD61-5µl	CD203c 5 µl	CD34 5 µl	CD117 5 µl	CD123 5 µl	CD4 2 µl
7	HLA-DR 2 µl	CD45 2 µl	CD41 5 µl	CD25 5 µl	CD34 5 µl	CD117 5 µl	CD42b 5 µl	CD9 5 µl

Immunofenotypning vid AML

- Screening → ej B, ej T
- Utvidgad analys i vissa fall obligat för diagnos i frånvaro av återkommande genetiska förändringar
- Komplement till morfologi & cytokemi (monocytära, erytroid, megakaryocytär, basofil)
- Vissa immunofenotyper är associerade med återkommande genetiska förändringar och kan påskynda riktad genetisk utredning
- Karaktärisering av leukemicellerna inför analys av MRD inför stamcellstransplantation/under behandling

AML

Acute myeloid leukemia and related neoplasms

Acute myeloid leukemia with recurrent genetic abnormalities

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*

APL with *PML-RARA*

AML with t(9;11)(p21.3;q23.3); *MLLT3-KMT2A*

AML with t(6;9)(p23;q34.1); *DEK-NUP214*

AML with inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2); *GATA2, MECOM*

AML (megakaryoblastic) with t(1;22)(p13.3;q13.3); *RBM15-MKL1*

Provisional entity: AML with BCR-ABL1

AML with mutated *NPM1*

AML with biallelic mutations of *CEBPA*

Provisional entity: AML with mutated RUNX1

Specifika genetiska
förändringar

Acute myeloid leukemia with myelodysplasia-related changes

Therapy-related myeloid neoplasms

Acute myeloid leukemia, NOS

AML with minimal differentiation

AML without maturation

AML with maturation

Acute myelomonocytic leukemia

Acute monoblastic/monocytic leukemia

Pure erythroid leukemia

Acute megakaryoblastic leukemia

Acute basophilic leukemia

Acute panmyelosis with myelofibrosis

Myeloid sarcoma

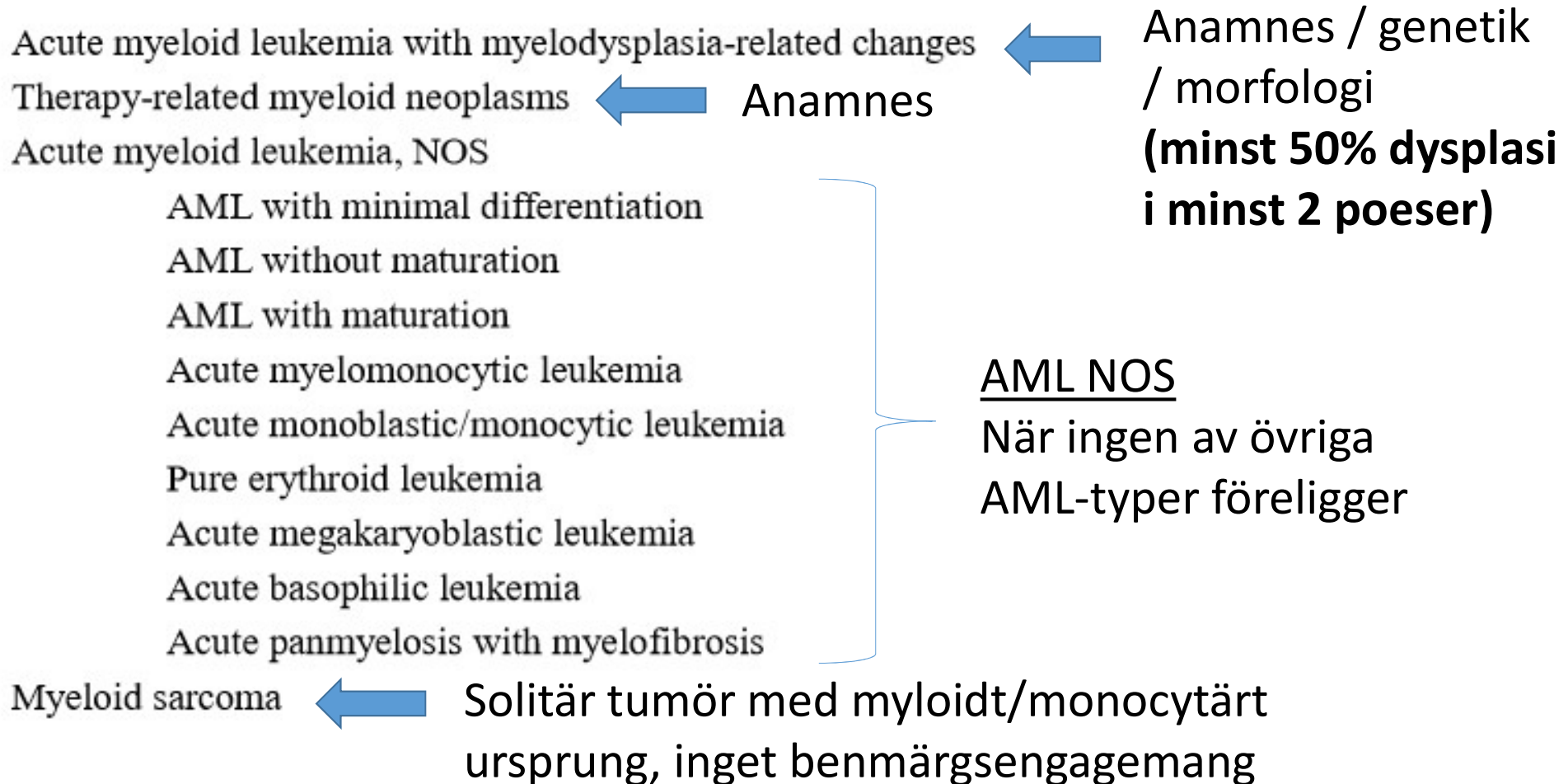
Myeloid proliferations related to Down syndrome

Transient abnormal myelopoiesis

Myeloid leukemia associated with Down syndrome

Barn

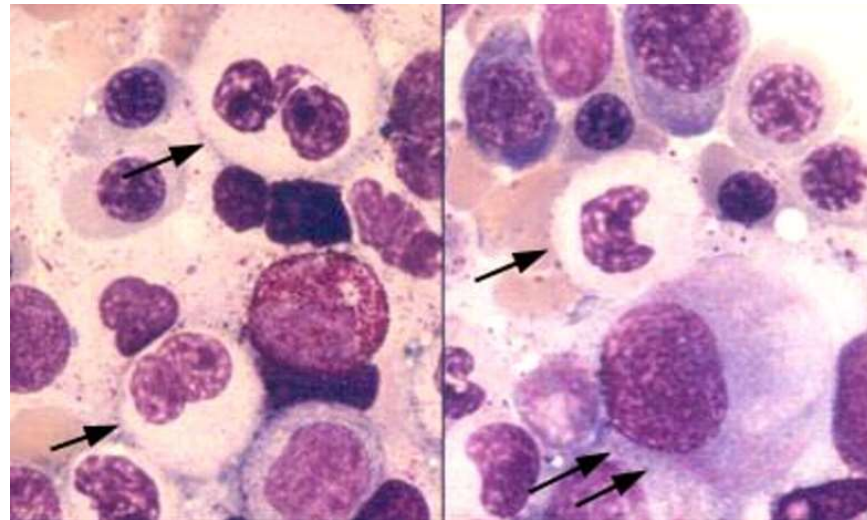
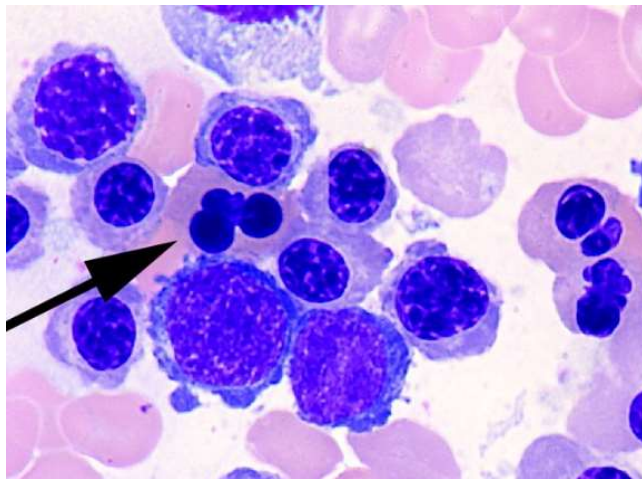
AML, forts.



Dysplasier

Specifika morfologiska avvikelser i

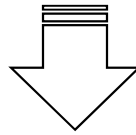
- erytropoesen
- myelopoesen
- megakaryopoesen



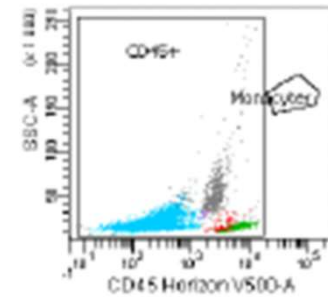
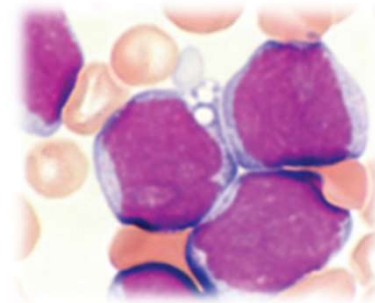


Immunofenotypning

Akuteleukemi?



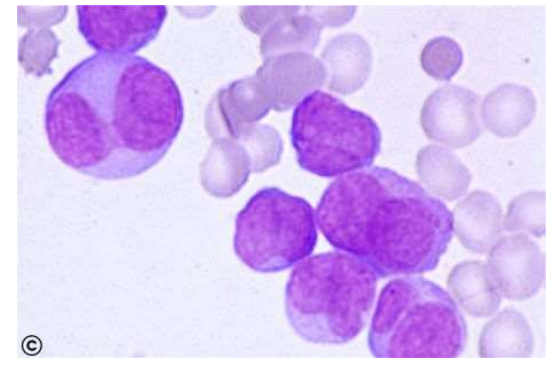
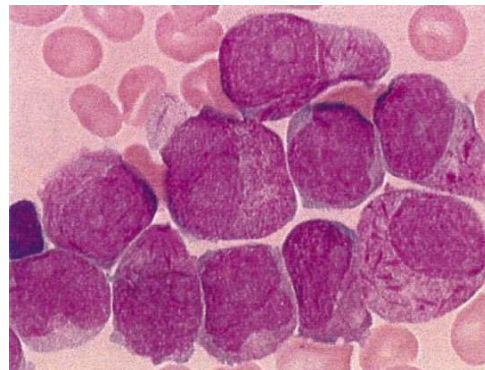
AML

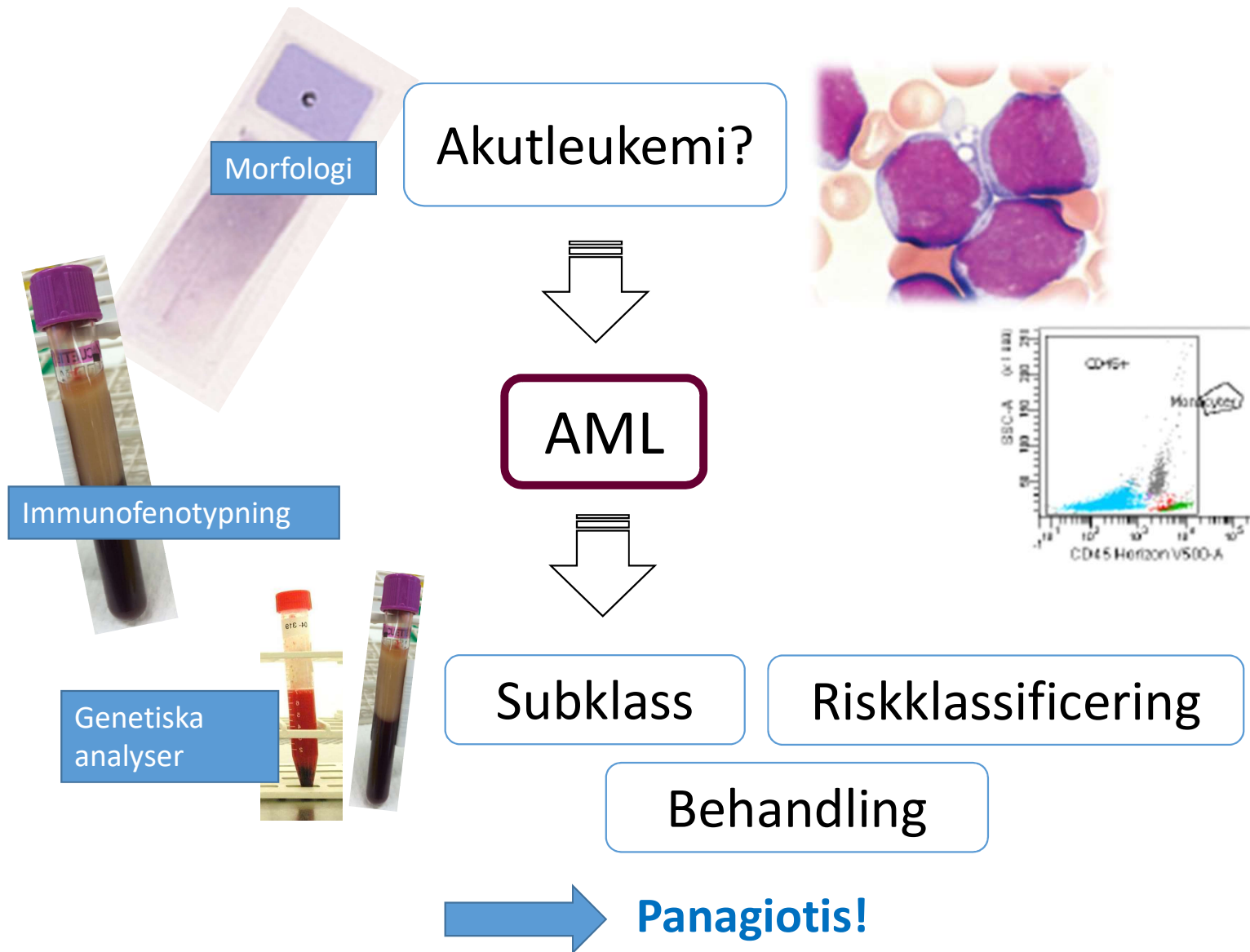


Viktig fråga:

Kan det vara APL (akut promyleocytleukemi)?

- Klassisk form?
- Hypogranulerad?





Morfologisk subklassificering AML

AML with minimal differentiation	FAB M0 – AML, men ingen av nedanstående, baseras på antigenuttryck
AML without maturation	FAB M1 – AML där blasterna är positiva för myeloperoxidas (MPO)
AML with maturation	FAB M2 – myeloiska blaster + utmognande myelopoies (>10%)
Acute myelomonocytic leukemia	FAB M4 – myeloiska/monocytära blaster, myelopoies >20%, monopoies > 20%
Acute monoblastic/monocytic leukemia	FAB M5 – monocytära blaster, utan/med promono/mono
Pure erythroid leukemia	FAB M6 (modifierad) – erytroida blaster
Acute megakaryoblastic leukemia	FAB M7 – megakaryoblaster
Acute basophilic leukemia	Blaster med basofila markörer
Acute panmyelosis with myelofibrosis	Myeloiska blaster + fibros men uppfyller ingen annan kategori

The French-American-British (**FAB**) **classification** of AML was developed in the 1970s by a group of French, American, and British **leukemia** experts.