Optische genoommapping voor hematologische maligniteiten

08-03-2022

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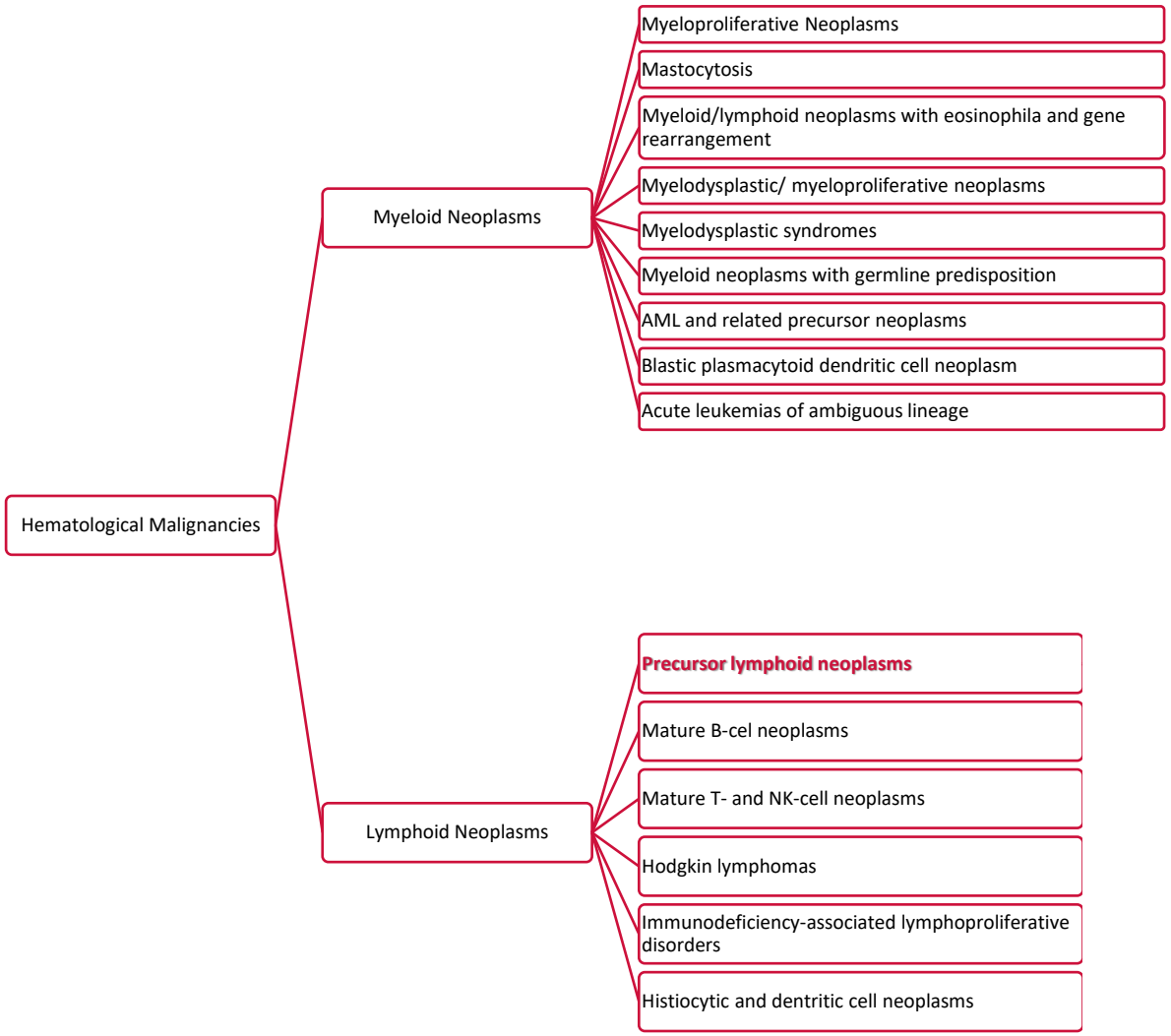
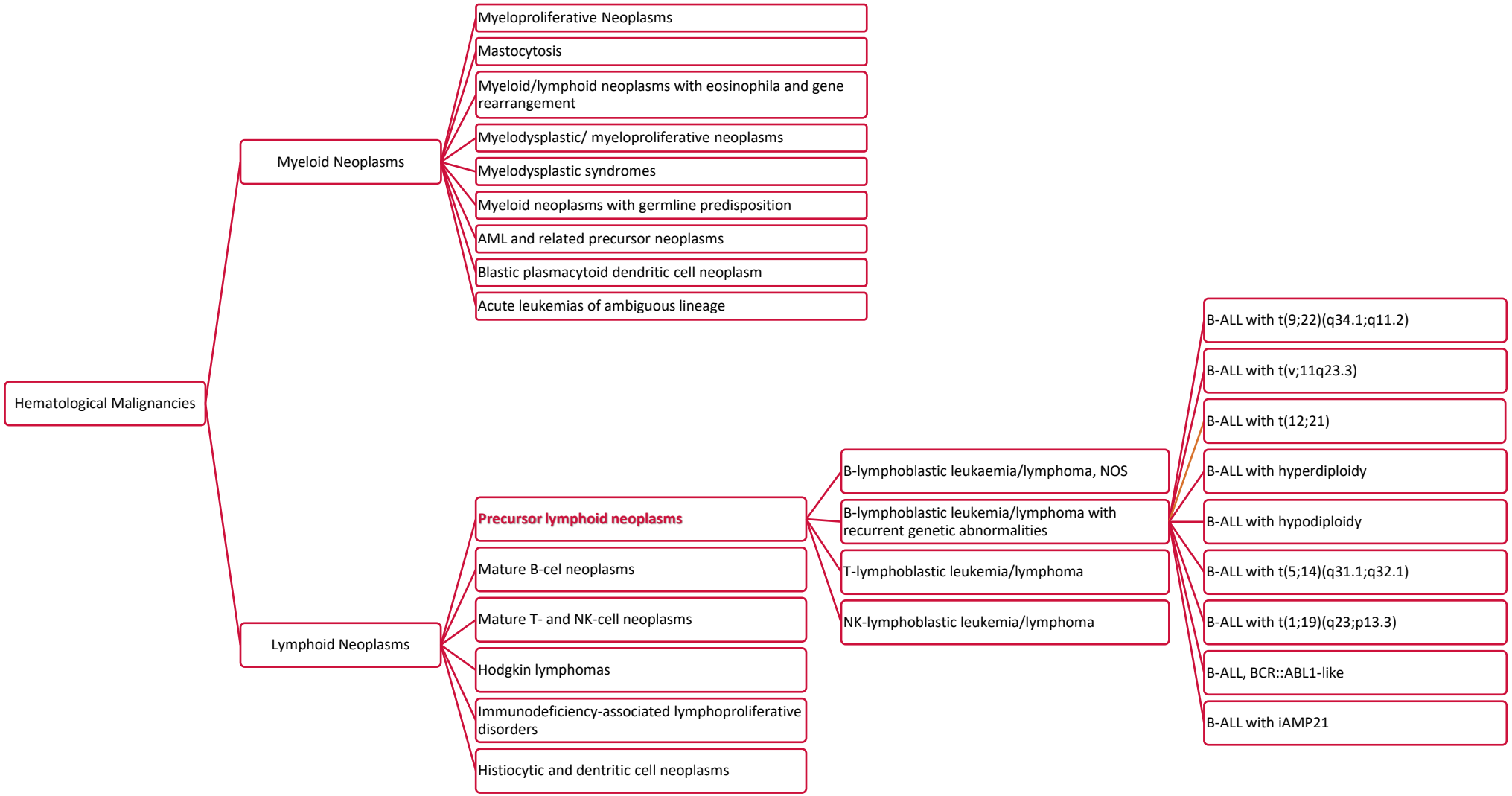
Overzicht

- Inleiding WHO classificatie van hematologische maligniteiten: ALL en AML
- Diagnostische tools binnen de genetica
- OGM werkingsmechanisme
- OGM praktische voorbeelden
- Conclusie

2016 WHO classificatie van hematologische maligniteiten



2016 WHO classificatie van hematologische maligniteiten

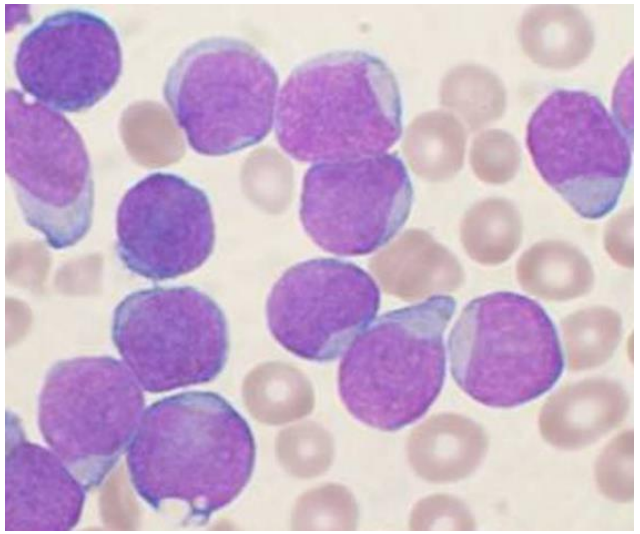


Wat is acute lymfatische leukemie (ALL)?

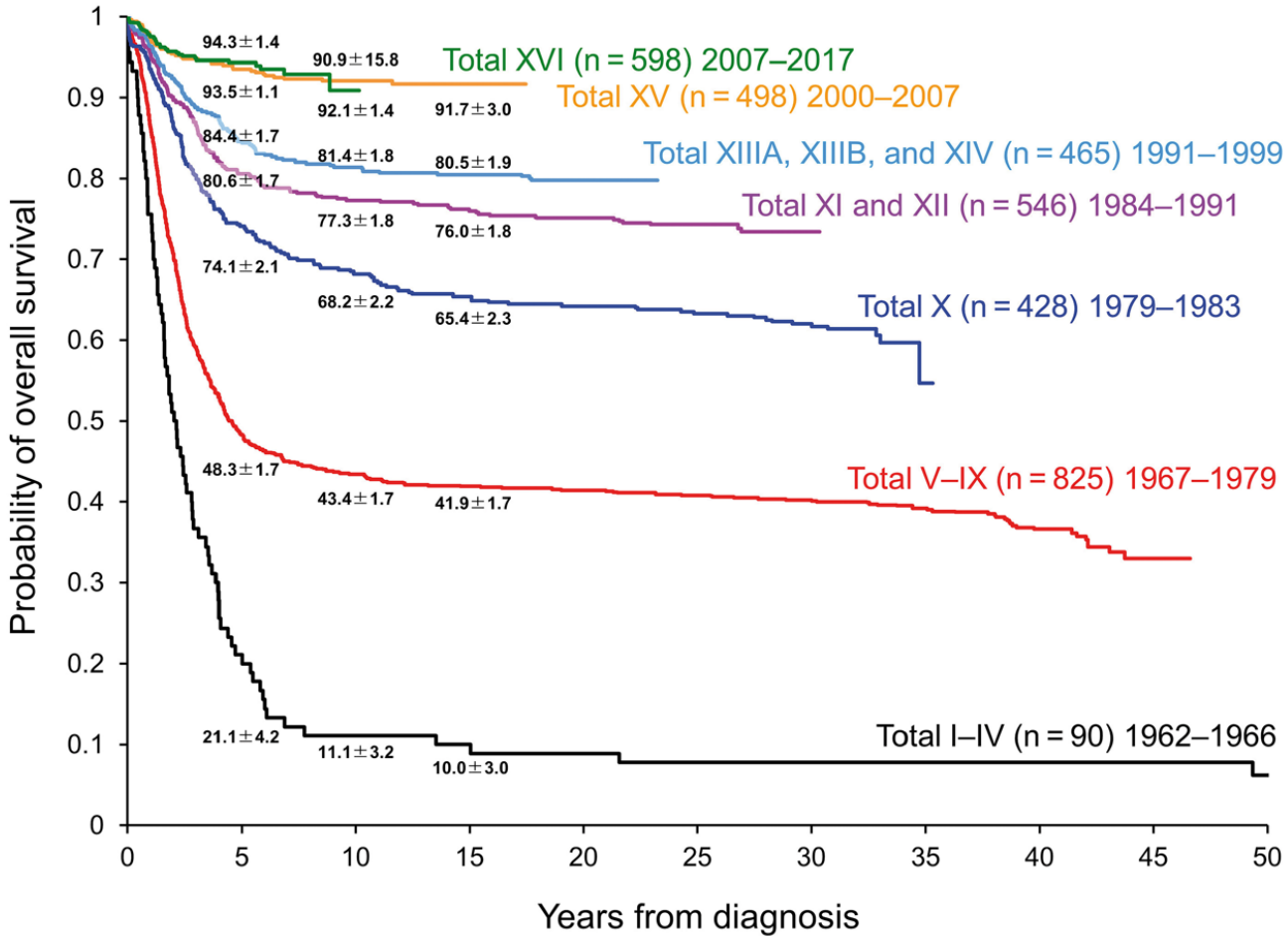
- Accumulatie van maligne, immature lymfoïde cellen in bloed en beenmerg
- 85% B-ALL, 15% T-ALL

- Symptomen: vermoeidheid, herhaalde infecties, botpijn, petechiën,…
- Diagnose: bloed en beenmergonderzoek: morfologie, immuunfenotypering, histologie en genetica

- Incidentie: 3-4 kinderen per 100 000 per jaar (<1 voor volwassenen)
- Prognose: 5-jaarsoverleving kinderen >90% (50% voor volwassenen), 1/5 ondervindt herval (slechte prognose)

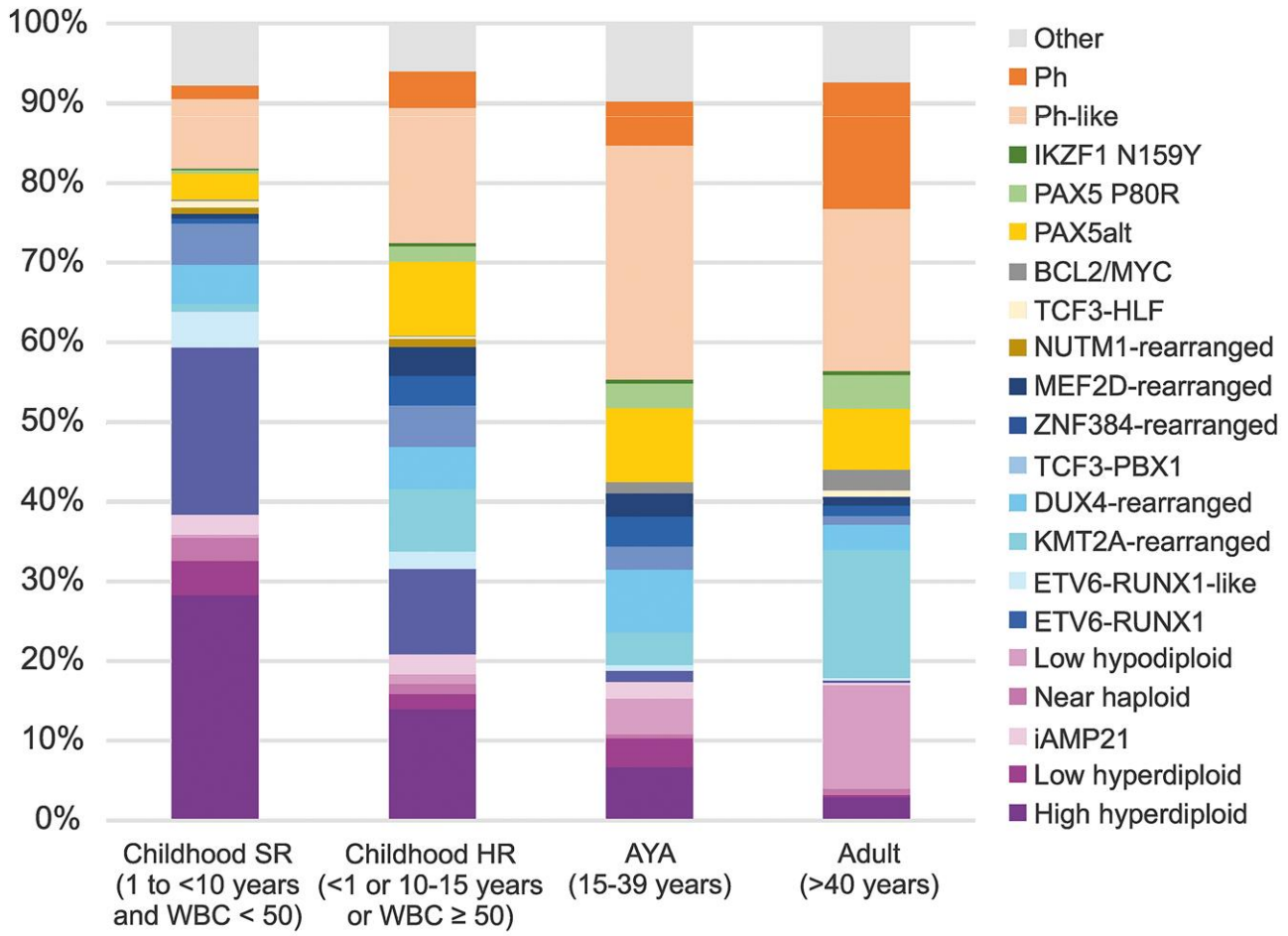


Verbeterde overleving van patiënten met ALL



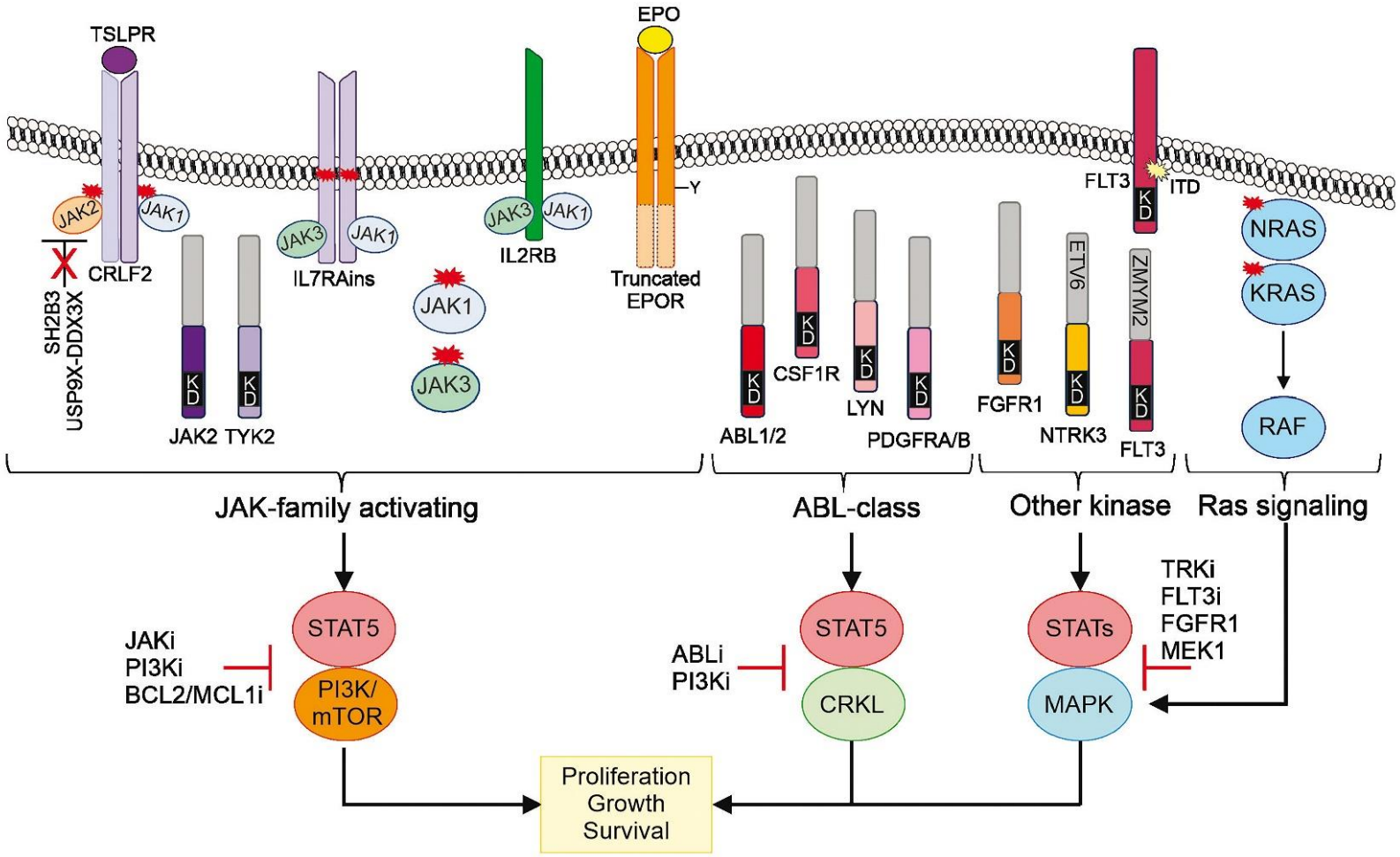
Inaba and Mullighan. Pediatric acute lymphoblastic leukemia. Haematologica 2020.

Genetische risicofactoren in B-ALL



Inaba and Mullighan. Pediatric acute lymphoblastic leukemia. Haematologica 2020.

Afwijkingen die leiden tot Ph-like ALL



Inaba and Mullighan. Pediatric acute lymphoblastic leukemia. Haematologica 2020.

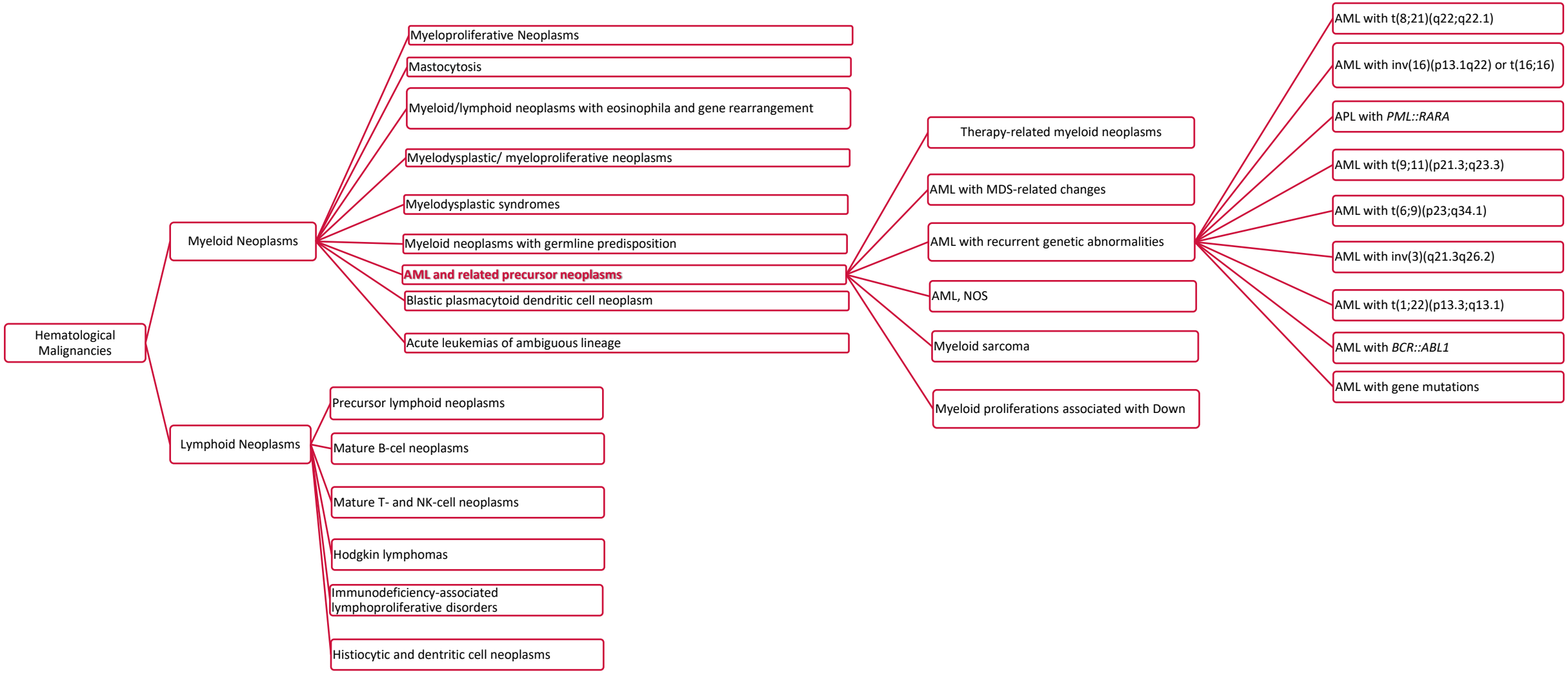
Klinisch relevante afwijkingen in ALL

Good risk abnormalities	*Standard risk abnormalities*	*Intermediate risk abnormalities*	*High risk abnormalities*
High hyperdiploidy (>50 chr)	t(1;19)(q23;qp13.3) *PBX1, TCF3*	t(X;14)(p22;q32)/t(Y;14)(p11q32) *IGH, CRLF2*	Near haploidy (25-29 chr)
TAL1 abnormalities*	15q13-15 rearrangements	del(X)(p22.33)/del(Y)(p11.32) *P2RY8, CRLF2*	Low hypodiploidy (30-39 chr)
t(2;8)(p11;q24) *IGK, MYC*			High hypodiploidy (<44, poor)
t(7;10)(q34;q24) *TRB, TLX1**			Trisomy 5
t(8;14)(q24;q32) *MYC, IGH*			del(5)(q32q33,3) *EBF1, PDGFRB*
t(8;14)(q24;q11) *MYC, IGL*			t(5;9)(q22;q34) *SNX2, ABL1*
dic(9;12)(p11~12;p11~13) *PAX5, ETV6*			t(5;14)(q35;q32) *TLX3, BCL11B*
t(10;14)(q24;q11) *TLX1, TRA/TRD**			del(7p12.2) *IKZF1*
t(12;21)(p13;q22) *ETV6, RUNX1*			t(7;19)(q34;p13) *TRB, LYL1*
del(21)(q22.2) *ERG*			dic(9;20)(p13;q11) *PAX5*
			del(9)(p23.3) *CDKN2A°*
			t(9;22)(q34;q11) *ABL1, BCR^*
			10p12 aberrations *MLLT10*
			11q23 aberrations *KMT2A*
			t(14;18)(q32;q21) *IGH, BCL2*
			t(17;19)(q22;p13) *HLF, TCF3^*

*better than other T-ALL
°prognosis variable
^extremely poor
chromosomal aberrations are shown on the left-hand side and involved genes on the right-hand side

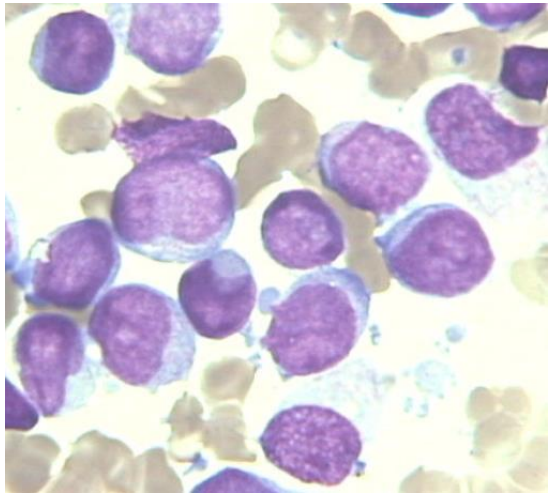
Table 1
Adeapted from: Cancer cytogenetics: Chromosomal and Molecular Genetic Aberrations of Tumor Cells, Foutrh Edition. Page 202-204.

2016 WHO classificatie van hematologische maligniteiten



Wat is acute myeloïde leukemie (AML)?

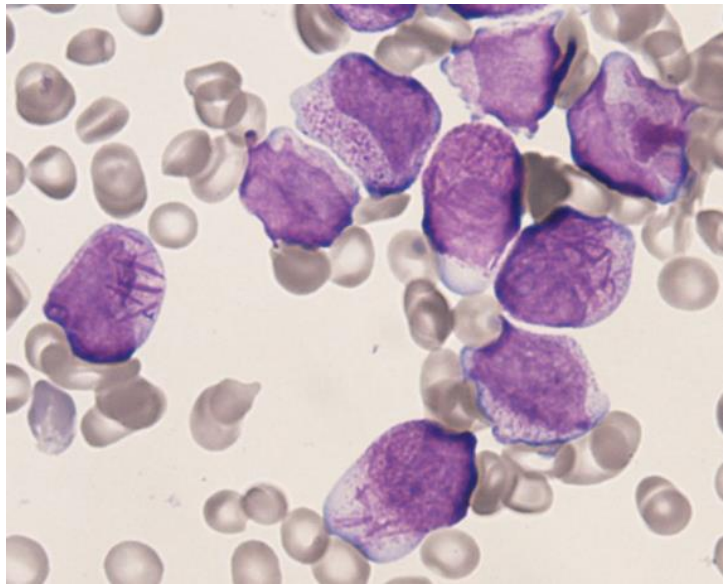
- Accumulatie van maligne, immature myeloïde cellen in bloed en/of beenmerg
- Minstens 20% blasten in bloed of beenmerg TENZIJ t(8;21), inv(16) of t(16;16) en t(15;17)
- Aanwijzingen: cytopenieën, voorgeschiedenis (chemo/radiotherapie, MDS), vermoeidheid, herhaalde infecties,…
- Diagnose: bloed en beenmergonderzoek: morfologie, immuunfenotypering, histologie en genetica

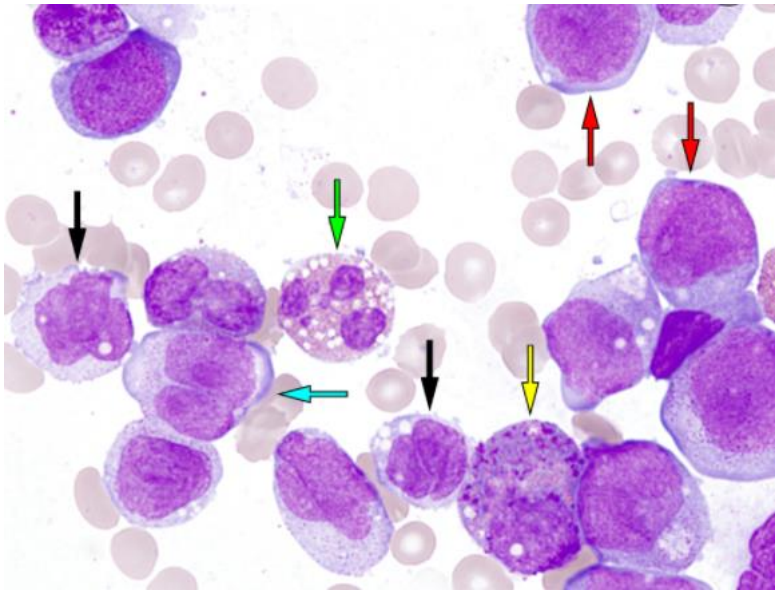


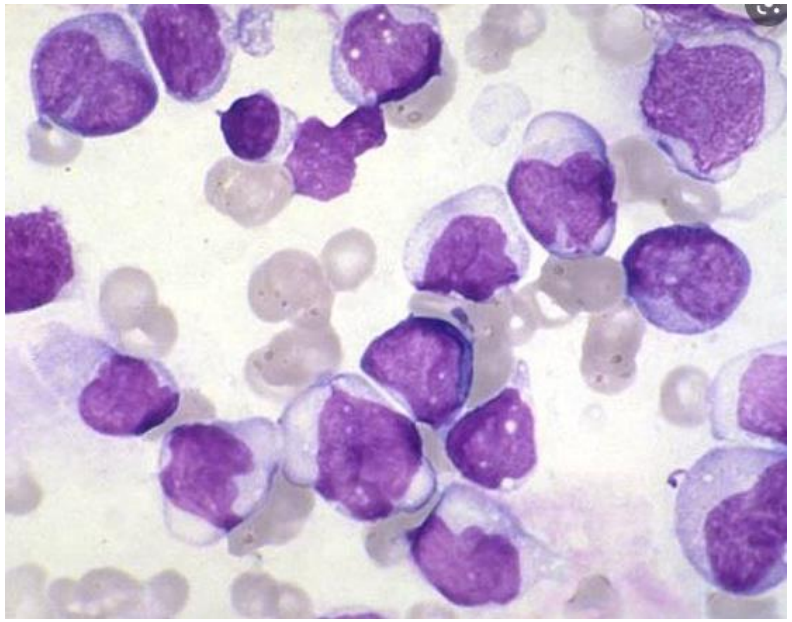
- Prevalentie: 0.5%
- Incidentie: 3.6 per 100 000 per jaar (meest frequente acute leukemie (70%) in volwassenen), mediane leeftijd ~70 jaar
  -> veel minder frequent in kinderen (5x meer ALL)

- Prognose: afhankelijk van type AML

AML: link genetica - morfologie







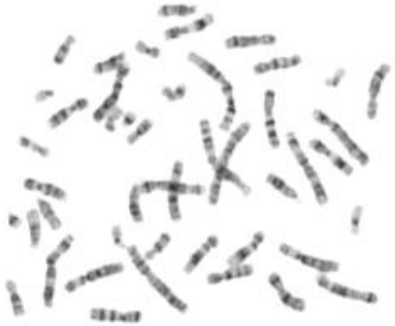
Klinisch relevante afwijkingen in AML

Table 4. 2017 ELN Risk Stratification by Genetics

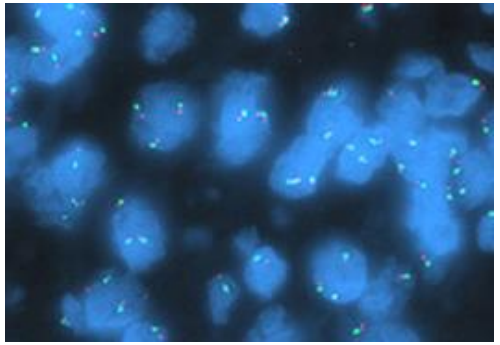
Risk Category*	Genetic Lesion
Favorable	t(8;21)(q22;q22.1); *RUNX1-RUNX1T1*
	inv(16)(p13.1q22) or t(16;16)(p13.1;q22); *CBFB-MYH11*
	Mutated *NPM1* without *FLT3*-ITD or with FLT3-ITD^{low}†
	Biallelic mutated *CEBPA*
Intermediate	Mutated *NPM1* and *FLT3*-ITD^{high}†
	Wild-type *NPM1* without *FLT3*-ITD or with *FLT3*-ITD^{low}† (without adverse-risk gene mutations)
	t(9;11)(p21.3;q23.3); *MLLT3-KMT2A*‡
	Cytogenetic 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*
	inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2); *GATA2, MECOM(EVI1)*
	−5 or del(5q); −7; −17/abn(17p)
	Complex karyotype,§ monosomal karyotype‖
	Wild-type *NPM1* and *FLT3*-ITD^{high}†
	Mutated *RUNX1*¶
	Mutated *ASXL1*¶
	Mutated *TP53*#

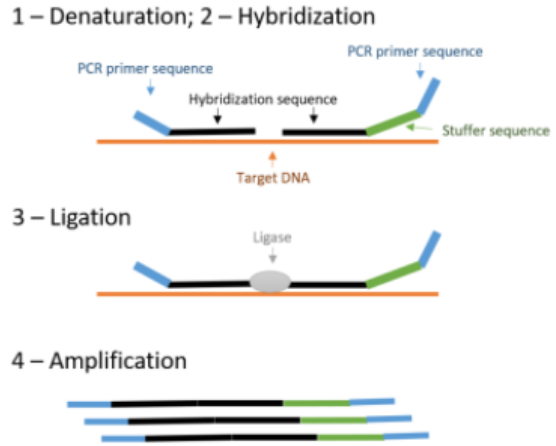
Bullinger et al. Genomics of Acute Myeloid Leukemia Diagnosis and Pathways. J Clin Oncol 2017.

Over welke diagnostische tools beschikt de dienst genetica?

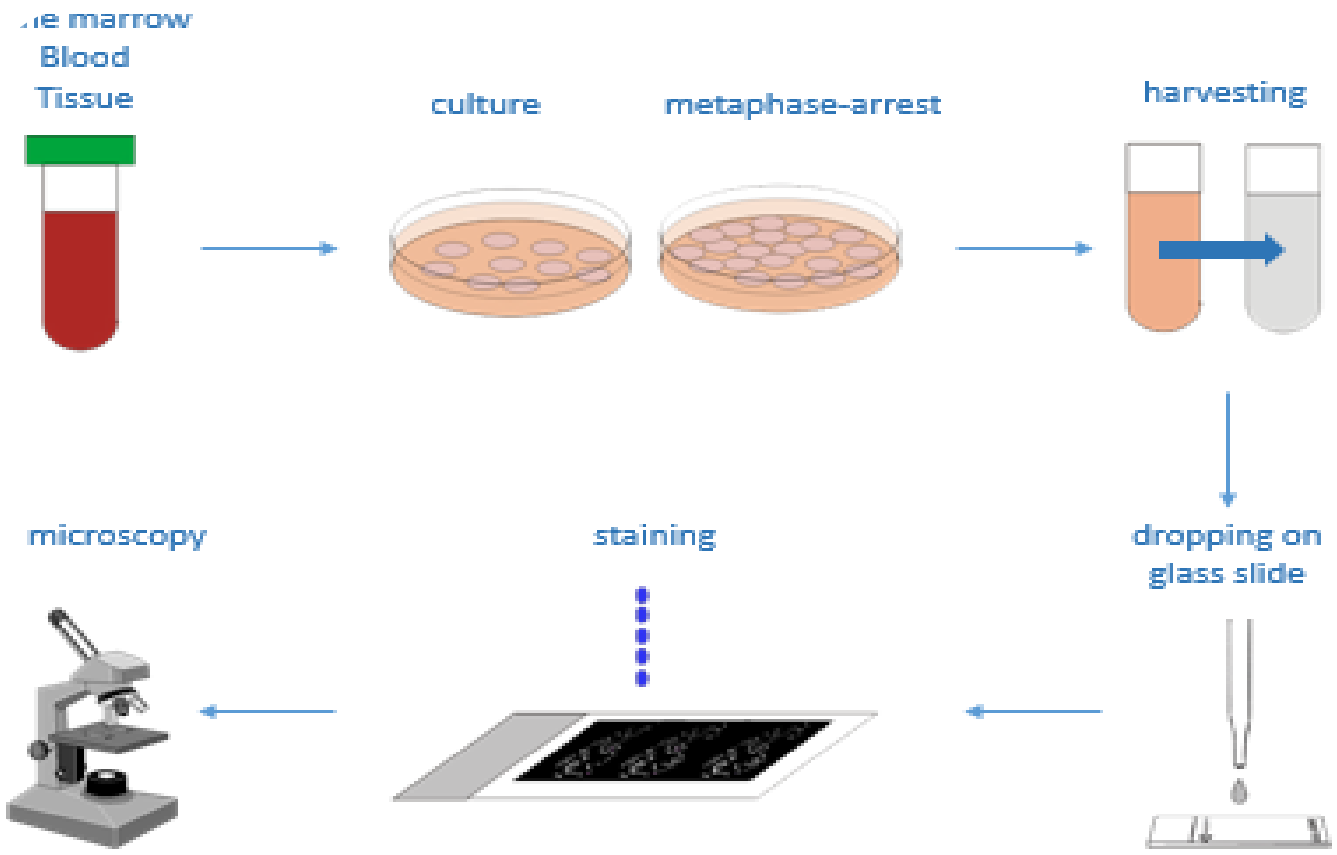


- Karyotypering
- FISH
- MLPA
- PCR-gebaseerde technieken
- NGS

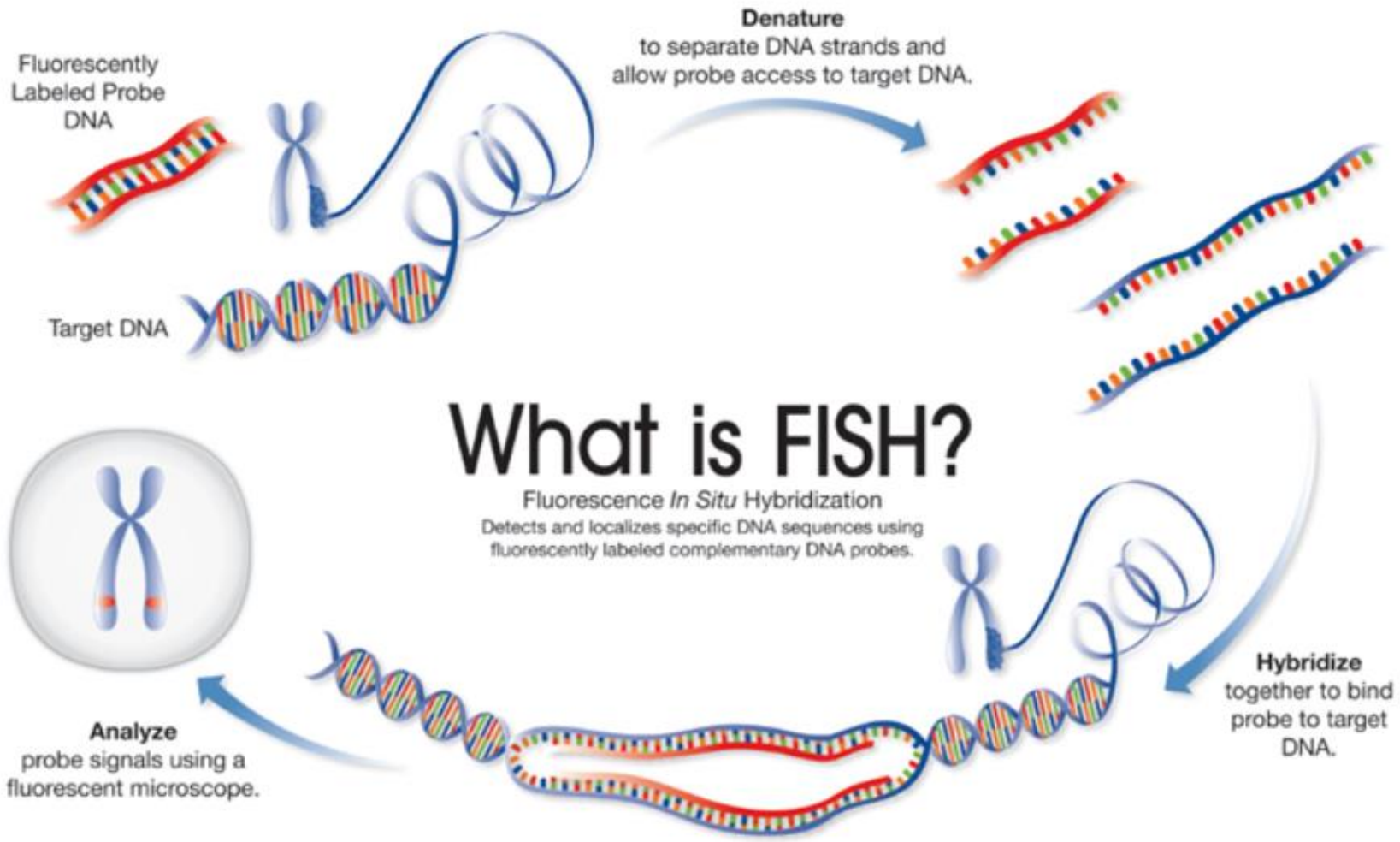




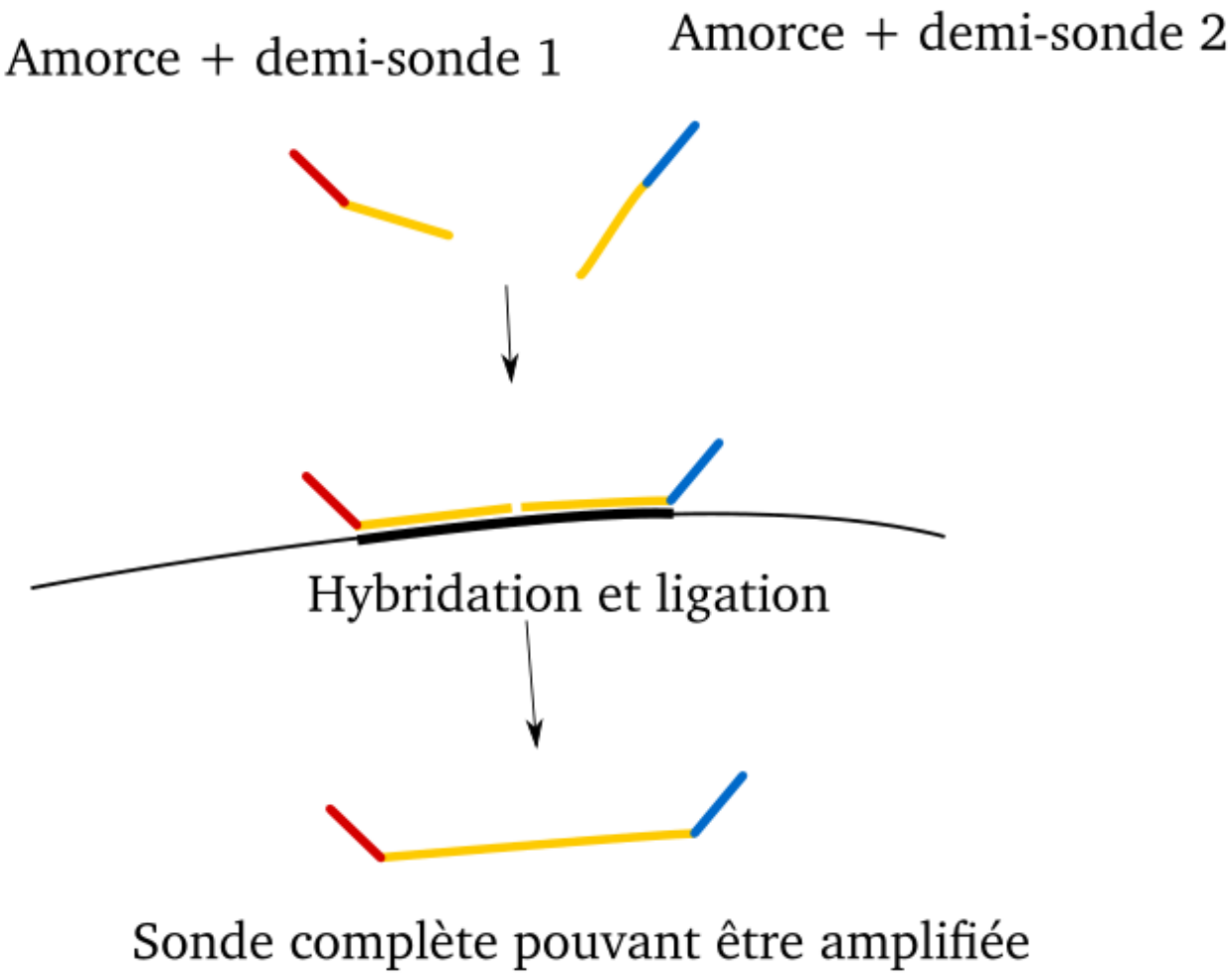
Karyotypering



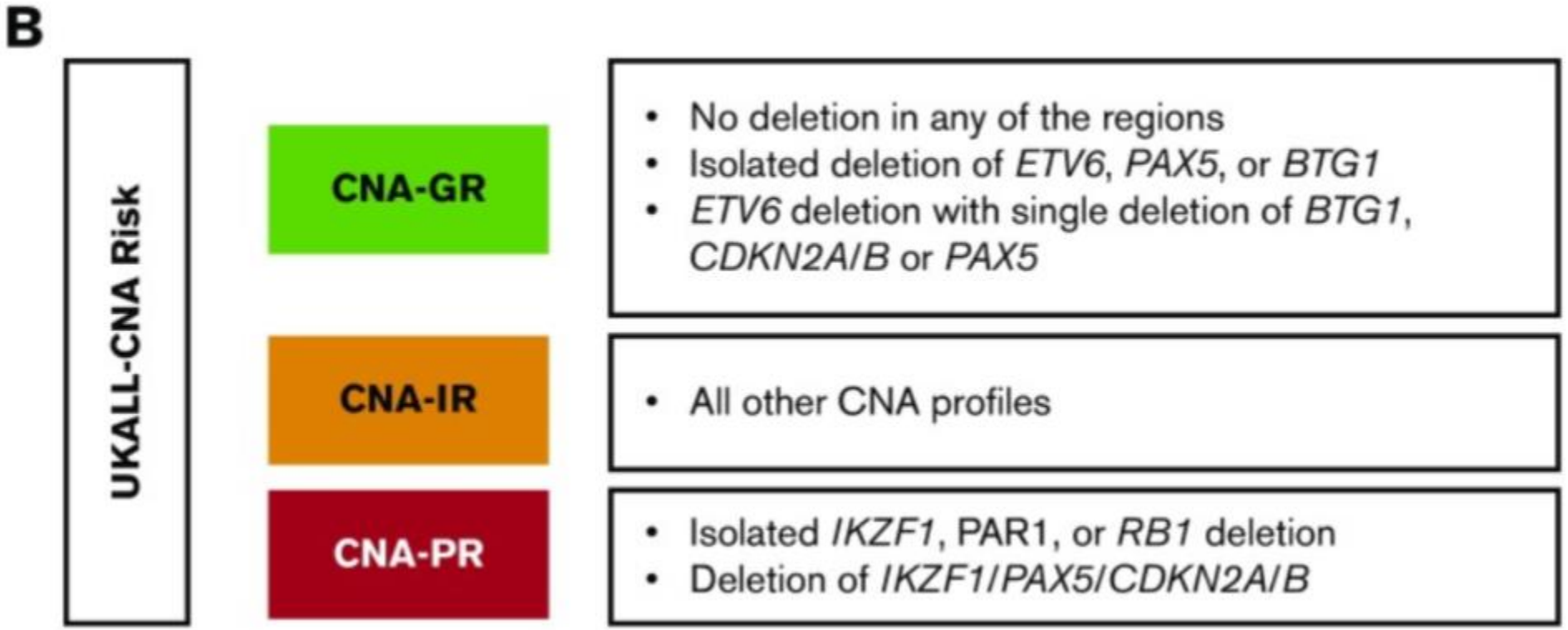
Fluorescent in-situ hybridisation



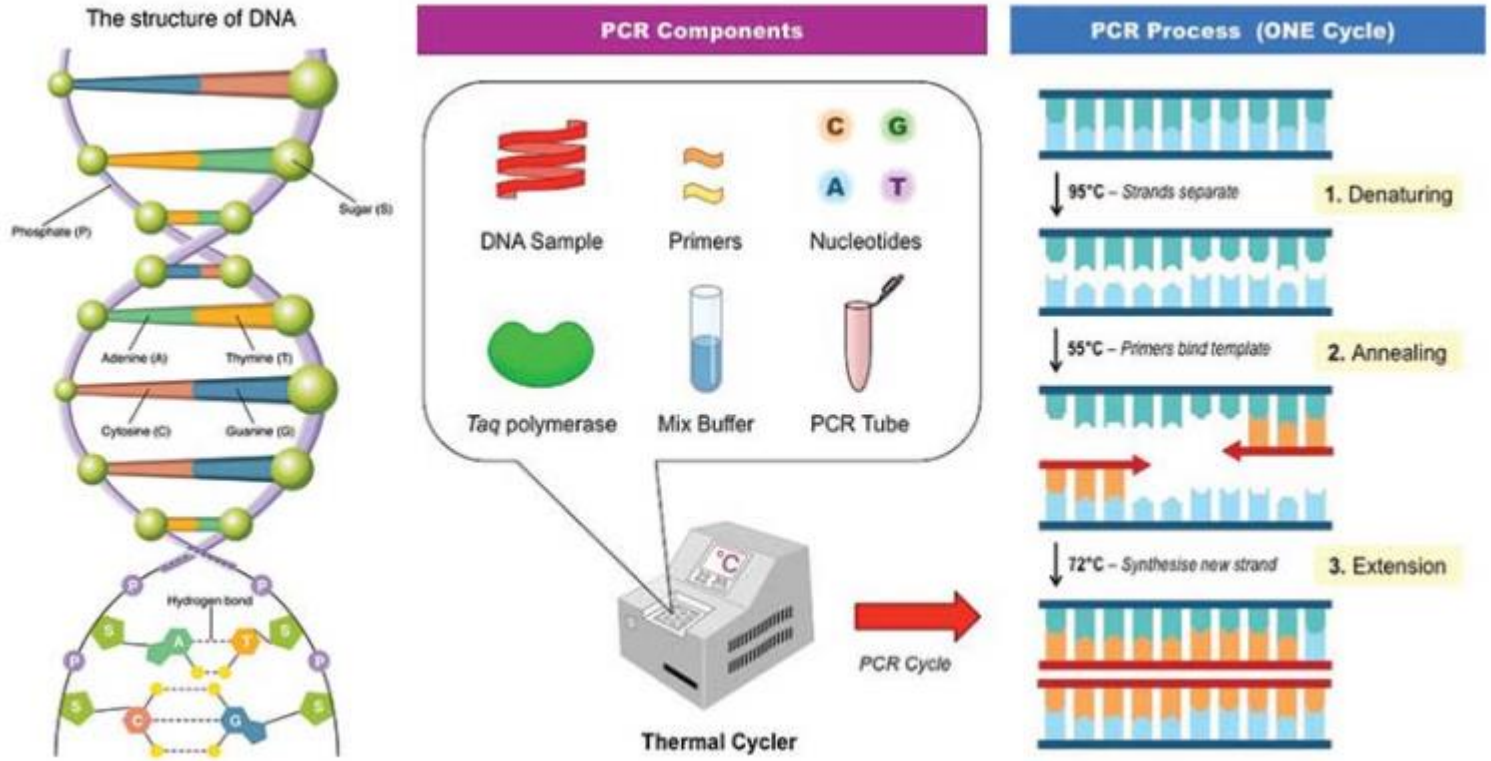
Multiple ligation probe amplification



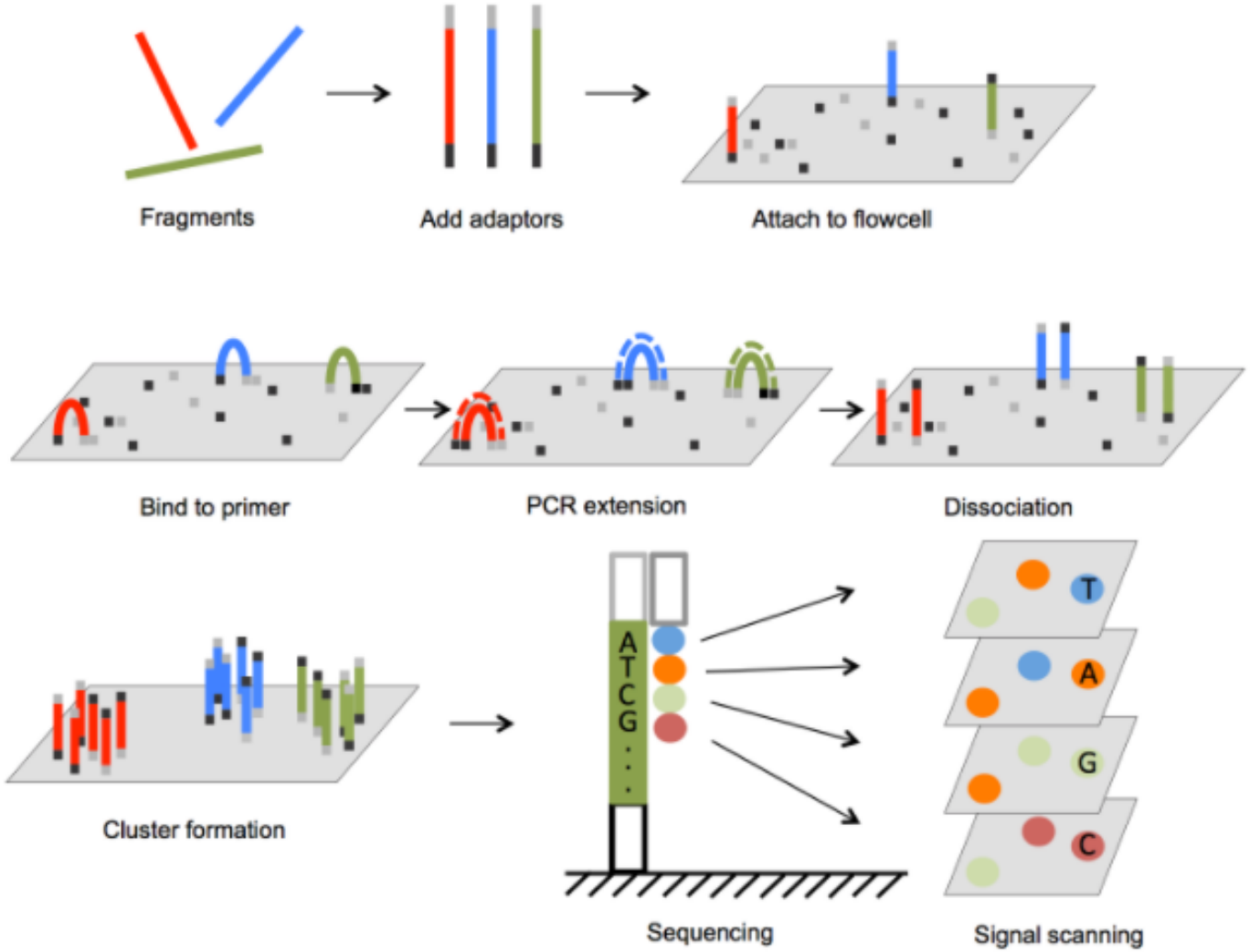
MLPA in B-ALL


B
UKALL-CNA Risk
CNA-GR
CNA-IR
CNA-PR
• No deletion in any of the regions
• Isolated deletion of ETV6, PAX5, or BTG1
• ETV6 deletion with single deletion of BTG1, CDKN2A/B or PAX5
• All other CNA profiles
• Isolated IKZF1, PAR1, or RB1 deletion
• Deletion of IKZF1/PAX5/CDKN2A/B

Polymerase chain reaction



Next generation sequencing



Diagnostische procedure

Acute lymfoblastische leukemie

- Karyotyperen: heparinestaal beenmerg, 1 dag niet gestimuleerde kweek
- FISH:

BCR::ABL1	*NUP214::ABL1*
ETV6::RUNX1 (< 25 jaar)	*TRA/TRD/TRB*
KMT2A	*KMT2A*
TCF3	*TLX3*
CRLF2	*TLX1*
iAMP21	*TAL1*
ABL-class fusions (*ABL1, ABL2, CSF1R, PDGFRB*)	*LMO2*
	PICALM
	MYC

- MLPA: del/dup in genen met prognostische relevantie in B-ALL

Diagnostische procedure

Acute myeloïde leukemie

- Karyotyperen: heparinestaal beenmerg, 2 dagen gestimuleerde kweek
- FISH: *MECOM*, *KMT2A*
- Prognostisch panel: *CBFB::MYH11*, *FLT3*-ITD/PM, *KMT2A*-PTD, *MECOM* expressie
- NGS

Problemen met deze workflow

Sterk gespecialiseerd personeel

Slechte kwaliteit chromosomen

Geen/onvoldoende mitosen

Overgroei normale cellen

Beperkte resolutie (>5Mb)

FISH/MLPA: Hogere resolutie maar beperkt tot specifieke (probe)regio's

Hogere TAT

Studieprotocollen vereisen snelle stratificatie van patiënten

Cascade FISH

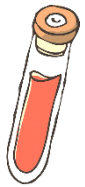
Combinatie testen

Optische genoommapping

CURRENT EXTENSIVE PANEL OF TESTS	NEW TECHNOLOGIES
• Classical cytogenetics • FISH (up to 10 experiments) • SNP arrays or MLPA • PCR-based techniques	OPTICAL GENOME MAPPING 1 assay: detects numerical and structural abnormalities simultaneously
Costly	Less expensive
Laborious (up to 12 tests)	Simplified workflow (1 test)
Lengthy TAT (up to 4 weeks)	Short TAT (1 week)
	Increased diagnostic yield

OGM procedure

Count WBC and take 1,5 million cells



Bone marrow or blood (if invaded)

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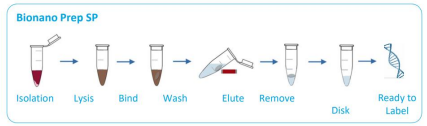
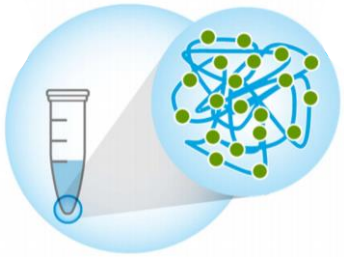
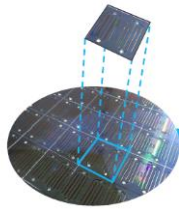
Extraction of UHMW DNA

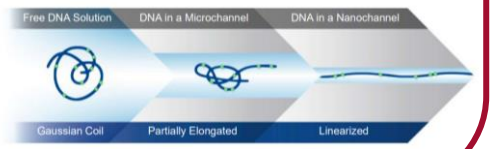


Label DNA at specific motifs

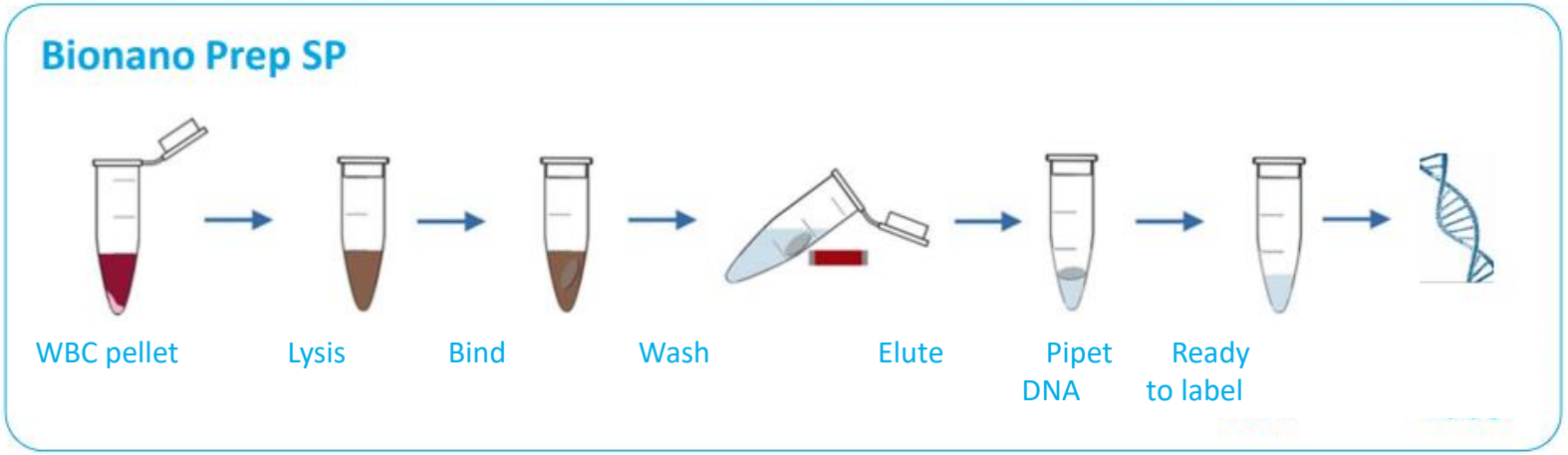


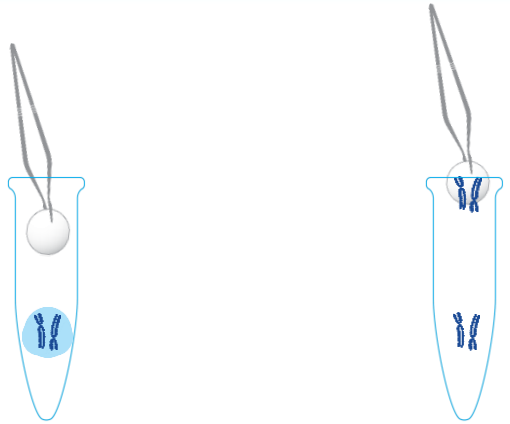
Transfer labeled DNA into Chip





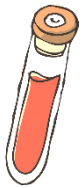
DNA extractie





OGM procedure

Count WBC and take 1,5 million cells



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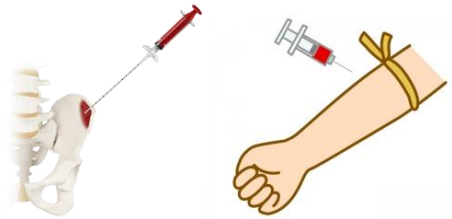

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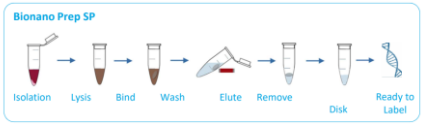

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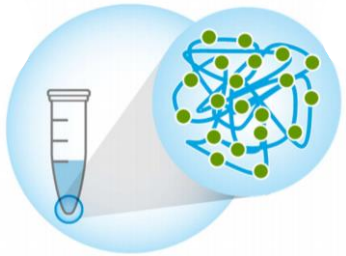

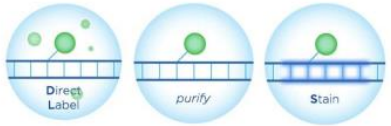

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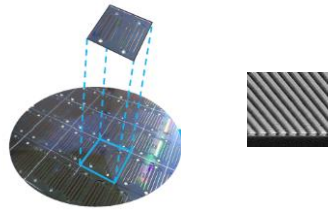
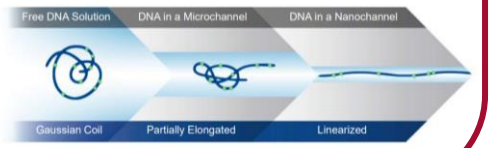
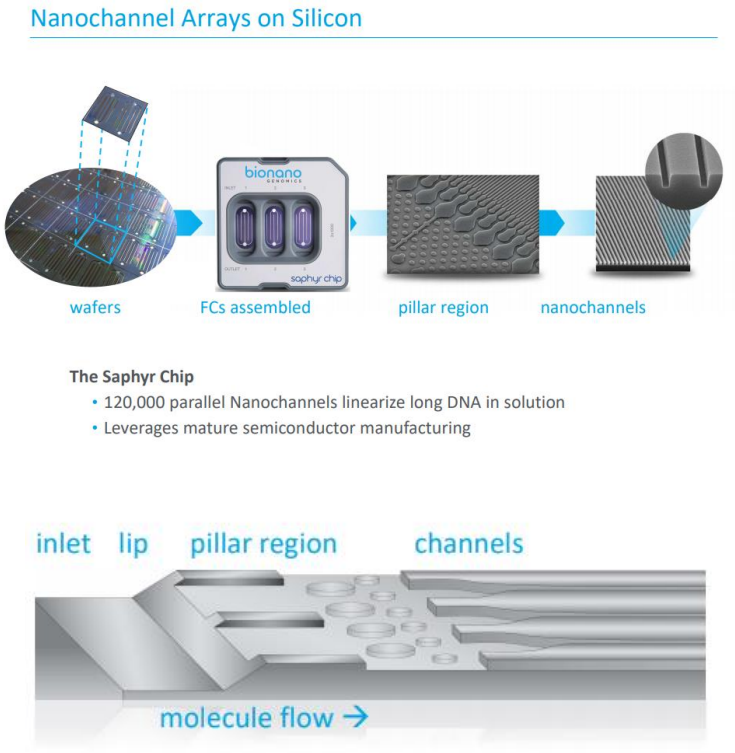
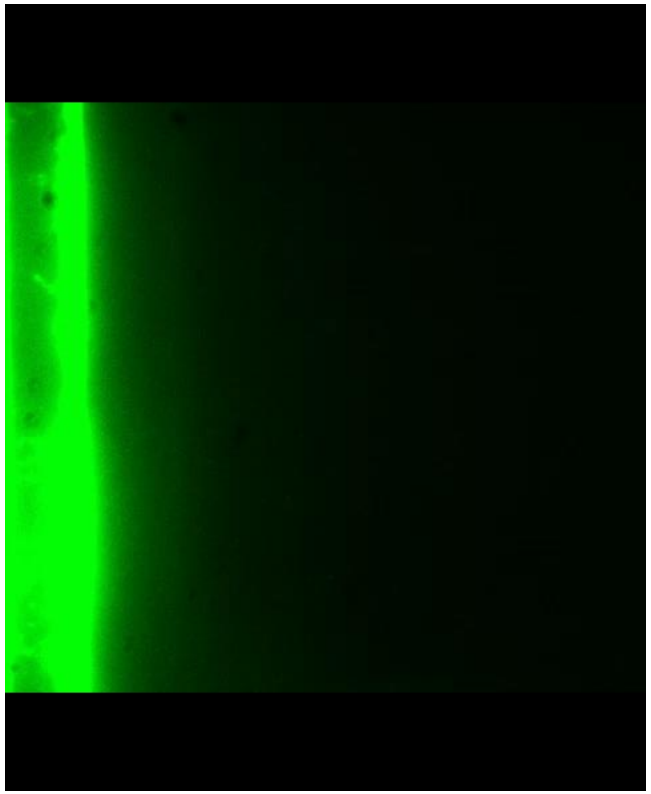


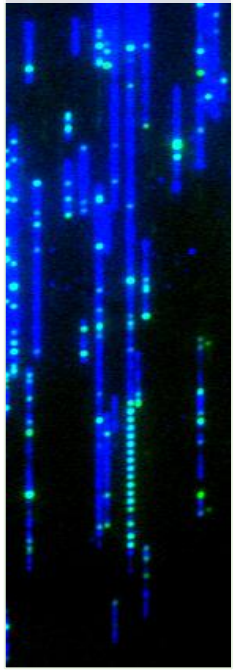


Visualisatie van de fluorescente labels



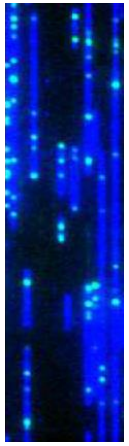


DNA counterstaining
DLS labels

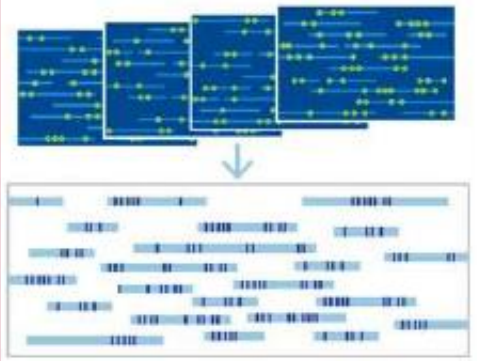


Data analyse

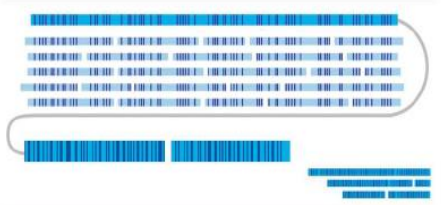
Raw Image Data:
Direct observation of
DLS labels on long
DNA molecules
(150 kb up to 3 Mb)



Algorithms convert the raw images into .bnx files
=> population of molecules

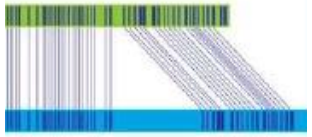


Algorithms align different molecules for constructing Consensus Genome Maps (.cmap files)



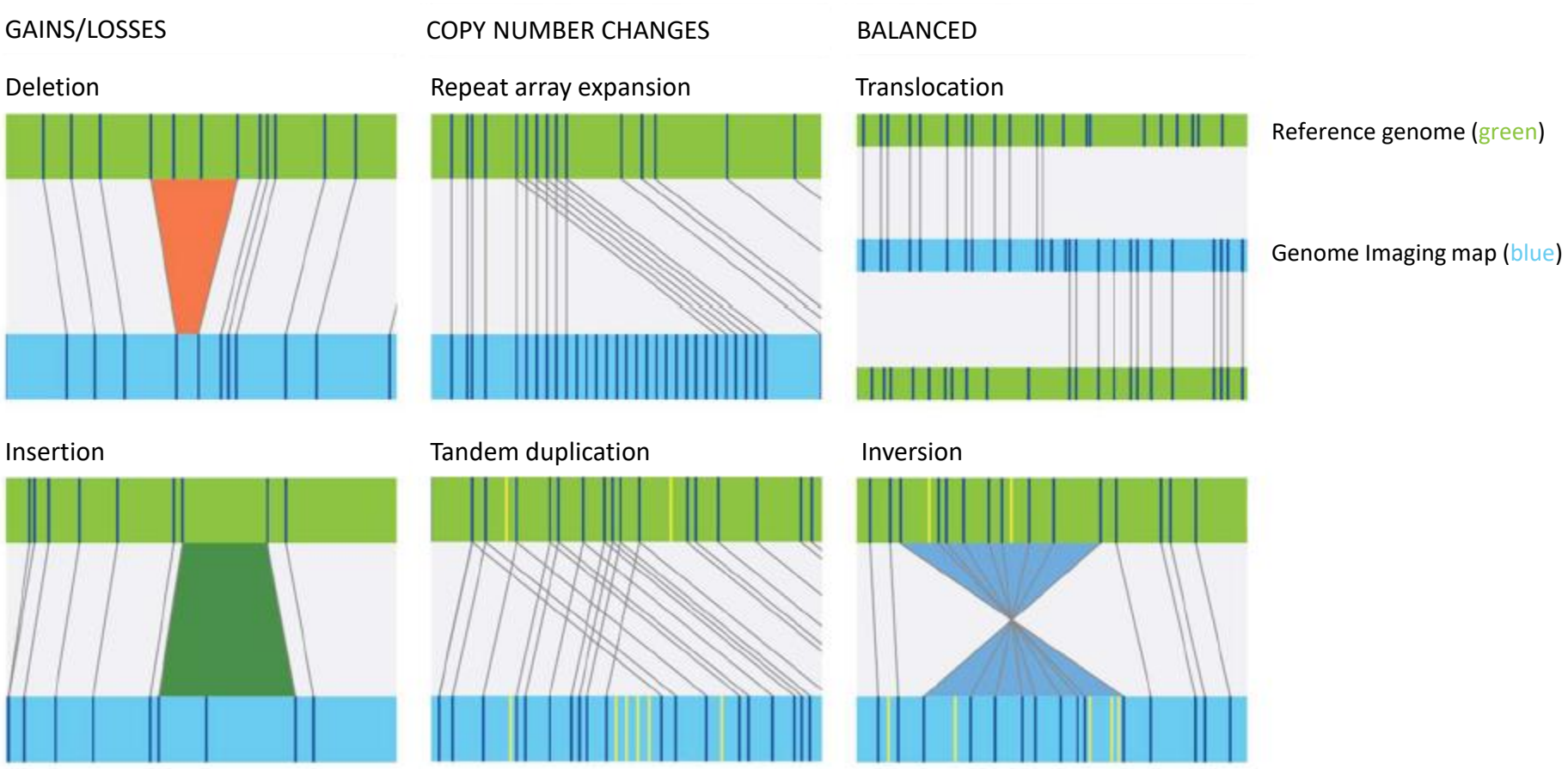
Cross mapping across a Reference

- CNA profile
- SA



insertion

Data analyse



OGM in de praktijk

- Gevalideerd voor diagnostische ALL en AML stalen
- 3 bio-informatische pipelines:
    - *De Novo* Assembly Pipeline (150X effective coverage, enkel voor ALL)
    - Rare Variant Pipeline (300X effective coverage)
    - Copy Number Analysis Pipeline
- Filter voor kopijnummerafwijkingen met hoge confidence en > 5Mb
- Submicroscopische structurele varianten van 500 bp – 5 Mb worden enkel gerapporteerd wanneer zij een klinisch relevante locus omvatten

Conclusie

- OGM is een geschikte diagnostische test voor ALL en AML

  \+ informatieve resultaten werden bekomen voor elke casus
  \+ excellente concordantie met standaard testpanel voor recurrente translocaties en submicroscopische afwijkingen
  \+ excellente concordantie voor winst/verlies van ganse of partiële chromosomen

- OGM is gebaseerd op ‘bulk’ DNA

  \- ploïdie & aanwezigheid van sub-klonen niet steeds correct gedetermineerd

Conclusie

- Turn-around-time van 1-2 weken
  - \+ geschikt voor studieprotocols met snelle risico stratificatie, zoals ALLtogether
  - \- FISH *BCR::ABL1* blijft noodzakelijk

- Interpretatie kan complex zijn en vergt grondige kennis van de cytogenetica
  - \- breekpunten niet exact: welke genen zijn betrokken? Onschuldige variant?
  - \- karyotypering blijft vooralsnog onmisbaar

Bedankt voor uw aandacht