AZ Sint-Jan Brugge

MB&C of AML

Dr.B.Cauwelier 2024

Goede zorg laat niemand achter

Diagnosis

Female, VS (‘62)

- Medical history : melanoma in situ (2020)
- Presents with leucocytosis, anemia and thrombopenia (& ecchymoses and mucosal bullae)
  Peripheral blood : 2/2022

Naam		Resultaat	Vorig resultaat	Eenheid	Referentiewaarden
Algemene hematologie					
Hemoglobine **	↓	**9,4**		g/dl	11,7 - 16,0
Hematocriet **	↓	**0,26**		ratio	0,35 - 0,47
Rode bloedcellen **	↓	**2,99**		10.E12/l	3,8 - 5,8
MCV **		87,9		fl	81 - 101
MCH **		31,4		pg	27 - 34
MCHC **		35,7		g/dl	31 - 36
RDW (RBC distribution width) **		13,5		%	12,3 - 17,7
Bloedplaatjes **	↓	**16**		10.E9/l	150 - 450
MPV (Mean platelet volume) **		7,9		fl	7,9 - 10,8
Witte bloedcellen **	↑	**47,9**		10.E9/l	4,5 - 11,0
Blasten	↑	**86**		%	0 - 0
Neutrofielen **	↓	**6**		%	40 - 75
Eosinofielen **		0		%	0 - 6
Basofielen **	↑	**3**		%	0 - 1
Lymfocyten **	↓	**4**		%	15 - 40
Monocyten **	↓	**2**		%	4 - 12
Neutrofielen absoluut **		3,1		10.E9/l	1,8 - 7,7
Eosinofielen absoluut **		0,0		10.E9/l	0,0 - 0,5
Basofielen absoluut **	↑	**1,3**		10.E9/l	0 - 0,2
Lymfocyten absoluut **		1,7		10.E9/l	1 - 4,8
Monocyten absoluut **	↑	**0,9**		10.E9/l	0,0 - 0,8
Normoblasten **		0		/ 100 wbc	< 1
Normoblasten absoluut **		0,00		10.E9/l	0,00 - 0,02

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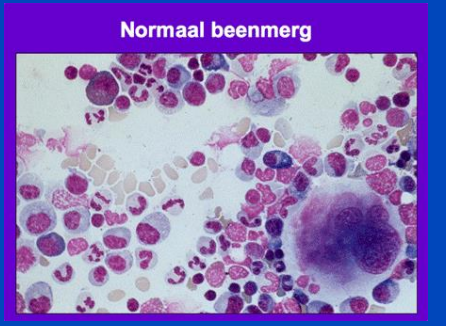
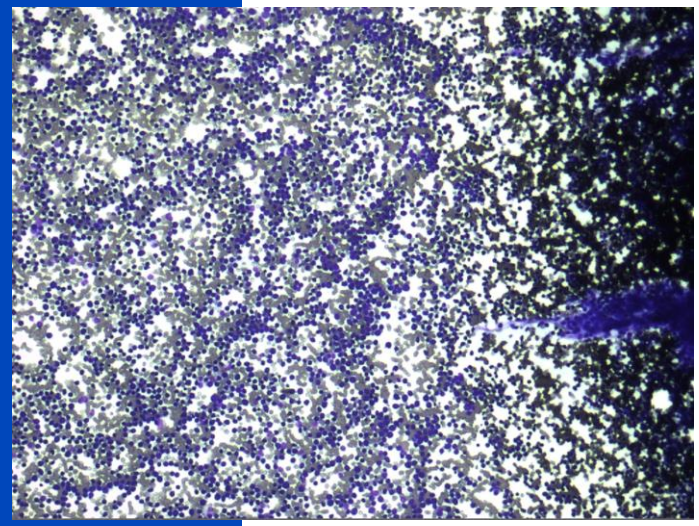
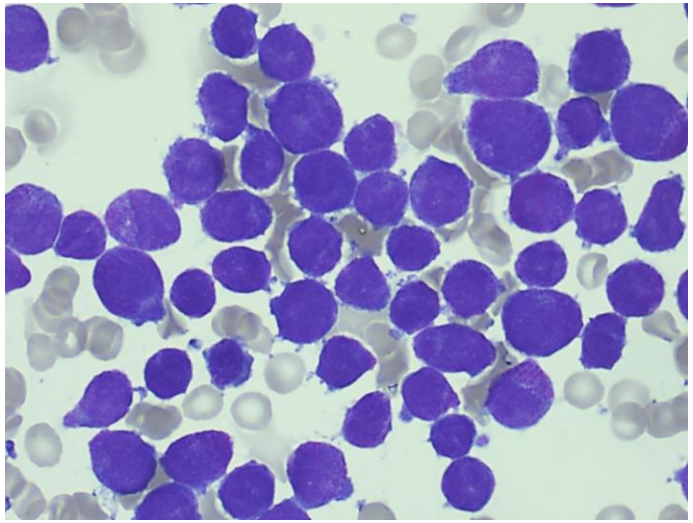
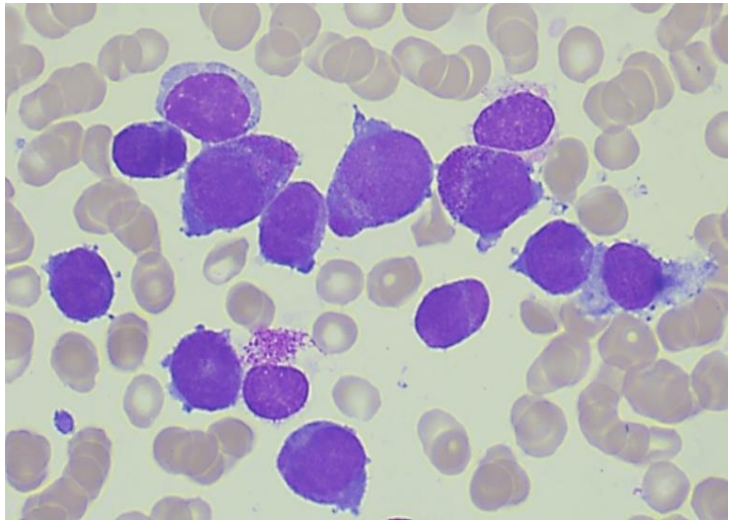


Diagnosis

Bone marrow



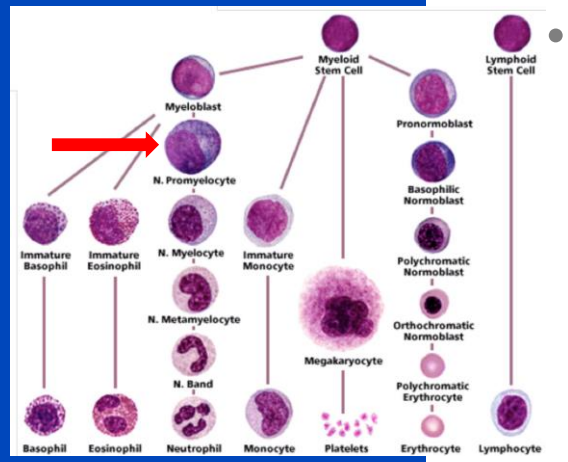




- **93% blasts**: 2-4 rbc large, moderate to often high N/C ratio, irregular sometimes bilobar nucleus, surrounded by fine to moderate border of moderately basophilic cytoplasm, often containing numerous **dense azurophilic granulations**, sometimes with Auer rod, but no clear faggot cells , zz with blebs.

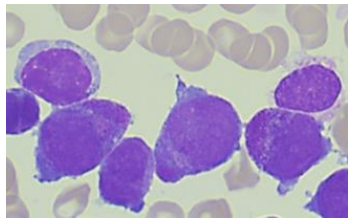
- Hypercellular bone marrow with 93% blasts. Morphological picture of acute myeloid leukemia, type **Acute Promyelocytic Leukemia**.

Acute promyelocytic leukemia (APL)

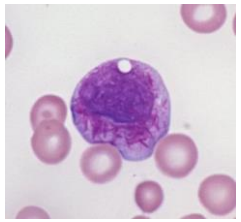


Normal hematopoiesis

- Maturation arrest at **promyelocytic stage**



Hypergranular APL



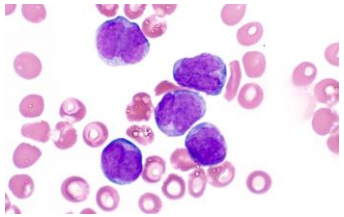
fagott cell

Hypergranular (typical) APL

>>hypergranular promyelocytes, Auer rods, sometimes fagott-cells, low WBC count

Hypogranular APL

>> hypo-agranular promyelocytes, sometimes Auer rods, bilobal nucleus, high WBC count



Hypogranular APL

- Often coagulation disorders (DIC, diffuse intravascular coagulation) !!
- 5-8% of AML

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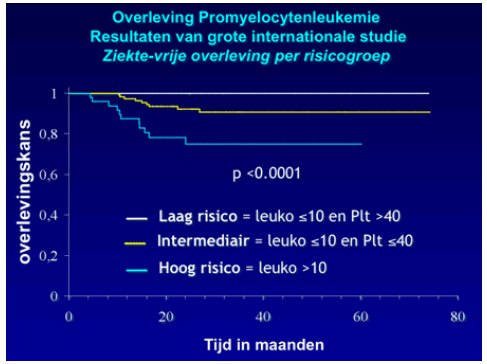
Acute promyelocytic leukemia (APL)

Diagnostic : t(15;17)(q22;q11) / *PML::RARA* or *RARA* variants

Good prognosis >>



URGENT diagnosis needed!

APL cells sensitive to **ATRA (all-trans retinoic acid) /ATO (arseentrioxide)** therapy needs to be started asap < 24 h

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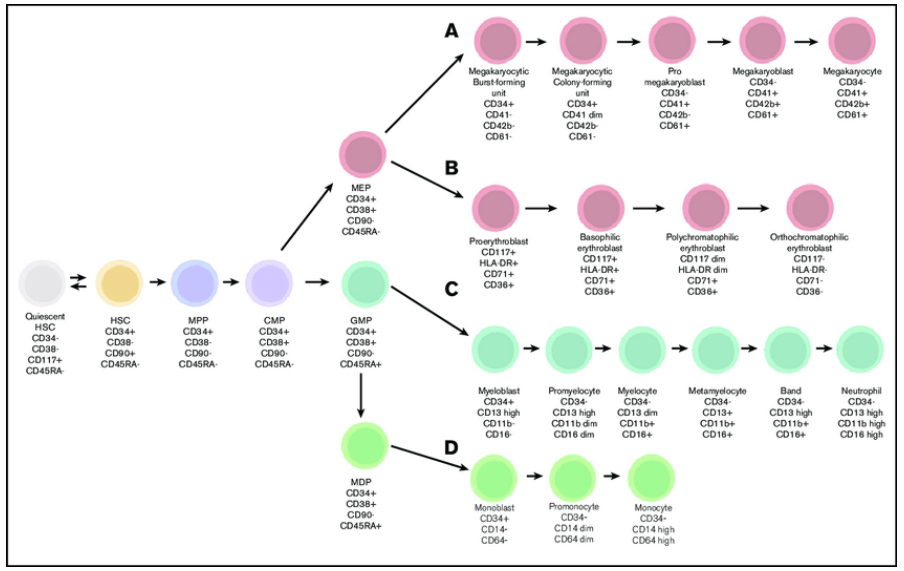
Diagnostic work-up

- Flowcytometry

>> immunophenotype analysis >> lineage assessment/diagnostic



Overview of the different stem cell compartments and progenitors in the BM; erythropoiesis (A), megakaryopoiesis (B), myelopoiesis (C), and monopoiesis (D)

Lineage	Markers
B lineage	
Strong CD19* and	≥1 marker expression CD10, CD22, or CD79a
Weak CD19 and	≥2 strongly expressed: CD10, CD22, CD79a
Consider immunohistochemical stains for B lineage	PAX5, OCT2, BOB1
T lineage	
CD3 (surface or cytoplasmic)	—
Myeloid lineage	
MPO or	—
Monocytic differentiation	NSE, CD64, CD11c, CD14, or lysozyme

The immunophenotypic criteria described here are for cases of suspected MPAL and are not required for straightforward cases of AML or ALL.

*Expression should be at least similar to that seen in stage I B-cell precursors or mature B cells.

WHO2016 &ICC2022

- Molecular biology/cytogenetics

>> diagnostic / prognostic /therapeutic

Diagnosis

Flowcytometry BM

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WHO2016 &ICC2022

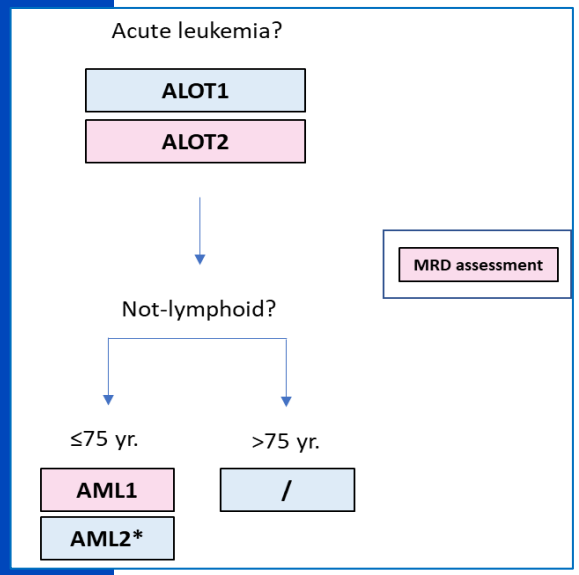

Figure 2: Flow chart for the diagnostic approach of acute myeloid leukemia.

ALOT1	BV711	BV786	BV605	HV450	HV500	FITC	PE	PerCP-Cy5.5	PE-Cy7	APC	APC-H7	APC-R700
Marker	/	/	cyCD22	cyCD3	CD45	cyMPO	cyCD79a	CD34	CD19	CD3	/	CD10
Clone			HIB22	UCHT1	HI30	MPO-7	HM57	8G12	J3-119	SK7		HI10A
Vol. (/test)			5µL	7µL	5µL	3µL	5µL	10µL	5µL	3µL		5µL

ALOT2	BV711	BV786	BV605	HV450	HV500	FITC	PE	PerCP-Cy5.5	PE-Cy7	APC	APC-H7	APC-R700
Marker	CD117	CD300e	CD33	HLADR	CD45	CD35	CD64	CD34	CD7	CD11b	CD14	/
Clone	Yb5.B8	UP-H2	P67.6	L243	HI30	E11	10.1	8G12	M-T701	D12	MφP9	
Vol. (/test)	5µL	5µL	10µL	1µL	5µL	5µL	20µL	10µL	2µL	5µL	5µL	

ALOT2: to confirm myeloid lineage (CD64,CD14) cfr lineage specific criteria

AML1 (tube 1)	BV711	BV786	BV605	HV450	HV500	FITC	PE	PerCP-Cy5.5	PE-Cy7	APC	APC-H7	APC-R700
Marker	CD117	CD13	CD33	HLADR	CD45	TdT	NG2	CD34	CD16	CD15	CD38	CD56
Clone	Yb5.B8	L138	P67.6	L243	HI30	HT-6	7.1	8G12	B73.1	HI98	HB7	NCAM16.2
Vol. (/test)	5µL	7µL	10µL	1µL	5µL	10µL	10µL	10µL	20µL	1.25µL	3µL	5µL

AML2 (tube 2)	BV711	BV786	BV605	HV450	HV500	FITC	PE	PerCP-Cy5.5	PE-Cy7	APC	APC-H7	APC-R700
Marker	CD123	/	CD4	/	CD45	CD42a/61	CD105	CD34	cyTCL1	CD71	CD43	/
Clone	9F5		RPA-T4		HI30	HIP8, PL7F12	266	8G12	eBio1-21	M-A712	IG10	
Vol. (/test)	1µL		5µL		5µL	1/4 µL	5µL	10µL	2µL	2µL	2.5µL	

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Diagnosis

Flowcytometry BM: gatingstrategy

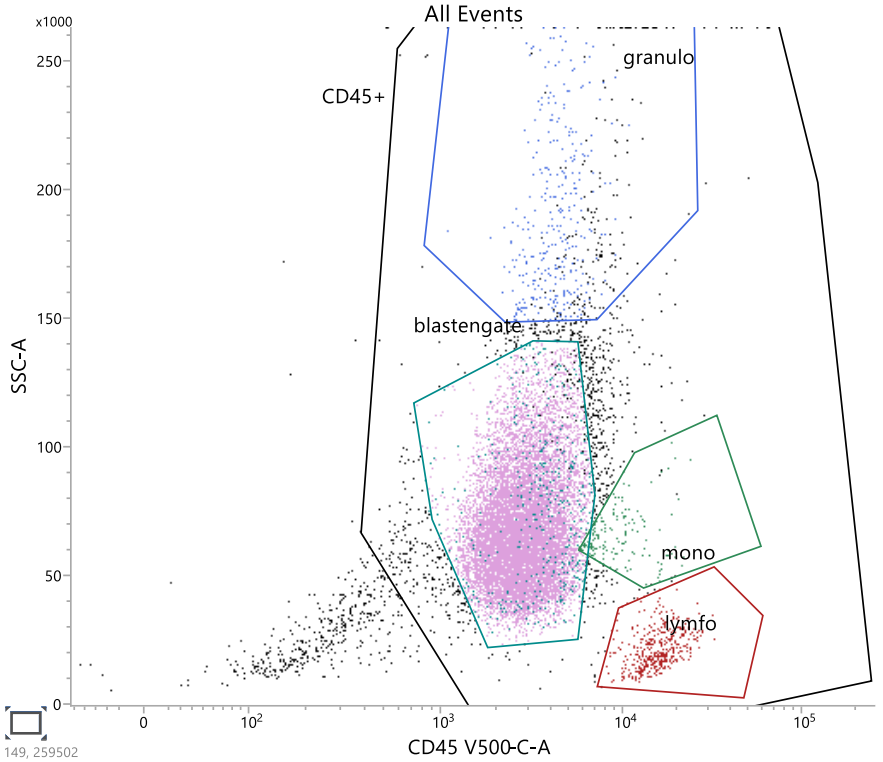
Show Statistical Gates/Populations

Gate Hierarchy

Population View

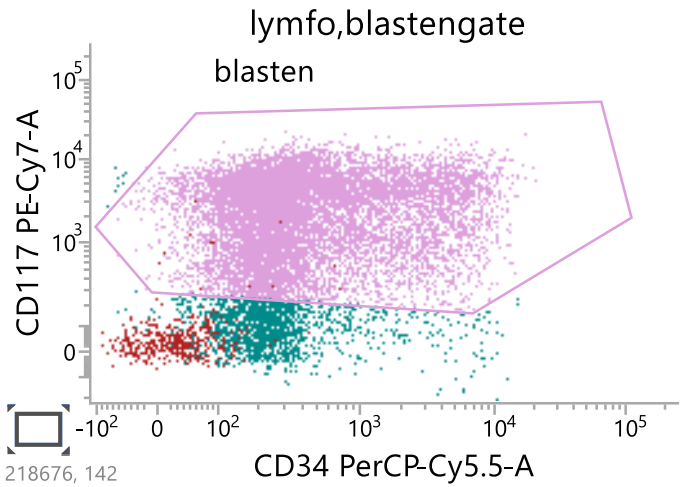
Show Population Statistics

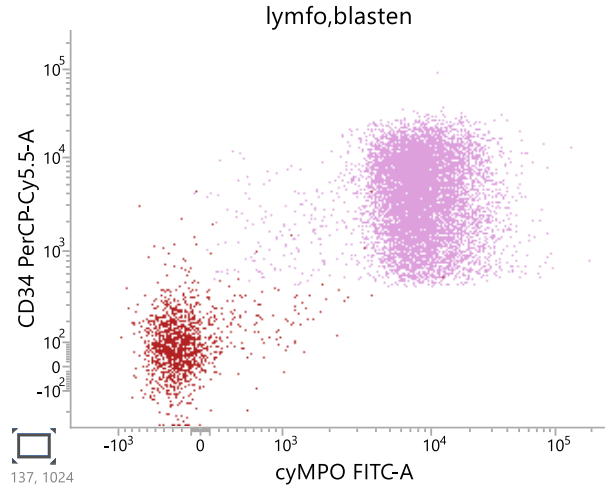
Name	Events	% Parent	% Grandparent	% Total
AML5				
All Events	15.396	***	***	100,00
in-time events	15.396	100,00	***	100,00
Singlet	15.043	97,71	97,71	97,71
Singlet2	15.021	99,85	97,56	97,56
Treshhold	15.017	99,97	99,83	97,54
CD45+	14.640	97,49	97,46	95,09
granulo	322	2,20	2,14	2,09
mono	162	1,11	1,08	1,05
lymfo	415	2,83	2,76	2,70
blastengate	13.130	89,69	87,43	85,28
blasten	11.420	86,98	78,01	74,18
CD34+	2.439	21,36	18,58	15,84
CD117+	10.034	87,86	76,42	65,17
CD33+	6.578	57,60	50,10	42,73



Diagnosis

Flowcytometry BM: results ALOT1&2 (lineage defining markers)



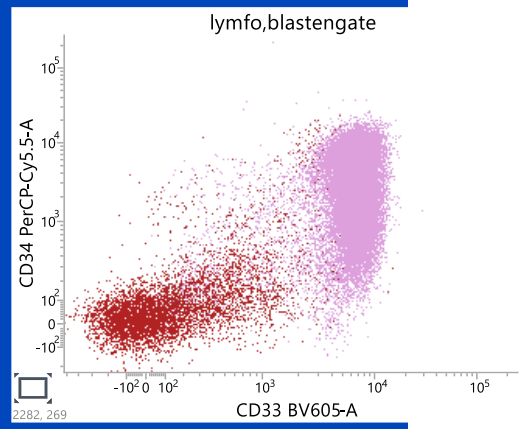


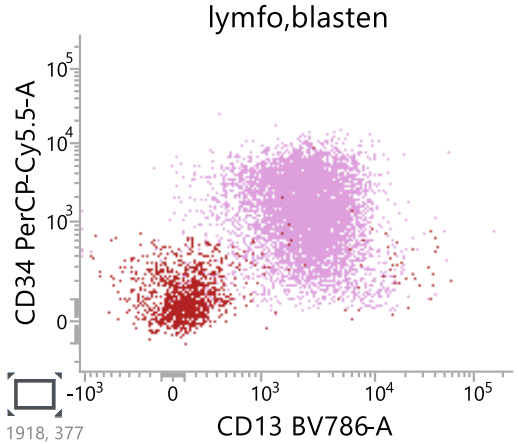
- cyCD3-/CD3-/CD10-/CD11b-/CD14-/CD19-/CD22-/CD34-mostly/CD45+/**CD64+weak**/cyCD79a-/CD117+/**cyMPO++.**

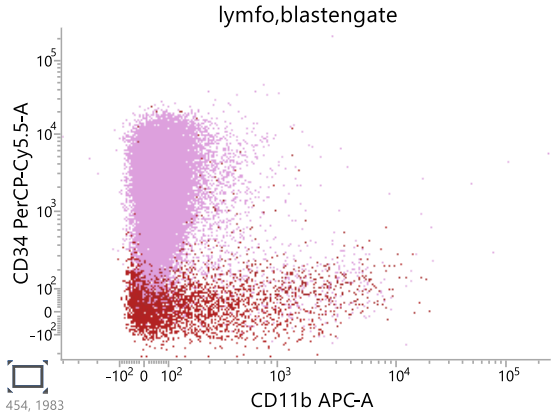
➡ AML

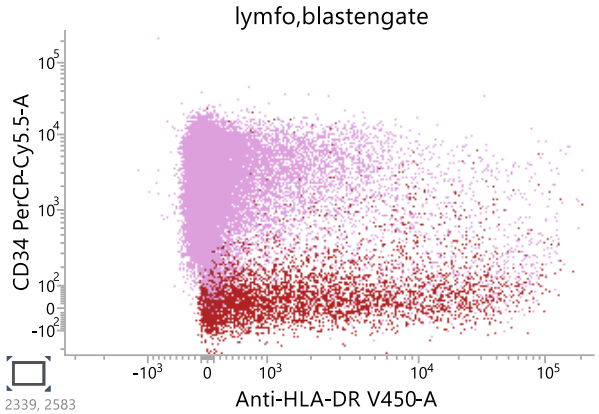
Diagnosis

Flowcytometry BM: additional markers









- IF shows presence of 91% myeloblasts, with fenotype: cyCD3-/CD3-/CD7-/CD10-/CD11b-/CD13+/CD14-/CD15-/CD16-/CD19-/CD22-/CD33+/**CD34-mostly/**CD35-/CD38+zwak/CD42aCD61-/CD45+/CD56-/CD64+zwak/CD71+weak/cyCD79a-/CD117+/CD300e-/**HLADR-/cyMPO+strong**/NG2-/TdT-.

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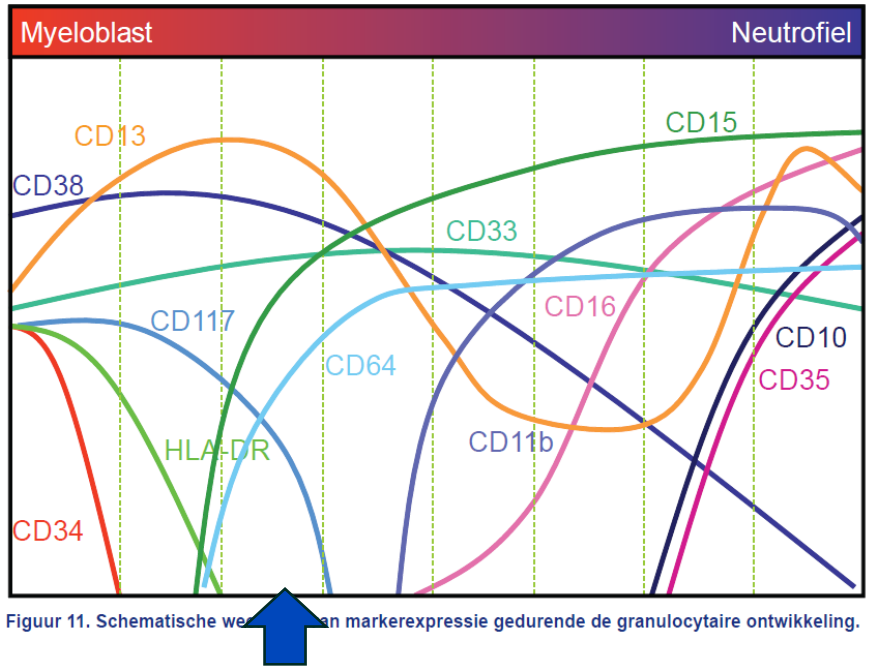
Diagnosis



Figuur 11. Schematische we[illegible]n markerexpressie gedurende de granulocytaire ontwikkeling.

- cyCD3-/CD3-/CD7-/CD10-/**CD11b-**/CD13+/CD14-/CD15-/CD16-/CD19-/CD22-/CD33+/**CD34-mostly**/CD35-/CD38+zwak/CD42aCD61-/CD45+/CD56-/**CD64+weak**/CD71+weak/cyCD79a-/CD117+/CD300e-/**HLADR-/cyMPO++**/NG2-/TdT-.

- **Final diagnosis together with flowcytometry : APL** (typical fenotype CD11b-/CD34-/CD64+/HLADR-/cy MPO++
- Cave : NPM1+ AML also often CD34-/HLADR- >> **molecular techniques necessary to confirm !!**

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AML molecular/cytogenetic investigation

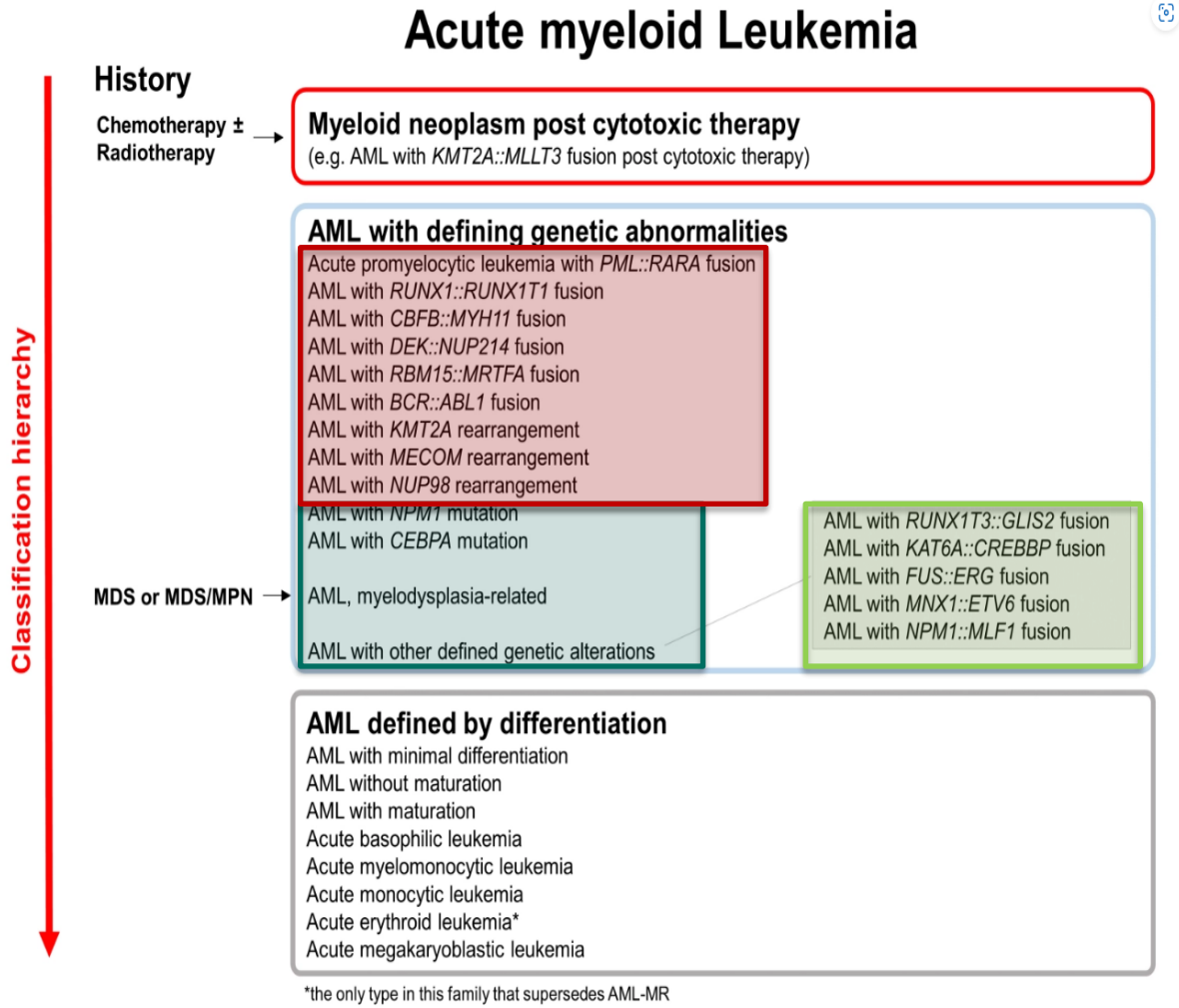
* Hemavision (acute leukemia gene rearrangements)
>>diagnostic / prognostic

* EVI1/MECOM overexpression
>>diagnostic / MRD
* MLL/KMT2A -PTD
>>prognostic

* Next Generation Sequencing myeloid panel
>> diagnostic / prognostic / therapeutic

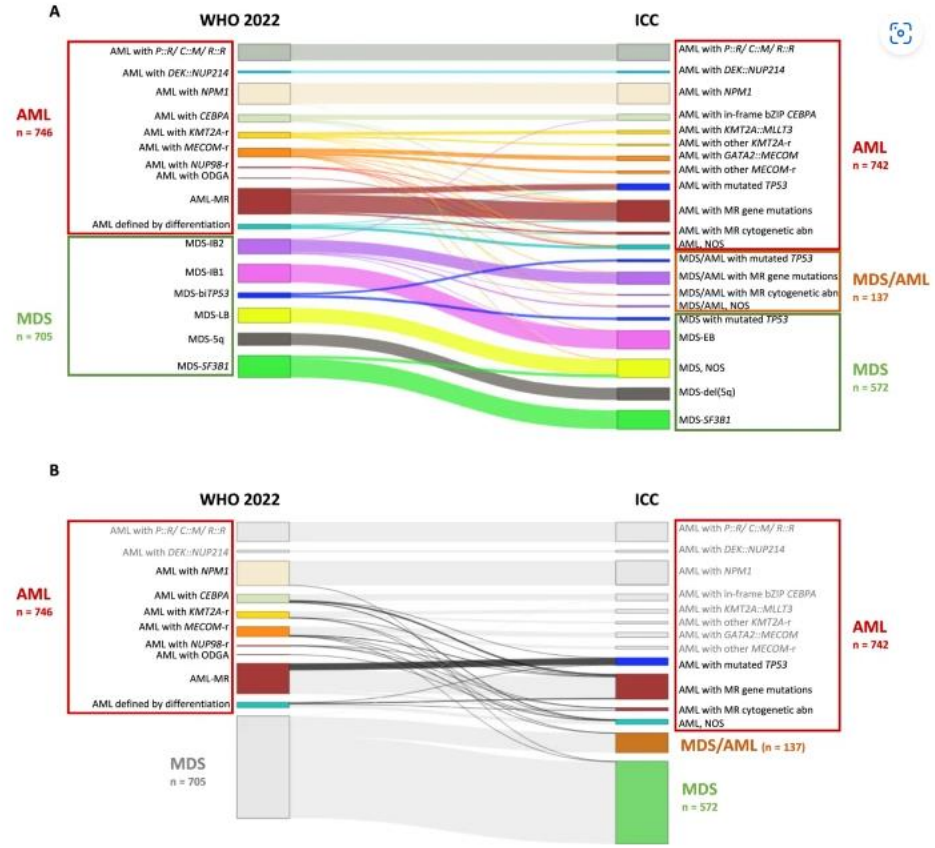
* Conventional karyotype
>> diagnostic / prognostic

* Optical Genome Mapping (Bionano)
>> diagnostic / prognostic
Also needed for defining AML, MDS related



WHO 2022 AML classification

AML classification WHO/ICC 2022



A Changes in specific MDS and AML diagnoses between WHO 2022 and ICC. **B** Major differences in AML diagnoses between WHO 2022 and ICC. *P::R = PML::RARA; C::M = CBFB::MYH11; R::R = RUNX1::RUNX1T1;* MR Myelodysplasia-related, NOS Not otherwise specified, EB Excess blasts, SLD Single lineage dysplasia, MLD Multilineage dysplasia, 5q/del(5q) Isolated 5q deletion, RS Ring sideroblasts, -r rearrangement, ODGA Other defined genetic alterations, IB Increased blasts, bi*TP53* Biallelic *TP53* inactivation, LB Low blasts, abn Abnormalities.

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AML molecular/cytogenetic techniques

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  >>diagnostic / prognostic

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* Conventional karyotype
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  Also needed for defining AML, MDS related

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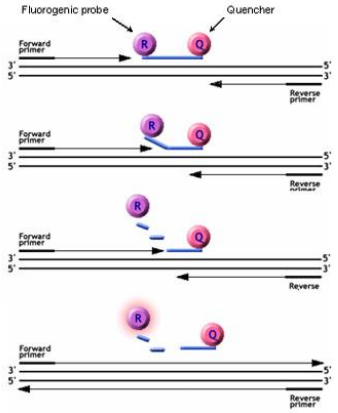
Hemavision

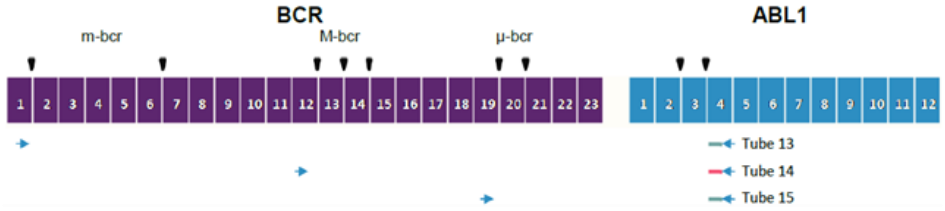
Tube	Translocation	Fusion Gene	Fw primer - Rev primer	Fluorochrome	
1	t(15;17)(q24;q21)	PML-RARA (bcr2, v)	PML ex5-RARA ex3	FAM	CY5
	inv(16)(p13;q22)	CBFB-MYH11	CBFB ex3-MYH11 ex30	ROX	CY5
2	t(8;21)(q22;q22)	RUNX1-RUNX1T1	RUNX1 ex6-RUNX1T1 ex3	ROX	CY5
3	t(15;17)(q24;q21)	PML-RARA (bcr1, L)	PML ex6a-RARA ex3	FAM	CY5
	t(9;11)(p21.3;q23.3)	KMT2A-MLLT3	KMT2A ex7-MLLT3 ex7	ROX	CY5
4	t(15;17)(q24;q21)	PML-RARA (bcr3, S)	PML ex3-RARA ex3	FAM	CY5
	t(9;11)(p21.3;q23.3)	KMT2A-MLLT3	KMT2A ex8-MLLT3 ex11	ROX	CY5
5	t(11;19)(q23.3;p13.3)	KMT2A-ELL	KMT2A ex7-ELL ex3	FAM	CY5
	t(16;21)(p11;q22)	FUS-ERG	FUS ex6-ERG ex12	ROX	CY5
6	t(12;22)(p13;q11-12)	ETV6-MN1	ETV6 ex2-MN1 ex2	FAM	CY5
7	t(9;9)(p13;q34)	DEK-NUP214	DEK ex9-NUP214 ex19	ROX	CY5
8	Reference gene	GUS	GUS ex11-GUS ex12	FAM	CY5
9	Reference gene	B2M	B2M ex2-B2M ex4	FAM	CY5
	t(1;11)(q21;q23.3)	KMT2A-EPS15	KMT2A ex8+9-EPS15 ex3	FAM	CY5
	t(8;11)(q27;q23.3)	KMT2A-AFDN	KMT2A ex8+9-AFDN ex2	ROX	CY5
10	t(1;19)(q23;p13)	TCF3-PBX1	TCF3 ex16-PBX1 ex3	FAM	CY5
	t(12;21)(p13;q22)	ETV6-RUNX1	ETV6 ex5-RUNX1 ex4b	ROX	CY5
11	t(11;19)(q23.3;p13.3)	KMT2A-MLLT1	KMT2A ex8+9-MLLT1 ex2	FAM	CY5
	t(4;11)(q21;q23.3)	KMT2A-AFF1	KMT2A ex8+9-AFF1 ex9	ROX	CY5
12	t(17;19)(q22;p13)	TCF3-HLF	TCF3 ex14-HLF ex4	FAM	CY5
	del(1)(p32)	STIL-TAL1	STIL ex1-TAL1 ex2	ROX	CY5
13	t(9;22)(q34;q11)	BCR-ABL1 (m-bcr, P190)	BCR ex1-ABL1 ex3	FAM	CY5
	t(9;9)(q34;q34)	SET-NUP214	SET ex9-NUP214 ex19	ROX	CY5
14	t(11;19)(q23.3;p13.3)	KMT2A-MLLT1	KMT2A ex7-MLLT1 ex9	FAM	CY5
15	t(9;22)(q34;q11)	BCR-ABL1 (M-bcr, P210)	BCR ex12-ABL1 ex3	ROX	CY5
	t(9;22)(q34;q11)	BCR-ABL1 (µ-bcr, P230)	BCR ex19-ABL1 ex3	FAM	CY5
16	t(1;17)(q21;q21)	ZBTB16-RARA	ZBTB16 ex3-RARA ex3	ROX	CY5
	Reference gene	ABL1	ABL1 ex2-ABL1 ex3	FAM	CY5
17	t(9;12)(q34;p13)	ETV6-ABL1	ETV6 ex2+5-ABL1 ex3	FAM	CY5
	t(5;12)(q33;p13)	ETV6-PDGFRB	ETV6 ex2+5-PDGFRB ex12	ROX	CY5
18	t(10;11)(p12;q23.3)	KMT2A-MLLT10	KMT2A ex8+9-MLLT10 ex10	FAM	CY5
	t(1;11)(q21;q23.3)	KMT2A-MLLT11	KMT2A ex8+9-MLLT11 ex2	ROX	CY5
19	t(X;11)(q13;q23.3)	KMT2A-FOXO4	KMT2A ex7-FOXO4 ex2	FAM	CY5
	t(11;17)(q23.3;q21)	KMT2A-MLLT6	KMT2A ex7-MLLT6 ex12	ROX	CY5
20	t(3;21)(q26;q22)	RUNX1-MECOM	RUNX1 ex6-MECOM ex2	FAM	CY5
	t(10;11)(p12;q23.3)	KMT2A-MLLT10	KMT2A ex7-MLLT10 ex7	ROX	CY5
21	t(3;17)(q25;q21)	NPM1-RARA	NPM1 ex6-RARA ex3	FAM	CY5
	t(3;5)(q25.1;q35)	NPM1-MLF1	NPM1 ex4-MLF1 ex3	ROX	CY5
22	t(10;11)(p12;q23.3)	KMT2A-MLLT10	KMT2A ex7-MLLT10 ex11	FAM	CY5
	t(3;21)(q26;q22)	RUNX1-MECOM	RUNX1 ex6-MECOM ex6	ROX	CY5
23	t(10;11)(p12;q23.3)	KMT2A-MLLT10	KMT2A ex8-MLLT10 ex10	ROX	CY5
24	-	-	-	-	-

- Multiplex real time PCR (HemaVision 28Q kit)
- Detection of **28 gene rearrangements** (with 145 breakpoints and splice variants)
- **Diagnostic** : WHO / ICC classification

BCR/ABL - t(9;22), PML/RARalfa - t(15;17), AML1/ETO - t(8;21), CBFB/MYH1 - inv(16), TEL/AML1 - t(12;21), different MLL (11q23) gene rearrangements

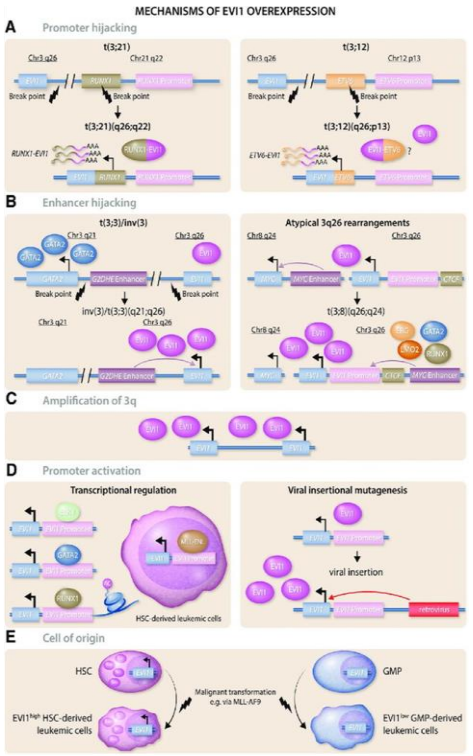
- **Prognostic :**
  - Good : PML/RARalfa - t(15;17), AML1/ETO - t(8;21), CBFB/MYH1 - inv(16) en TEL/AML1 - t(12;21)
  - Bad : DEK/NUP214 - t(6;9) en MLL/MLLT4 - t(6;11)
- Sensitivity : 1/1000
- Only at **diagnosis / relapse**





EVI1 overexpression

° EVI1 (Ecotropic Viral Integration Site 1) plays a crucial role in the regulation of gene expression and cellular differentiation.
° Overexpression of EVI1 has been identified in several malignancies
° **EVI1 overexpression** is observed in **5-10% of AML** and is often associated with a **poor prognosis**. Patients with AML and high levels of EVI1 expression may have a higher likelihood of treatment resistance, increased risk of relapse, and shorter overall survival.
° EVI1 overexpression can be associated with certain chromosomal abnormalities. One of the most well-known associations is with **3q26 rearrangements**, where the EVI1/MECOM gene is located. **MLL rearrangements** also lead to EVI1 overexpression
° EVI1 (3q26) overexpression is a molecular marker **(MRD)**
° **Real-time quantitative PCR** ; molecules EVI1 transcript / ABL gene ; cut-off for overexpression is 0.1.



EVI1-mediated Programming of Normal and Malignant Hematopoiesis
October 2023HemaSphere 7(10):e959

KMT2A-PTD

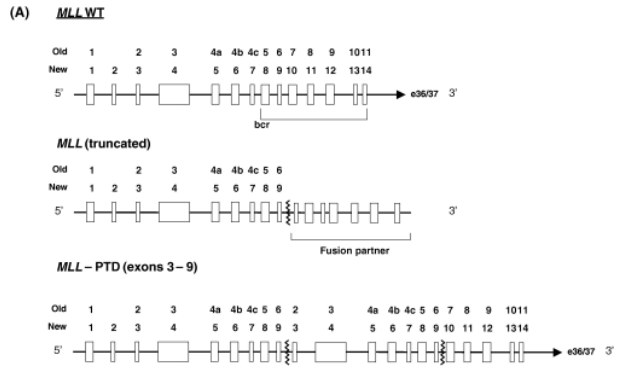
° **MLL** (Mixed Lineage Leukemia) partial tandem duplication (PTD) is a genetic abnormality associated with acute myeloid leukemia (AML) 5-10% . The MLL gene, also known as **KMT2A**, is located on chromosome 11q23, and it plays a crucial role in the normal development and regulation of blood cells.

° **MLL/KMT2A-PTD** is a duplication of exon 3 to exon 9 of the MLL gene (rarely exon 3 to exon 10 or exon 3 to exon 11) resulting in a fusion of e9/e3 (or e10/e3, e11/e3).

° **MLL/KMT2A-PTD is** mostly associated with de novo AML with a normal karyotype and is associated with **bad prognosis**. There is a strong association with **trisomy 11**. MLL-PTD is also described in secundary AML and ALL.

° MLL-PTD is detected by **real time quantitative PCR** at **diagnosis**

° Sensitivity is max 1/10 normal cells. Molecules MLL-PTD / ABL1; > **not suited for MRD**



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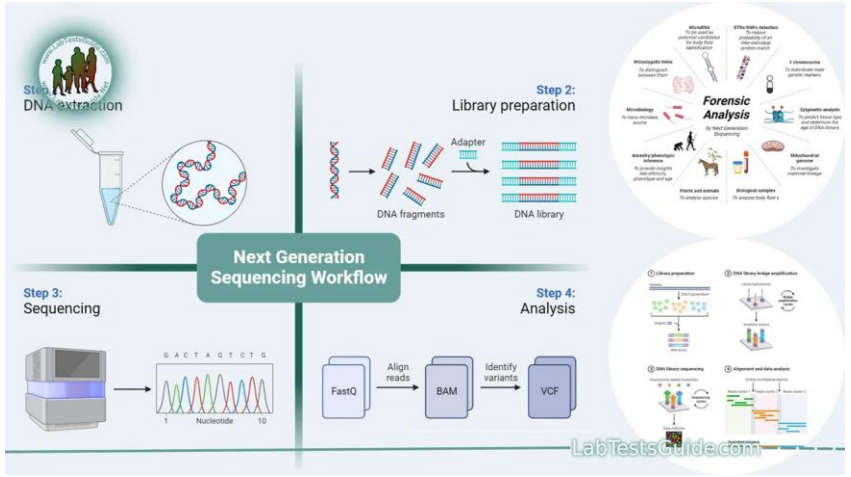
Next Generation Sequencing

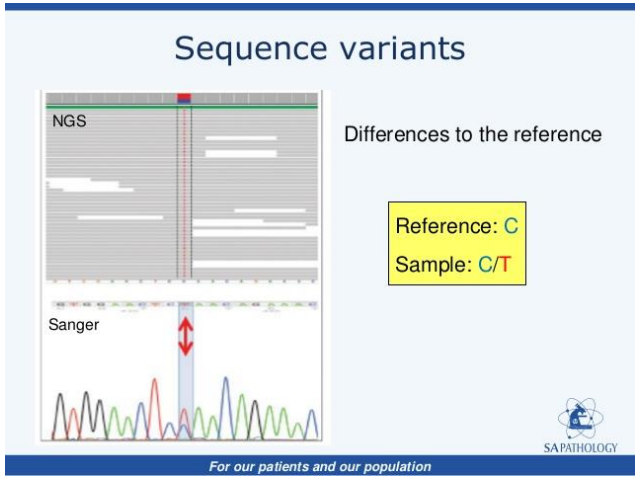
° Next-generation sequencing (NGS) is a technology used for DNA and RNA sequencing and **variant/mutation detection**. NGS can sequence hundreds and thousands of genes or whole genome in a short period of time.

° The sequence variants/mutations (**single nucleotide polymorphisms** (SNPs) and **small insertions** and **deletion** (indels) detected by NGS have been widely used for disease **diagnosis, prognosis, therapeutic** decision.

° 44 ComPerMed Gene panel conform guidelines of RIZIV NGS convention

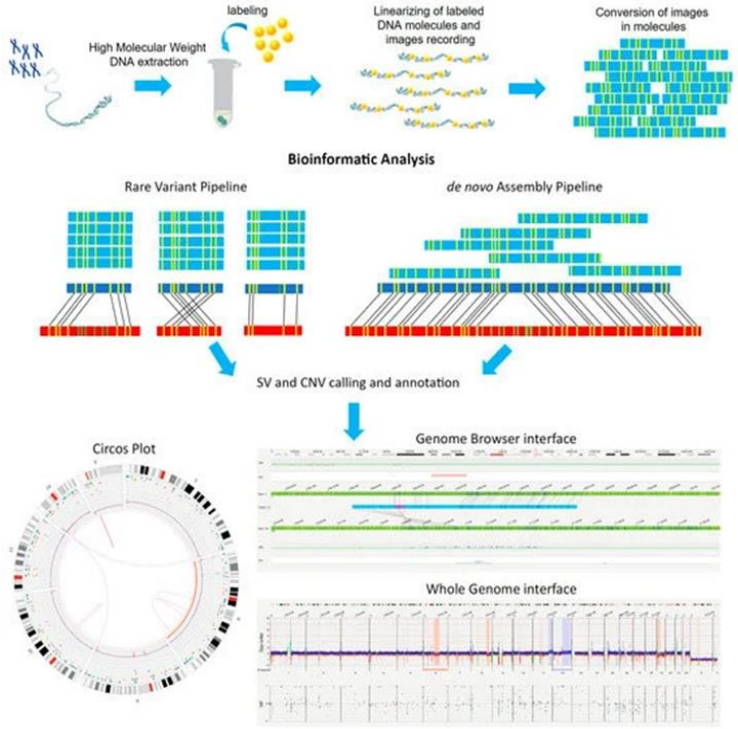
° Only pathogenic variants, possible pathogenic variants and variants of unknown significance (VUS) above 2% variant allel frequentie (VAF) are reported



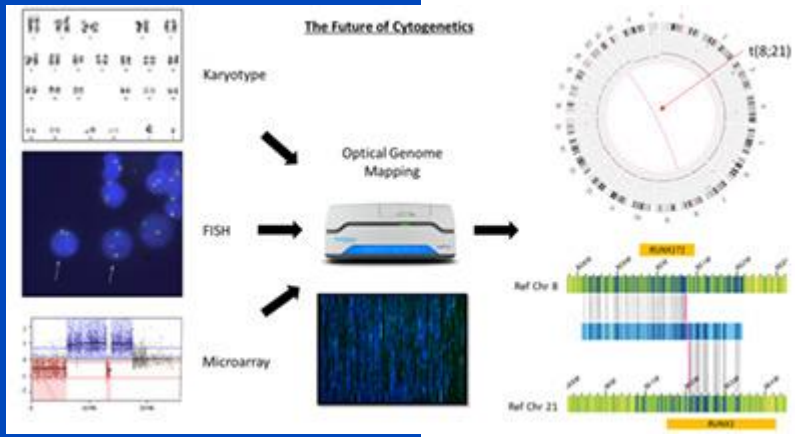


Optical Genome Mapping

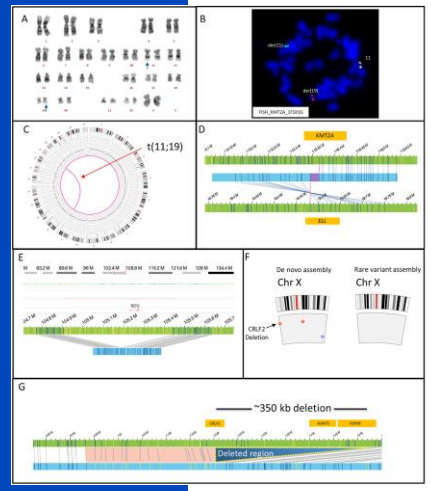
- Genome-wide DNA analysis for **diagnostic** purposes
- The molecular karyotype is obtained by performing Optical Genome Mapping (OGM) on peripheral blood or bone marrow.
- DNA is isolated, labeled and read on the Saphyr instrument (Bionano) and the OGM data was analyzed using two analysis pipelines:
  - i) the Rare Variant Analysis (>300x coverage) and
  - ii) the De Novo Assembly (80x coverage).
  - The reference genome GRCh37/hg19 is used in the above analysis pipelines. The final results are visualized with the Bionano Access software.



Optical Genome Mapping



- The following abnormalities are retained in the **molecular karyotype**:
  - i) **numerical and structural abnormalities ≥500kbp** independently of the genes involved, and
  - ii) **numerical and structural abnormalities** (including deletions, duplications, inversions and translocations) **<500kbp**, but not smaller than 500bp, involving genes with a known **diagnostic, prognostic or therapeutic impact** in hematological disorders. Regions in which complex rearrangements occur, such as chromotripsis and chromoplexis, are also explicitly mentioned in the molecular karyotype. The Phi-like pattern can be picked up in ALL patients.
- The molecular karyotype only contains **structural abnormalities** with a **variant allele frequency (VAF) of 5%** (i.e. 10% abnormal cells for heterozygous abnormalities), and **numerical abnormalities** with a variant allele frequency of **10%**. Regions in which loss of heterozygosity is detected are not reported. Copy neutral loss of heterozygosity and abnormalities



- <500bp cannot be detected with OGM.
- In routine for AML/ALL since 10/2023 ; Belac Accreditation granted

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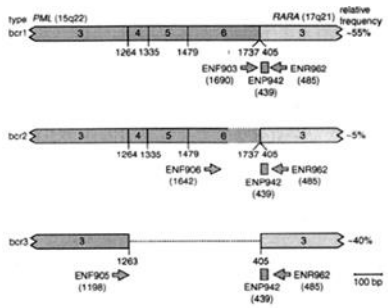
Results of molecular investigation

° **Hemavision** : **PML-RARA (bcr 3,S) , t(15;17)**

>> good **MRD marker** !

>> no LAIP (MRD by flow) needed



° MLL-PTD, EVI1 overexpression : absent

° **NGS** myeloid panel : FLT3,c.1715_1837 dup p.? : **pathogenic FLT3-ITD mutation (123 bp)**

FLT3,c.1790_1837+3dup p.? **pathogenic FLT3-ITD mutation (51 bp)**

MYE-LB081570

Terug naar overzicht stalen | Terug naar start | Uitloggen

v2.2.0

SeqNext

Gen	Nucleotidewijziging	Aminozuurwijziging	VAF	Klasse	MOLIS	COSMIC	Pop. freq.	SIFT/PolyPhen	Conserved
FLT3	c.1715_1837dup	p.?	39%	VUS	FLT3, c.1715_1837dup p.?, 39% VAF	0	0, 0, 0	-/-	-
TET2	c.2599T>C	p.(Tyr867His)	52%	(Vermoedelijk) benigne	TET2, c.2599T>C p.(Tyr867His), 52% VAF	7	0.0024, 0.005, 0.0114783	deleterious/probably_damaging	Nee
TET2	c.3797A>G	p.(Asn1266Ser)	47%	VUS Gescoord (MVY - 09-12-2020)	TET2, c.3797A>G p.(Asn1266Ser), 47% VAF	0	0, 0, 0	deleterious/probably_damaging	Ja
TET2	c.5167C>T	p.(Pro1723Ser)	47%	(Vermoedelijk) benigne	TET2, c.5167C>T p.(Pro1723Ser), 47% VAF	6	0.0024, 0.005, 0.0122161	tolerated/benign	Nee

Workbench

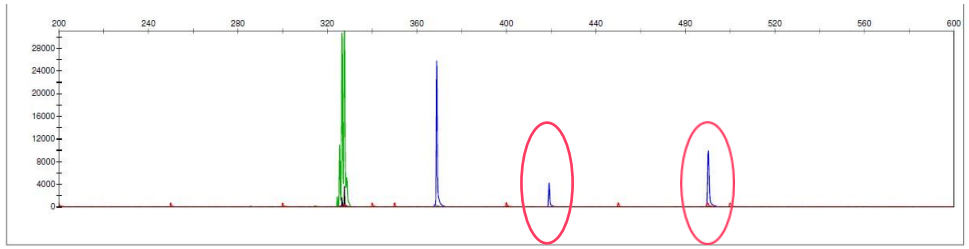
Gen	Nucleotidewijziging	Aminozuurwijziging	VAF	Reads
CEBPA	c.564_566del3	p.Pro189del	2.1%	10/480
TET2	c.2599T>C	p.Tyr867His	47%	560/1180
TET2	c.3797A>G	p.Asn1266Ser	46%	393/849
TET2	c.5167C>T	p.Pro1723Ser	46%	598/1307

Workbench - InDels and Structural Variants

Geen varianten gedetecteerd

FLT3ITDext

ITD lengte	Nucleotidewijziging	Aminozuurwijziging	AR	VAF	Aantal reads
123	c.1715_1837dup	p.612_613insD/573-612	0.1797	13.9	202
51	c.1790_1837+3dup	p.597_598insEYDLKWEFPRENLEFGN	0.1126	8.714	82



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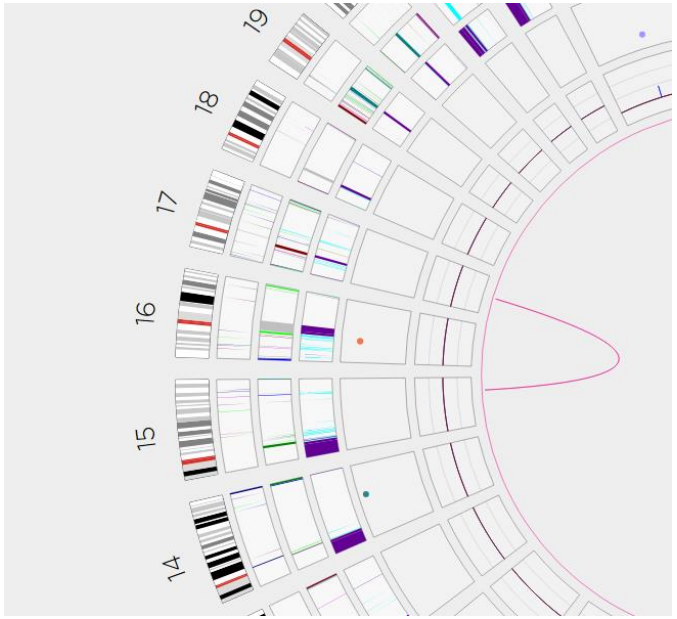
Final diagnosis

- **Conventional karyotype : t(15;17)(q24;q21) (10)**
- **Bionano :**



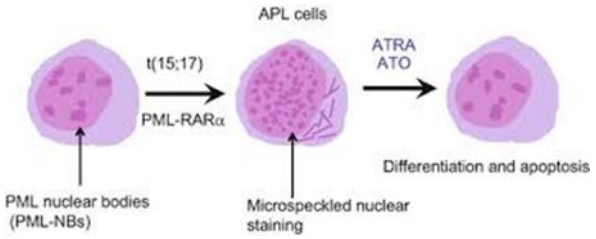
Acute promyelocytic leukemia with FLT3-ITD mutation

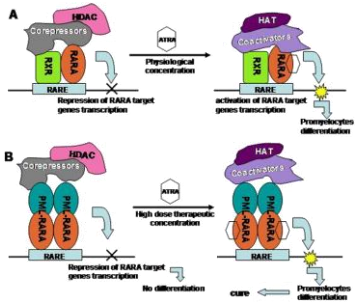
AZ Sint-Jan Brugge

Acute promyelocytic leukemia with FLT3-ITD mutation :

- **Acute promyelocytic leukemia (APL**) is a unique subtype of acute myeloid leukemia (AML) characterized by coagulopathy and the accumulation of morphologically aberrant promyelocytes carrying one of the rearrangements involving the *RARAα* gene, which encodes the retinoic acid receptor alpha located at 17q21.
- APL patients with ***FLT3*-ITD (13-40%)** or *FLT3*-D835 (8%) are more likely to present with elevated WBC counts and **poorer prognosis** than those without these mutations.
  - **Hypogranular morphology**
  - Associated with **BCR3 isoform**

- **Treatment** with a combination of all-*trans* retinoic acid (ATRA) and arsenic trioxide (ATO), which are associated with survival rates or more than 90% .





>> Follow up (MRD) with real-time quantitive PCR for **PML/BCR3 transcript**

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Follow-up

quantitative real-time PCR for PML/BCR3 transcript

■ PML/RARA bcr3 kwantitatief

Afnametijd	Materiaal	Waarde
29/12/2023 13:07	Beenmerg in grote EDTA tube	Zwak aanwezig, niet kwantificeerbaar (SENS-waarde 0.0035%) [1]
01/12/2023 10:54	Beenmerg in grote EDTA tube	Zwak aanwezig, niet kwantificeerbaar (SENS-waarde 0.020%) [1]
06/11/2023 12:30	Beenmerg in grote EDTA tube	2.7 E-1 (MRD - waarde 54%) [1]
05/09/2023 12:11	Beenmerg in grote EDTA tube	Geen transcript gedetecteerd (SENS-waarde 0.0014%) [1]
30/05/2023 16:54	Beenmerg in grote EDTA tube	Geen transcript gedetecteerd (SENS-waarde 0.0055%) [2]
14/04/2023 13:45	Beenmerg in grote EDTA tube	2.7E-3 (MRD-waarde: 0.62%) [2]
31/03/2023 11:58	Beenmerg in grote EDTA tube	3.2E-1 (MRD-waarde: 65.%) [2]
20/03/2023 16:30	Beenmerg in grote EDTA tube	1.5E-1 (MRD-waarde: 31.0%) [2]
31/01/2023 17:06	Beenmerg in grote EDTA tube	4.2E-2 (MRD-waarde: 8.8%) [2]
21/12/2022 12:39	Beenmerg in grote EDTA tube	1.4E-3 (MRD-waarde: 0.32%) [2]
02/11/2022 12:36	Beenmerg in grote EDTA tube	3.5E-4 (MRD - waarde 0.084%) [2]
18/07/2022 12:41	Beenmerg in grote EDTA tube	Zwak aanwezig, niet kwantificeerbaar (SENS-waarde 0.0013%) [2]
02/05/2022 13:23	Beenmerg in grote EDTA tube	Geen transcript gedetecteerd (SENS-waarde 0.0039%) [2]
11/03/2022 13:30	Beenmerg in grote EDTA tube	Zwak aanwezig, niet kwantificeerbaar (SENS-waarde 0.0037%) [2]

[1] Info: De real-time PCR kwantificeert het PML::RARA bcr3 junctietype. De gevoeligheid van de analyse wordt weergegeven als de SENS-waarde. Meer info: zie labogids.

[2] Info: De real-time PCR kwantificeert het PML-RARA bcr3 junctietype. De gevoeligheid van de analyse wordt weergegeven als de SENS-waarde. Meer info: zie labogids.

1/2023 : molecular relapse
18/7/22 : weak presence of transcript
2/5/22 : molecular remission

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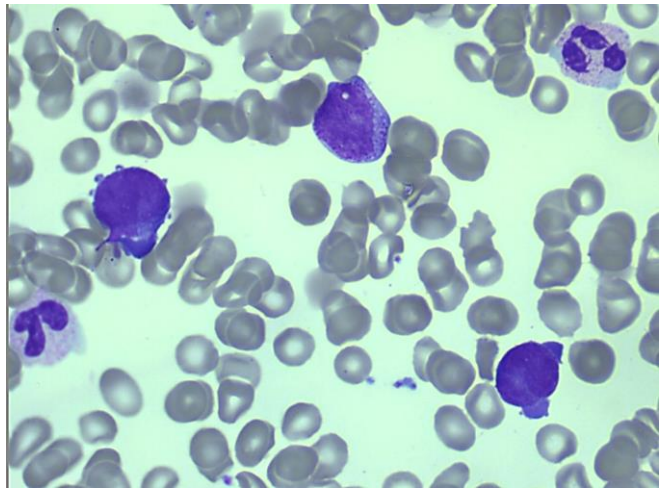
First relapse (3/2023)

Morfological relapse



Normocellular BM with dysplastic features of the myeloid lineage, 2.5% blasts and **22.5% aberrant promyelocytes**

Real-time quantitative PML/BCR3 : 1,5 E-1

No flowcytometry

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First relapse (3/2023)

NGS :

* **DNMT3A**, c.1717C>T p. (Gln573Ter), 2,6% VAF possibly pathogenic mutation : CHIP ?
* **FLT3-ITD c.1790_1837 + 3 dup (51 bp),** 9,3 % VAF >> loss of the longest FLT3-ITD

MYE-323033531419

Terug naar overzicht stalen | Terug naar start | Uitloggen

v2.2.0

SeqNext

Gen	Nucleotidewijziging	Aminozuurwijziging	VAF	Klasse	MOLIS	COSMIC	Pop. freq.	SIFT/PolyPhen	Conserved
DNMT3A	c.1717C>T	p.(Gln573Ter)	2,6%	VUS of wspat, diagnose?	DNMT3A, c.1717C>T p.(Gln573Ter), 2.6% VAF	1	0, 0, 0	-/-	-
TET2	c.3797A>G	p.(Asn1266Ser)	15%	VUS Gescoord (BF - 31-03-2023)	TET2, c.3797A>G p.(Asn1266Ser), 15% VAF	0	0, 0, 0	deleterious/probably_damaging	Ja

Workbench

Gen	Nucleotidewijziging	Aminozuurwijziging	VAF	Reads
DNMT3A	c.1717C>T	p.Gln573*	2.9%	75/2592
TET2	c.2599T>C	p.Tyr867His	50%	899/1806
TET2	c.3797A>G	p.Asn1266Ser	15%	202/1320
TET2	c.5167C>T	p.Pro1723Ser	48%	880/1852

Workbench - InDels and Structural Variants

Geen varianten gedetecteerd

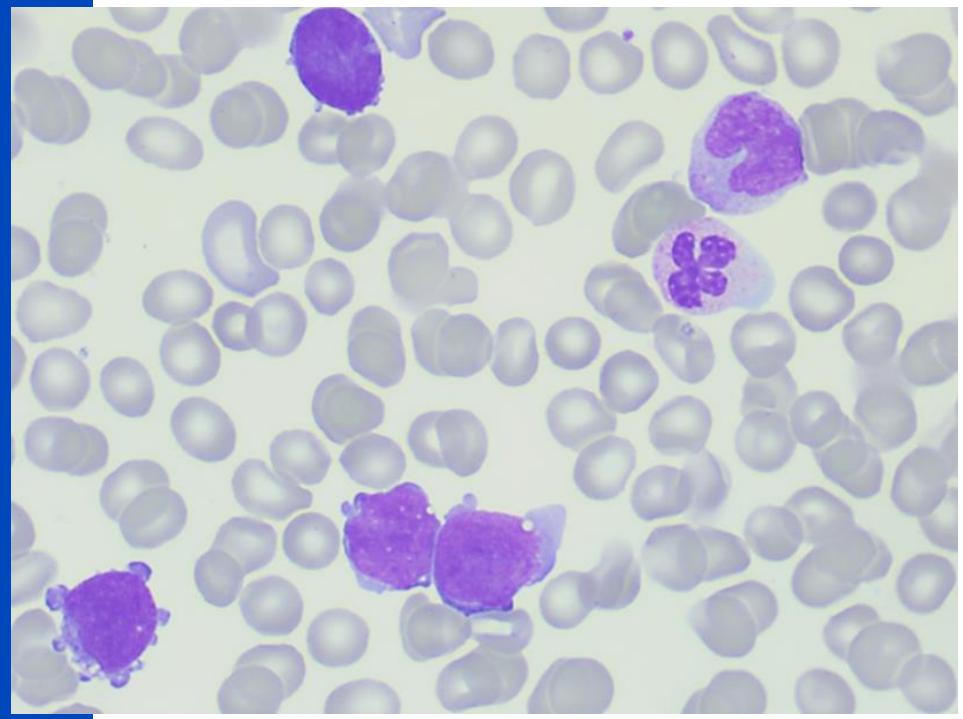
FLT3ITDext

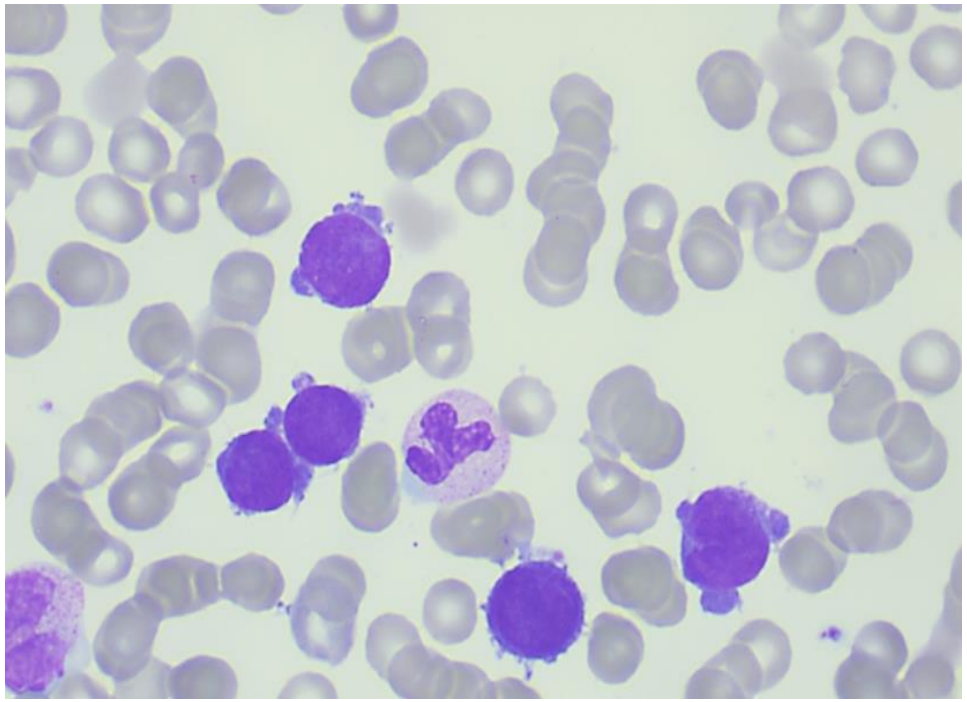
ITD lengte	Nucleotidewijziging	Aminozuurwijziging	AR	VAF	Aantal reads
51	c.1790_1837+3dup	p.597_598insEYDLKWEFPRENLEFGN	0.1026	9.304	157

>> Treatment with ATRA and ATO + autologous HSCT 7/2023

Second relapse

Relapse (11/23) after autologous HSCT (7/23)





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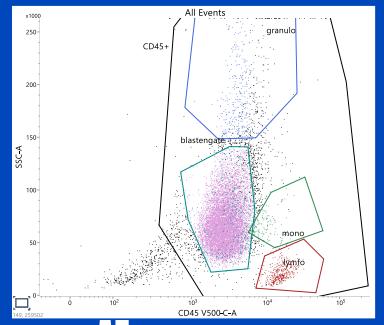
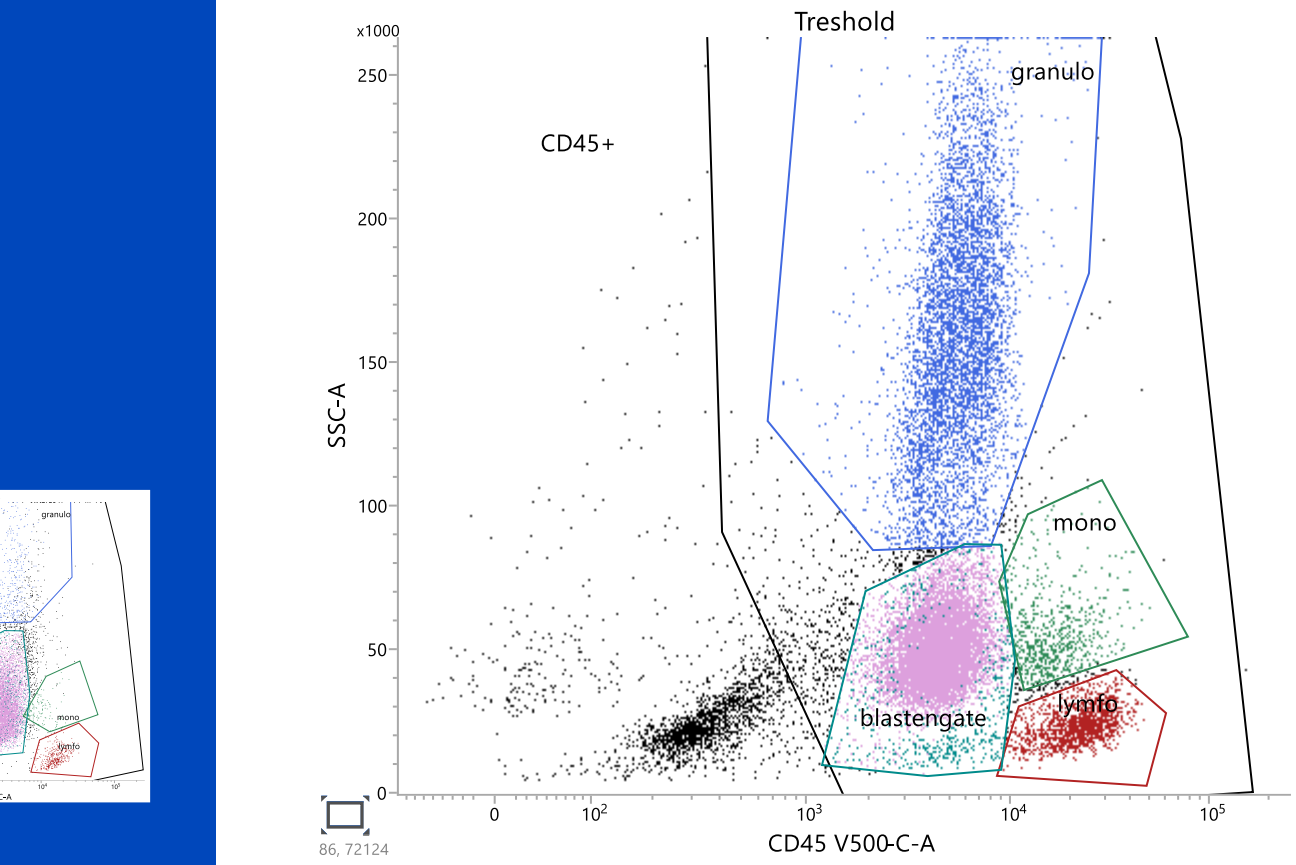
Relapse of APL, however **hypogranular variant** (itt diagnosis)

Second relapse

Flow BM





Show Statistical Gates/Populations

Gate Hierarchy

Population View

Show Population Statistics

Name	Events	% Parent	% Grandparent	% Total
ALOT1				
All Events	26.481	***	***	100,00
in-time events	26.481	100,00	***	100,00
Singlet	24.983	94,34	94,34	94,34
Singlet2	24.671	98,75	93,16	93,16
Treshold	22.110	89,62	88,50	83,49
CD45+	21.885	98,98	88,71	82,64
granulo	6.226	28,45	28,16	23,51
mono	525	2,40	2,37	1,98
lymfo	1.420	6,49	6,42	5,36
blastengate	11.468	52,40	51,87	43,31
blasten	10.039	87,54	45,87	37,91
CD3+	102	1,02	0,89	0,39
CD10+	103	1,03	0,90	0,39
CD19+	5.759	57,37	50,22	21,75
CD34+	8.938	89,03	77,94	33,75
cyCD3+	9.185	91,49	80,09	34,69
cyCD79a+	138	1,37	1,20	0,52
cyMPO+	9.968	99,29	86,92	37,64
CD22	117	1,17	1,02	0,44

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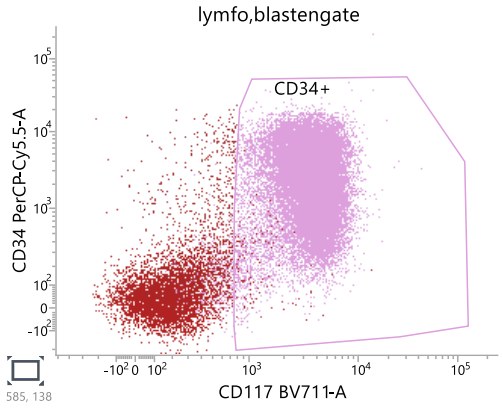
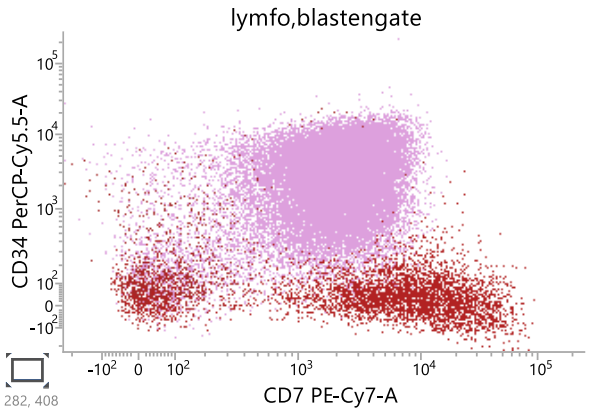
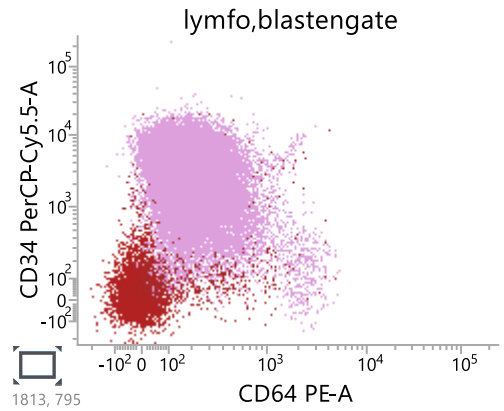
Second relapse

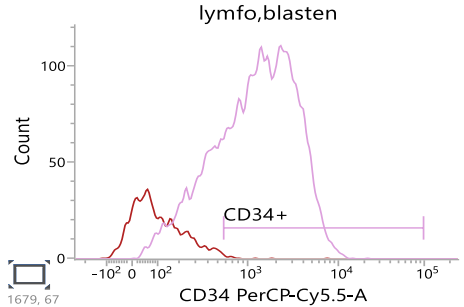
Flow BM

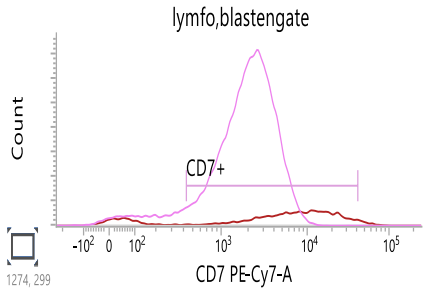
- 42% blasts with fenotype : cyCD3-/CD3-/**CD7+(itt diagnosis)**/CD10-/CD11b-/CD13+/CD14-/CD15-/CD16-/CD19-tot+zwak/cyCD22-/CD33+/**CD34+(itt diagnosis)/**CD35-/CD38-/CD45+/CD56-/**CD64-(itt diagnosis)/**cyCD79a-/CD117+/CD300e-/HLADR-/cyMPO+strong/NG2-/TdT-, compatible with myeloblasts/promyelocytes.

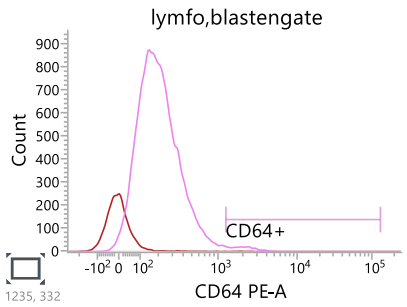












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Second relapse

NGS :

MYE-323110576514

Terug naar overzicht stalen | Terug naar start | Uitloggen

v2.2.0

SeqNext

Gen	Nucleotidewijziging	Aminozuurwijziging	VAF	Klasse	MOLIS	COSMIC	Pop. freq.	SIFT/PolyPhen	Conserved
DNMT3A	c.1717C>T	p.(Gln573Ter)	3.3%	VUS of wspat, diagnose?	DNMT3A, c.1717C>T p.(Gln573Ter), 3.3% VAF	1	0, 0, 0	-/-	-
FLT3	c.1791_1837+4dup	p.?	75%	VUS	FLT3, c.1791_1837+4dup p.?, 75% VAF	0	0, 0, 0	-/-	-
TET2	c.3797A>G	p.(Asn1266Ser)	28%	VUS Gescoord (BF - 31-03-2023)	TET2, c.3797A>G p.(Asn1266Ser), 28% VAF	0	0, 0, 0	deleterious/probably_damaging	Ja
TET2	c.5333A>G	p.(His1778Arg)	51%	(Vermoedelijk) benigne	TET2, c.5333A>G p.(His1778Arg), 51% VAF	0	0.0791, 0.0229, 0.19978	deleterious/possibly_damaging	Nee

Workbench

Gen	Nucleotidewijziging	Aminozuurwijziging	VAF	Reads
CUX1	c.4375_4377del3	p.Ser1459del	2.2%	22/985
DNMT3A	c.1717C>T	p.Gln573*	3.7%	56/1496
TET2	c.2599T>C	p.Tyr867His	47%	541/1143
TET2	c.3797A>G	p.Asn1266Ser	28%	232/843
TET2	c.5167C>T	p.Pro1723Ser	50%	569/1148

Workbench - InDels and Structural Variants

Geen varianten gedetecteerd

FLT3ITDext

ITD lengte	Nucleotidewijziging	Aminozuurwijziging	AR	VAF	Aantal reads
51	c.1790_1837+3dup	p.597_598insEYDLKWEFPRENLEFGN	0.2808	21.92	245

* **DNMT3A,** c.1717C>T p. (Gln573Ter), 2,6 >> 3,3 % VAF
* **FLT3-ITD c.1790_1837 + 3 dup (51 bp)** >> 21,5 %VAF

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Treatment

° Hydrea, cytarabine and idarubicine

° third line therapy with Gemtuzumab Ozogamicin complicated with TMA en VOD/SOS (R:eculizumab)

>> Allo HSCT with HLA-identical sibling 2/2024

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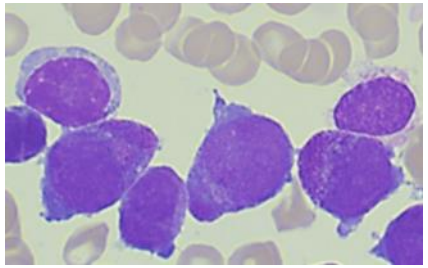
Conclusion

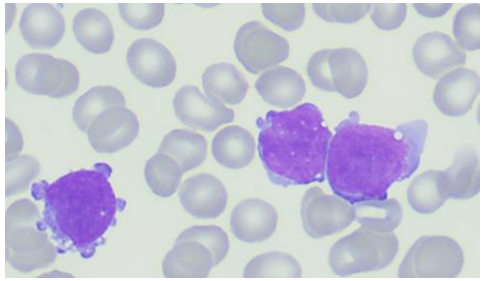
This case illustrates that

° despite the assumed good prognosis of **APL**, the presence of pathogenic variants (**FLT3-ITD** ic) detected by NGS can complicate the disease course profoundly

° relapse after ATRA therapy can show a **shift in immunophenotype and morfology**



Diagnosis :
hypergranular
CD7-/CD34-mostly/CD64+



Relapse:
hypogranular
CD7+/CD34+/CD64-

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Goede zorg laat niemand achter

Time for questions …