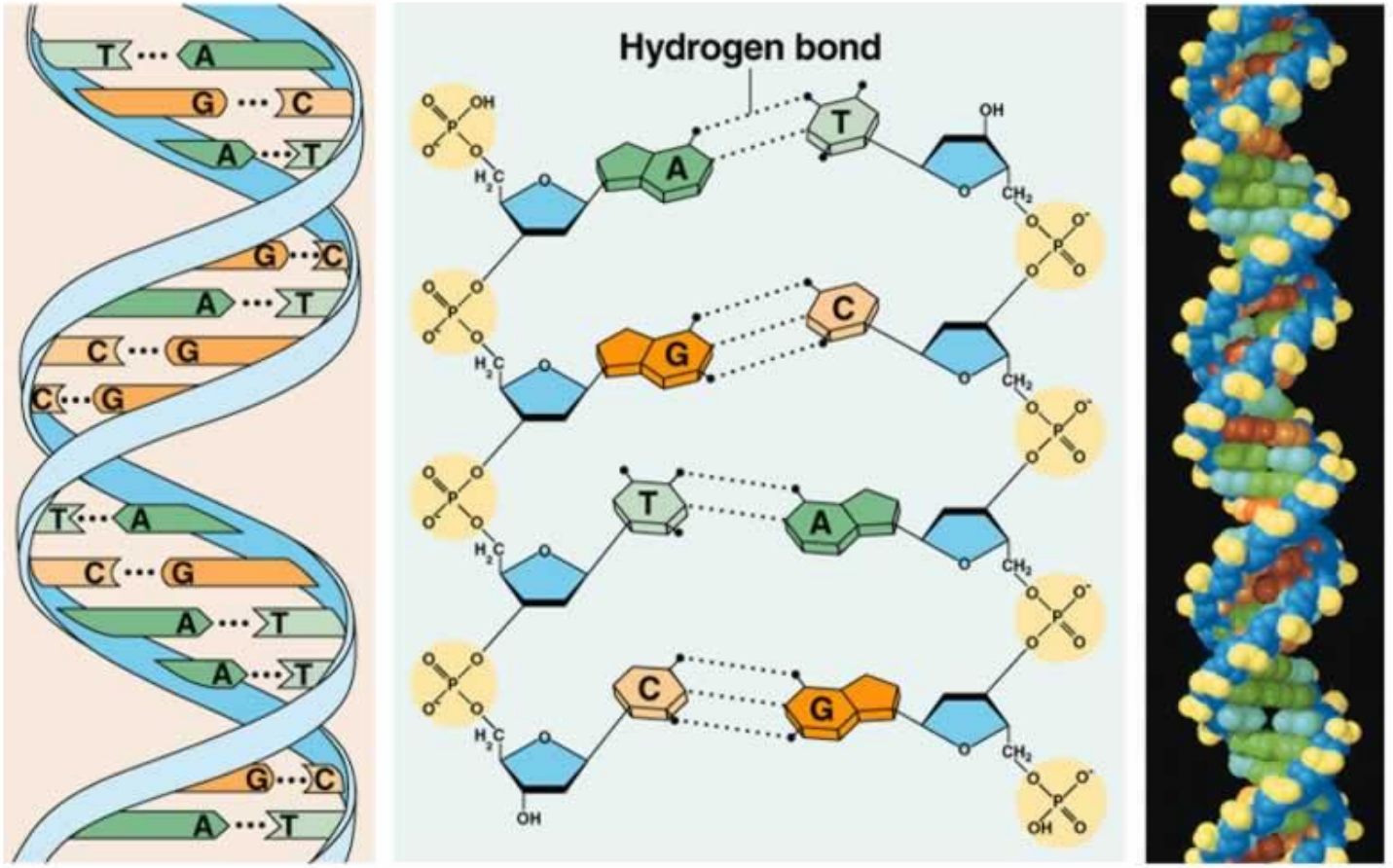
Moleculaire diagnostiek

André Mulder

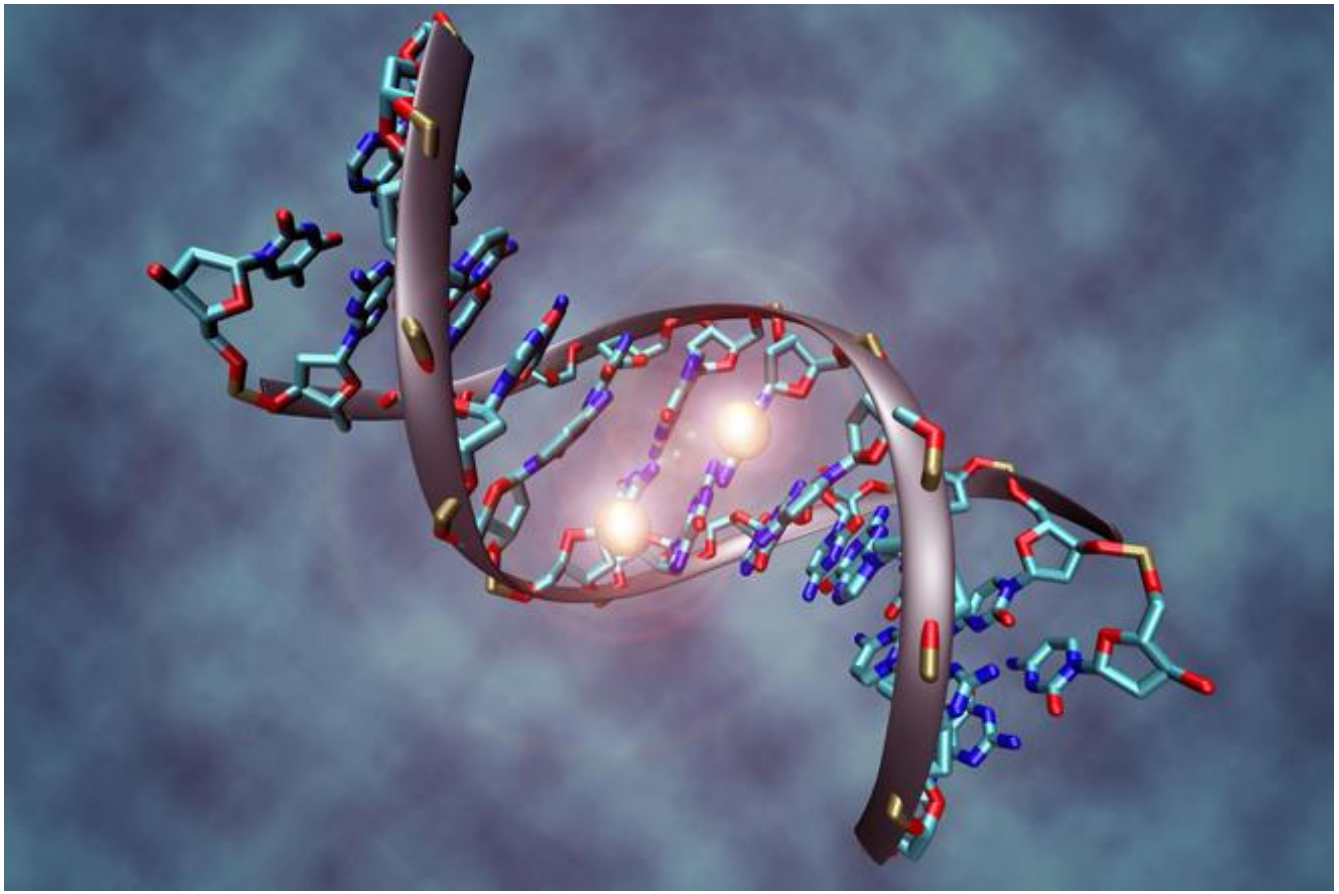
2024

Dubbelstrengs DNA

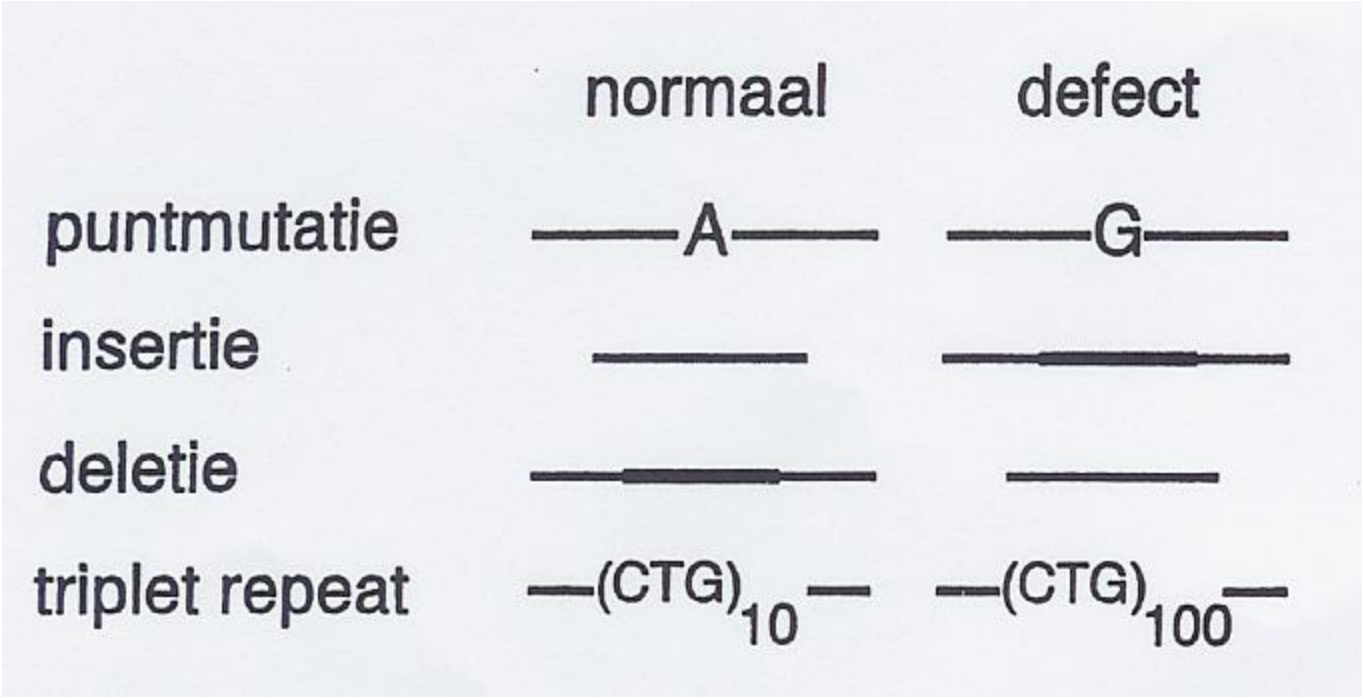


Humaan genoom:

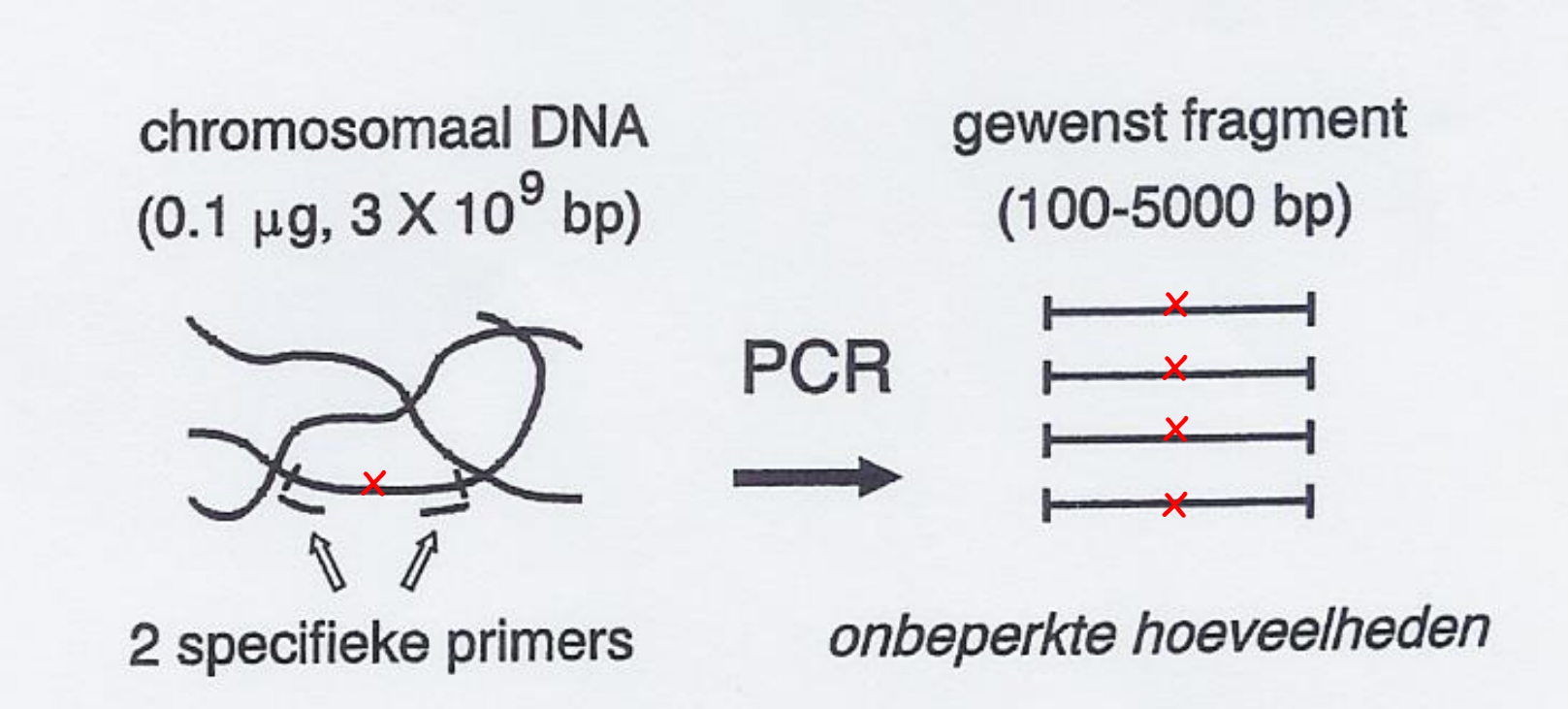
3.000.000.000 bp = 3 miljard baseparen



Kleine afwijkingen in DNA



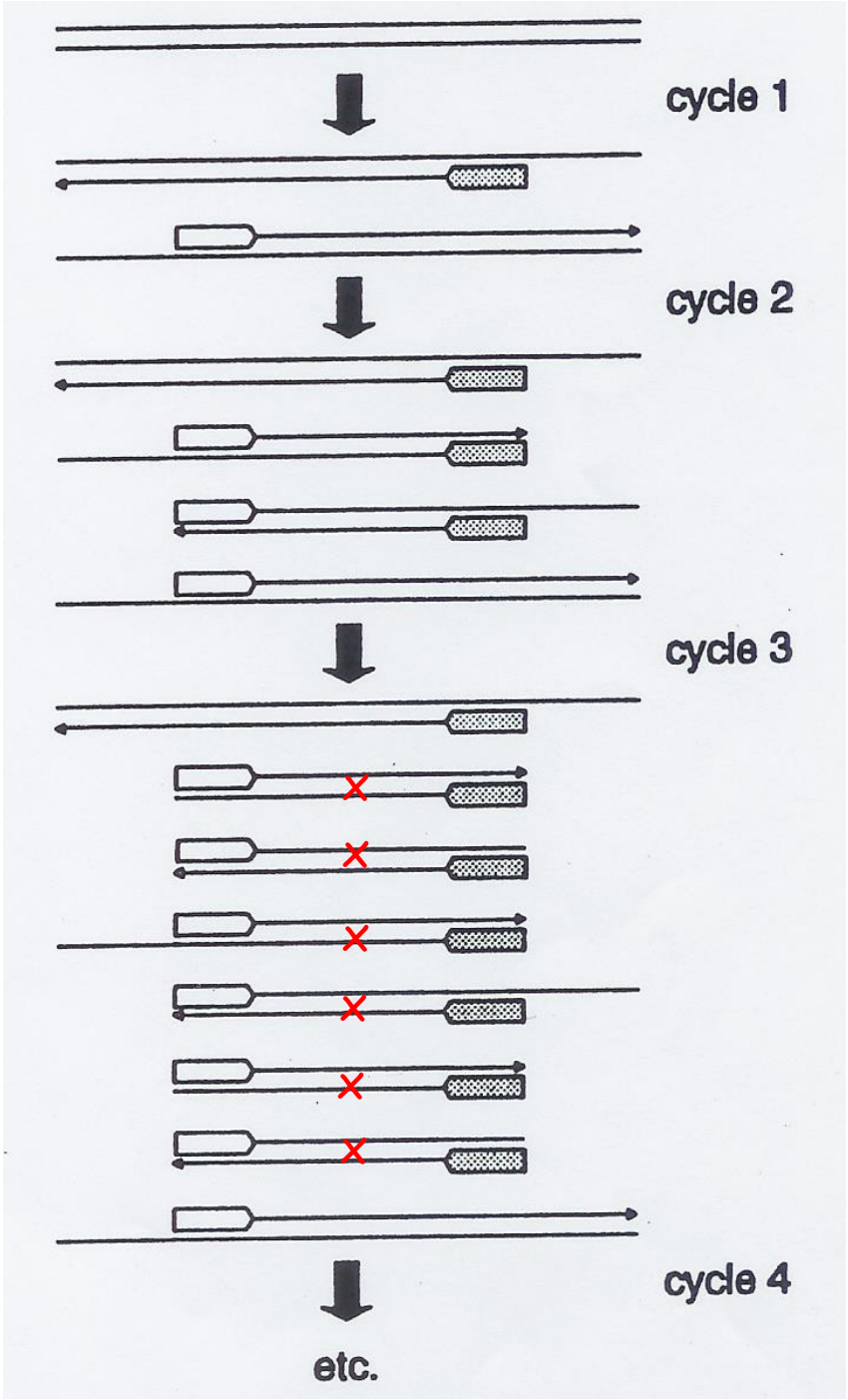
Polymerase ketting reactie
Polymerase chain reaction



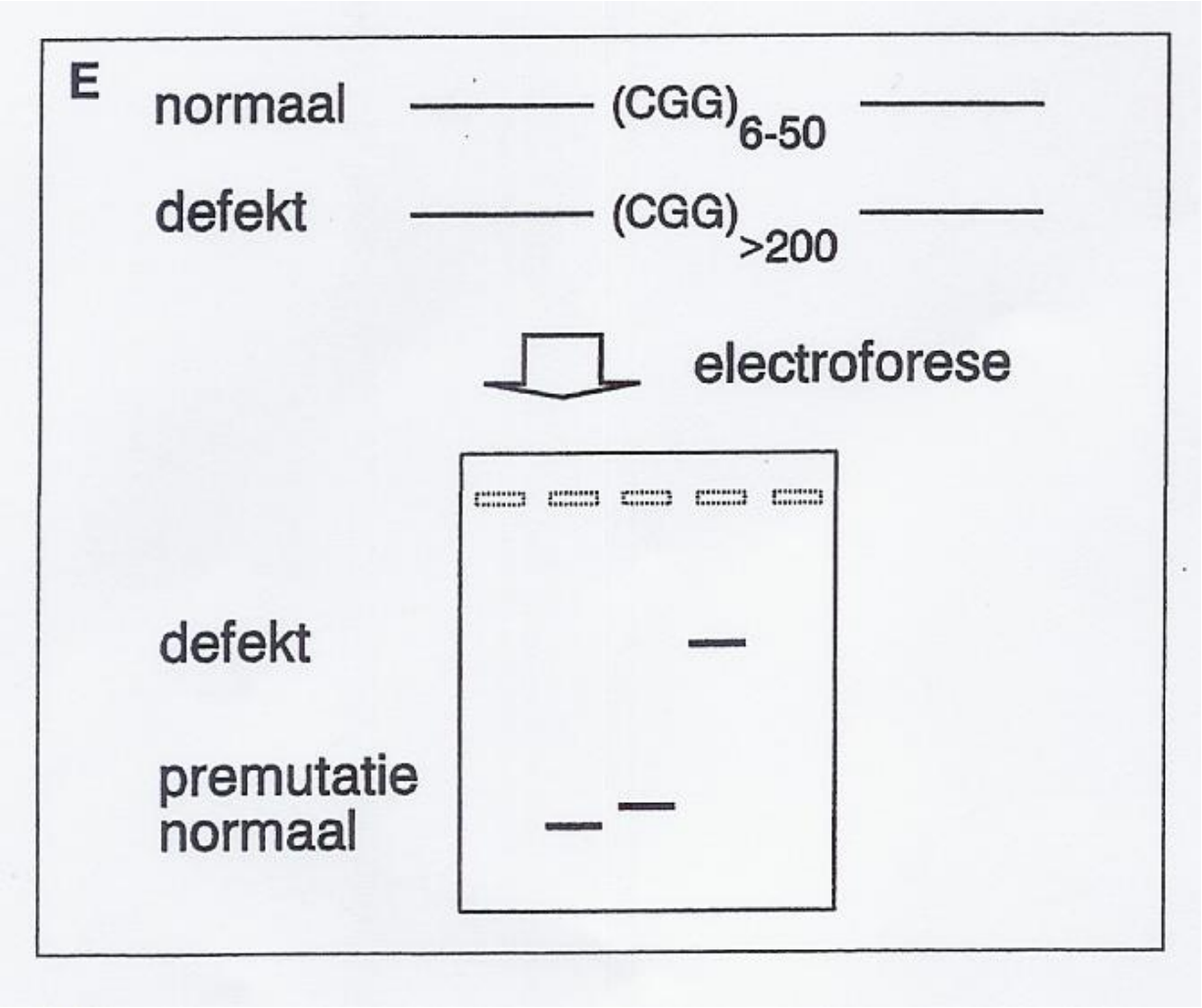
PCR:

- denaturatie: 95°C
- annealing: 55-65°C
  - 2 primers (± 20 nt)
- extension: 72°C:
  - Taq DNA polymerase:
    - Thermus aquaticus
    - heetwaterbronnen
  - losse nucleotiden (A,T,C,G)

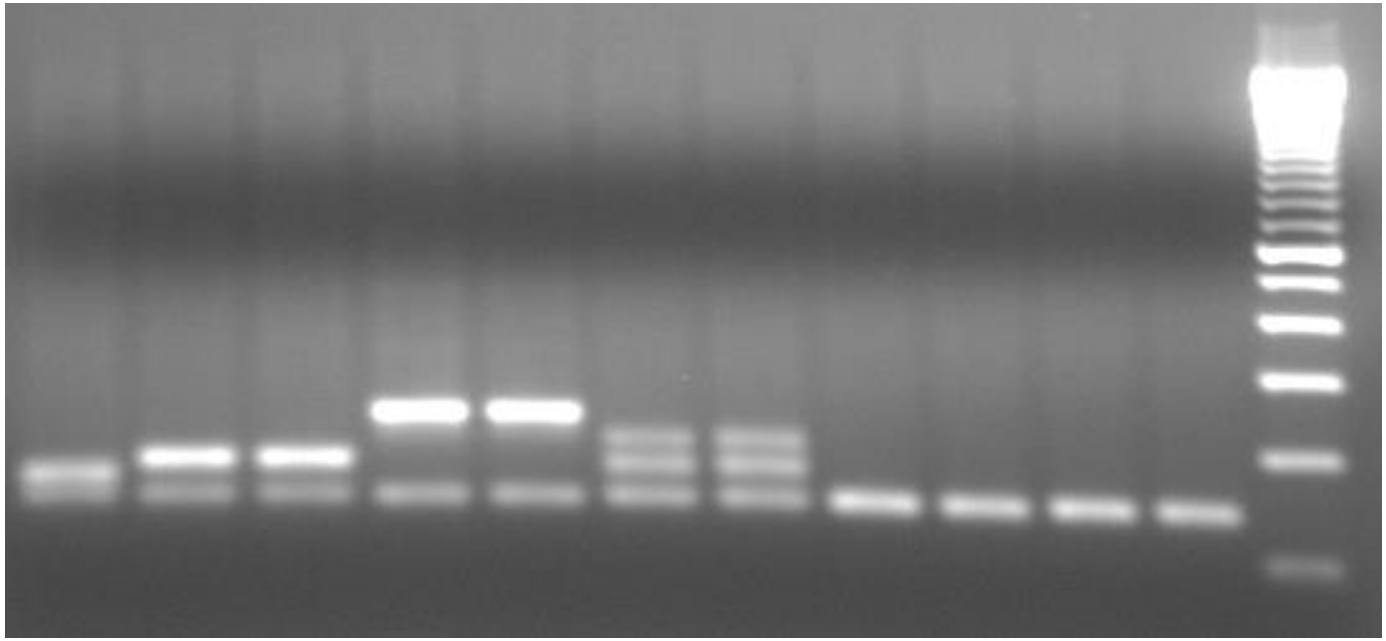
- 25 - 40 cycli (circa 2 uur)



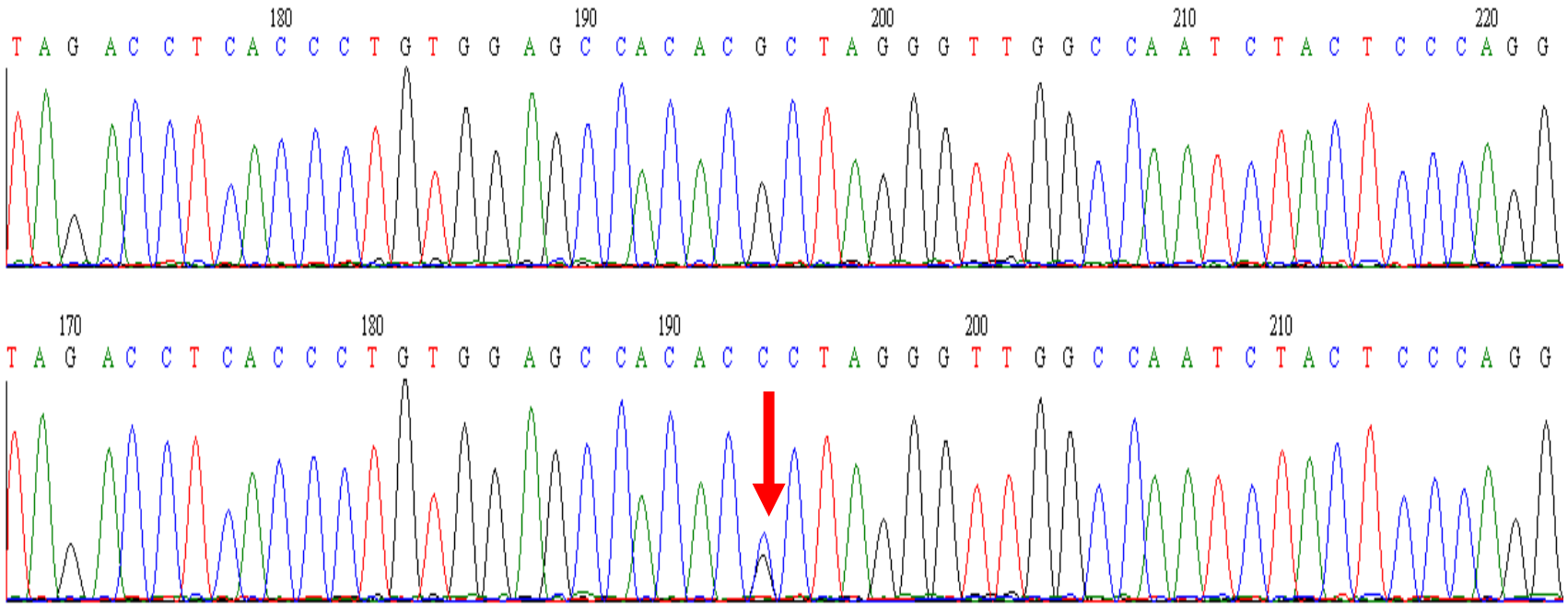
Gelelectroforese: grote afwijkingen



FLT3-ITD: internal tandemduplicates



Sanger DNA sequentie mutatie-analyse



Targeted Next Generation Sequencing: NGS: UMCG: 107 genen, gevoeligheid 1%

Whole exon sequencing: WES: gevoeligheid circa 10%

Whole genome sequencing: WGS: gevoeligheid circa 15%

SOPHiA GENETICS®

Next generation Sequencing (NGS): 107 genen (sinds begin 2021)

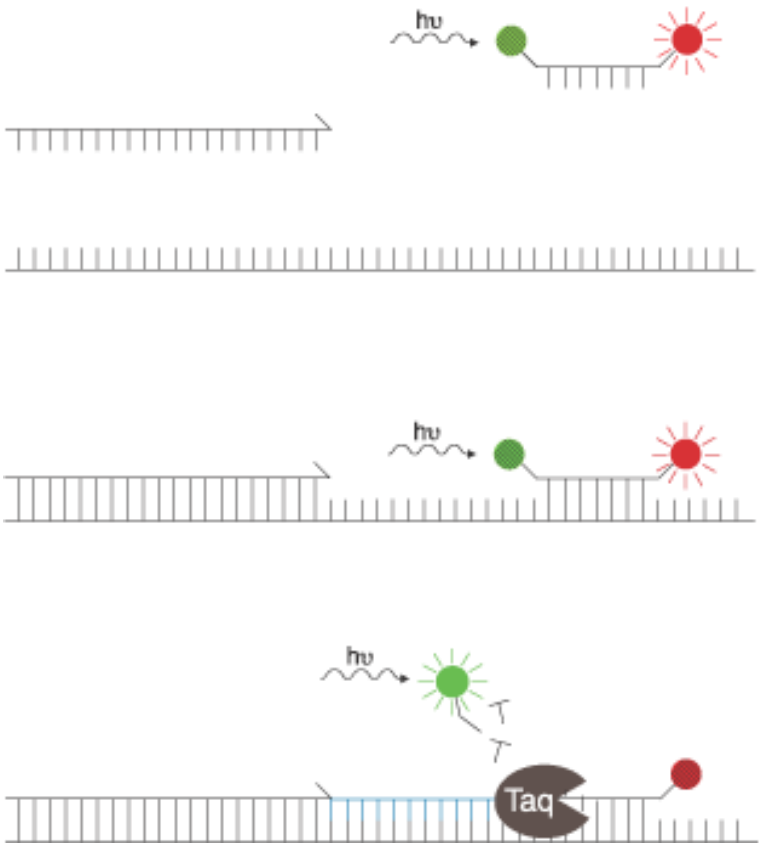
Myeloid Extended Solution - 98 genes

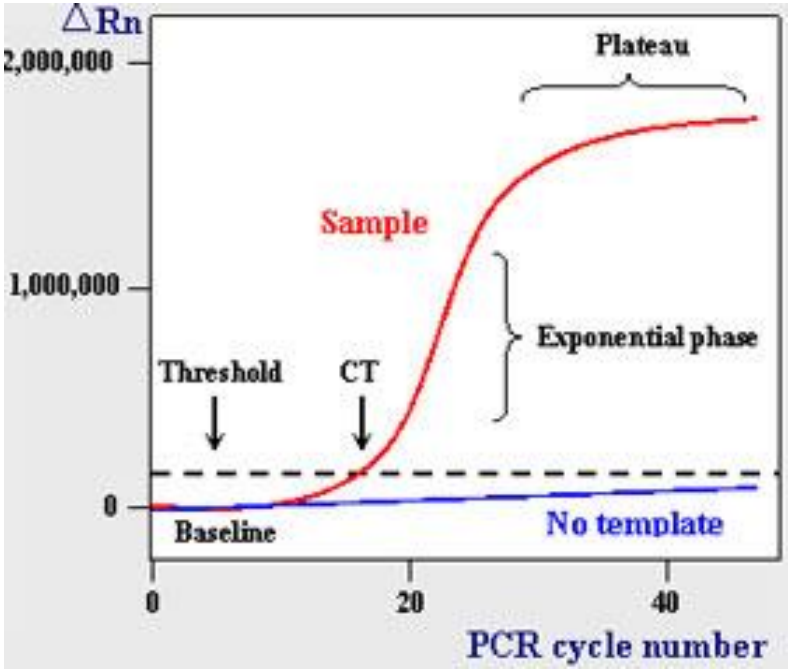
ABL1
ANKRD26
ASXL1
ASXL2
ATM
ATRX
BCOR
BCORL1
BRAF
BRCC3
CALR
CBL
CBLB
CBLC
CCND2
CDKN2A
CEBPA
CHEK2
CREBBP
CSF3R
CSMD1
CSNK1A1
CTCF
CUX1
DDX41
DHX15
DNMT3A
ELANE
ETNK1
ETV6
EZH2
FANCA
FANCL
FLT3
GATA1
GATA2
GNAS
GNB1
HNRNPK
HRAS
IDH1
IDH2
IKZF1
JAK1
JAK2
JAK3
KDM6A
KIT
KMT2A
KMT2D
KRAS
LUC7L2
MECOM
MET
MPL
MYC
NF1
NOTCH1
NOTCH2
NPM1
NRAS
PAX5
PDGFRA
PHF6
PIGA
PML
PPM1D
PTPN11
RAD21
RAF1
RB1
RBBP6
RPS19
RTEL1
RUNX1
SAMD9
SAMD9L
SBDS
SETBP1
SF3B1
SH2B3
SMC1A
SMC3
SOS1
SRP72
SRSF2
STAG1
STAG2
STAT3
STAT5B
TERC
TERT
TET2
TP53
U2AF1
WT1
ZBTB7A
ZRSR2

Extra 9 genen:
MYD88, FBXW7, PTEN, NFE2, BIRC3, IL2RG, SAMHD1, TCL1A, UBA1

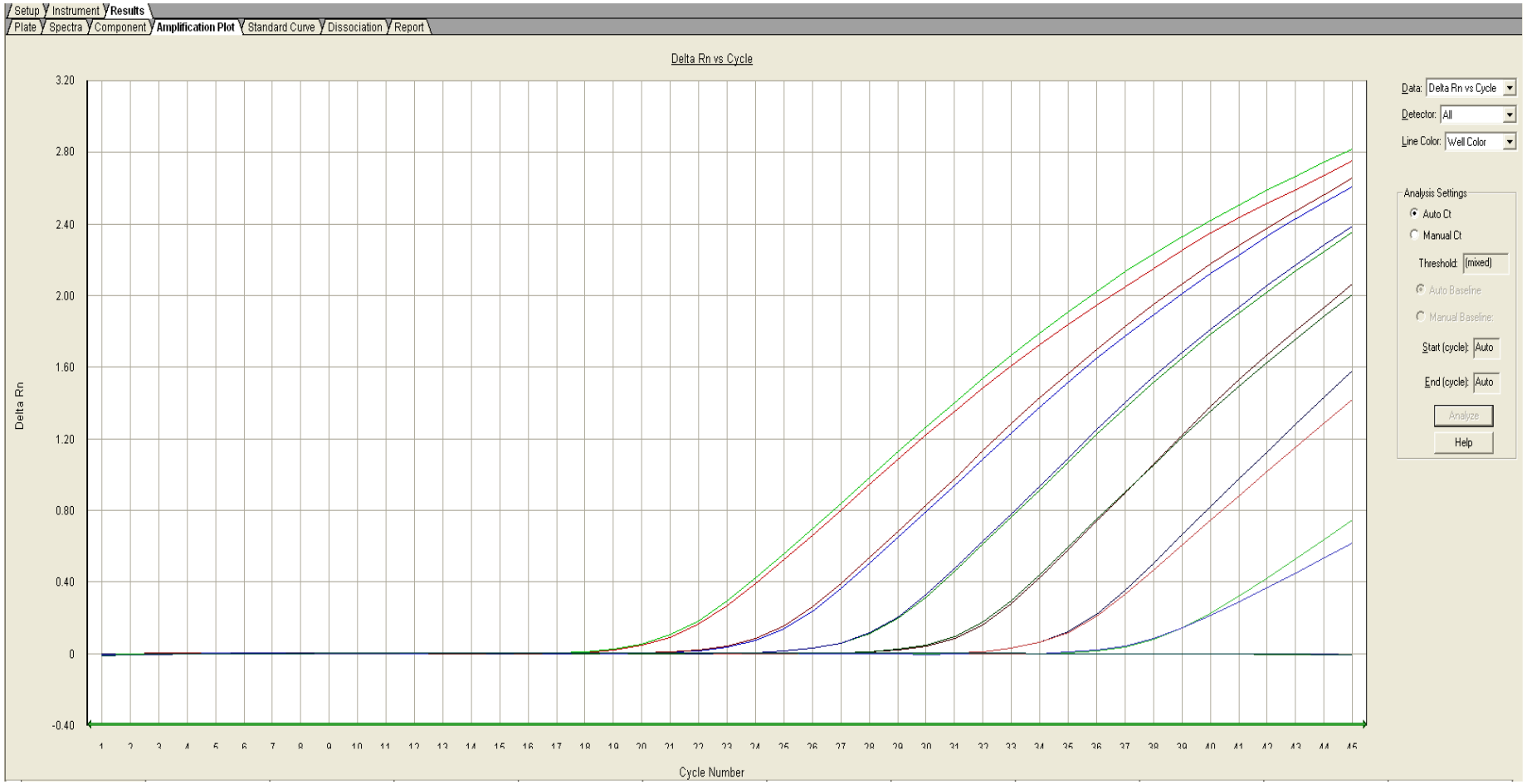
Restziekte activiteit?

Principe real time qPCR

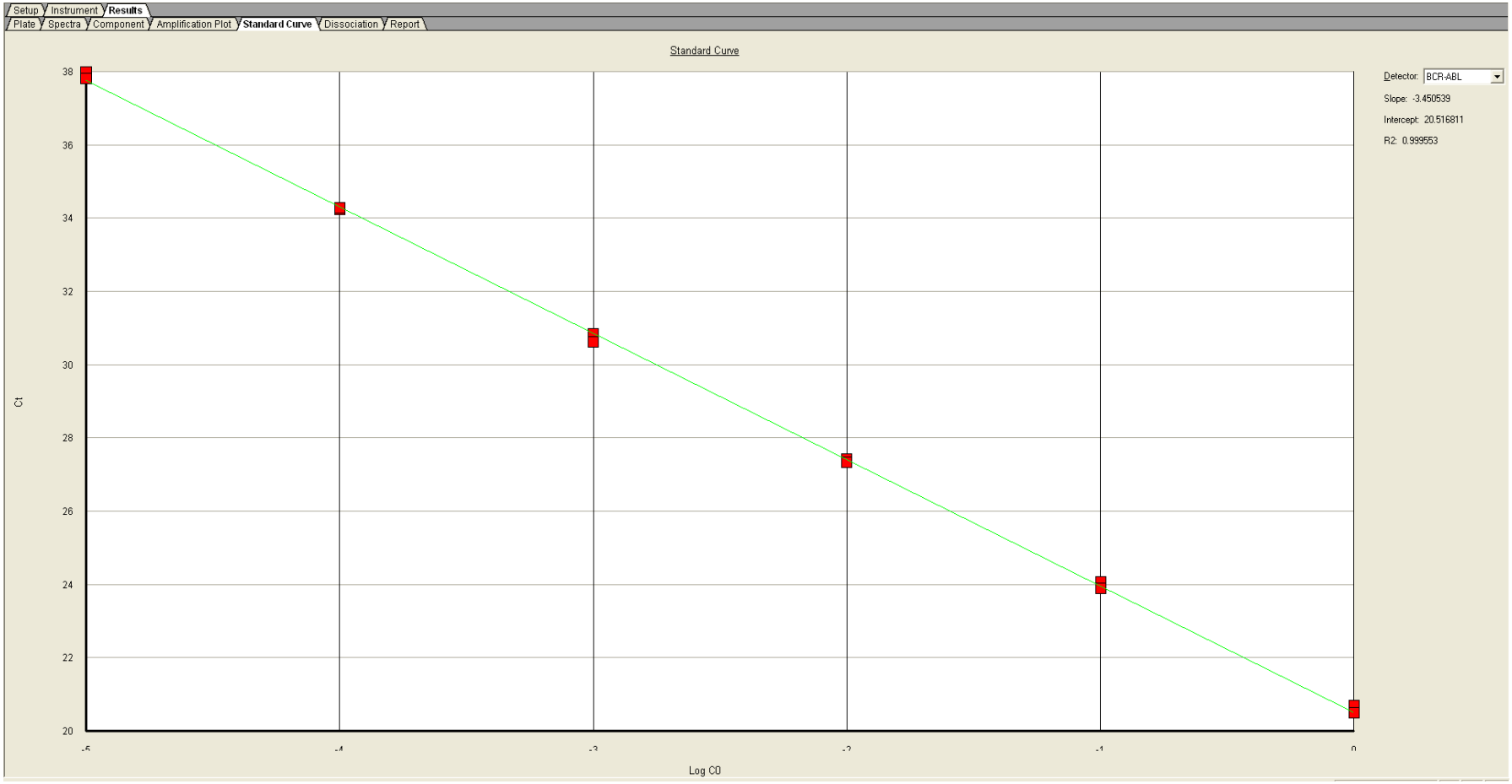




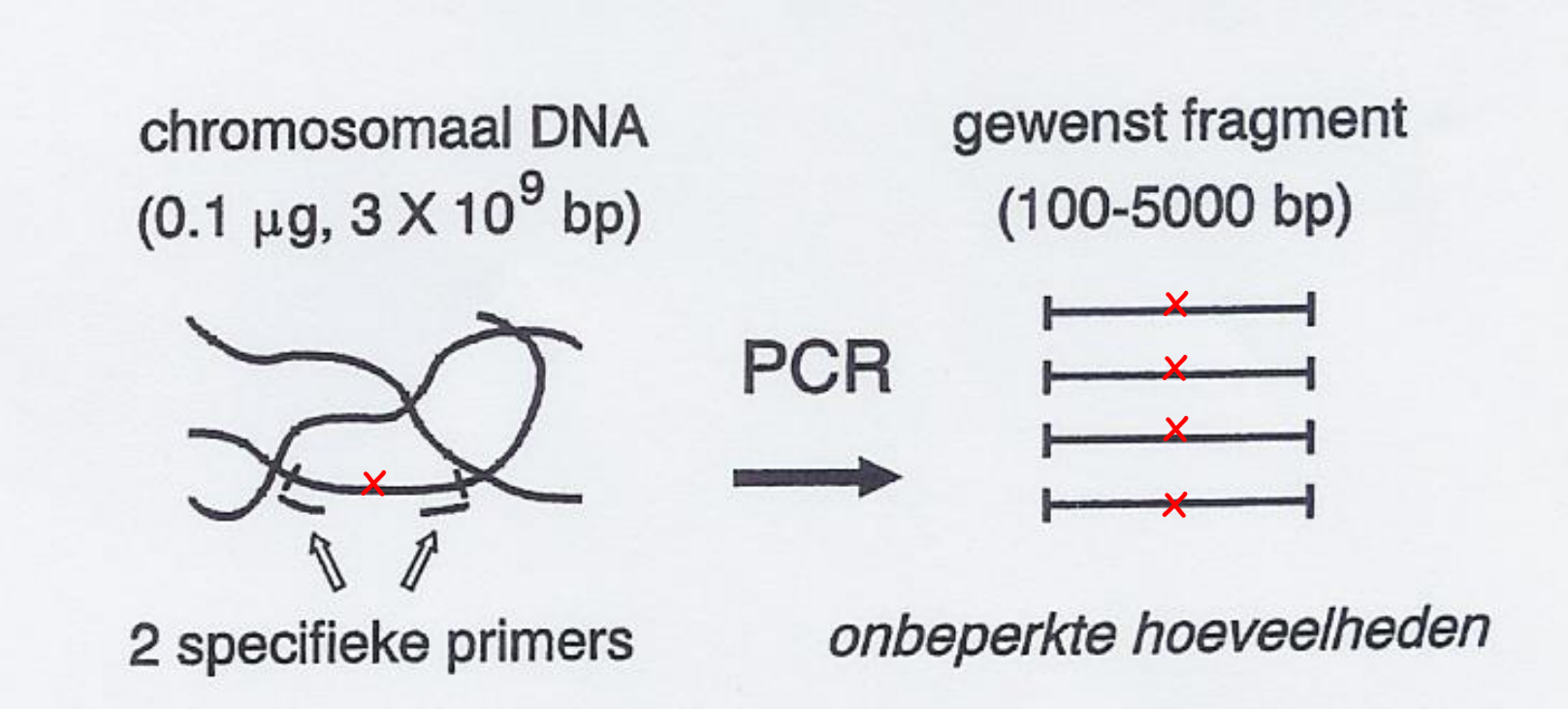
Positieve cellijn K562: *BCR::ABL1*



K562 ijklijn: *BCR::ABL1*

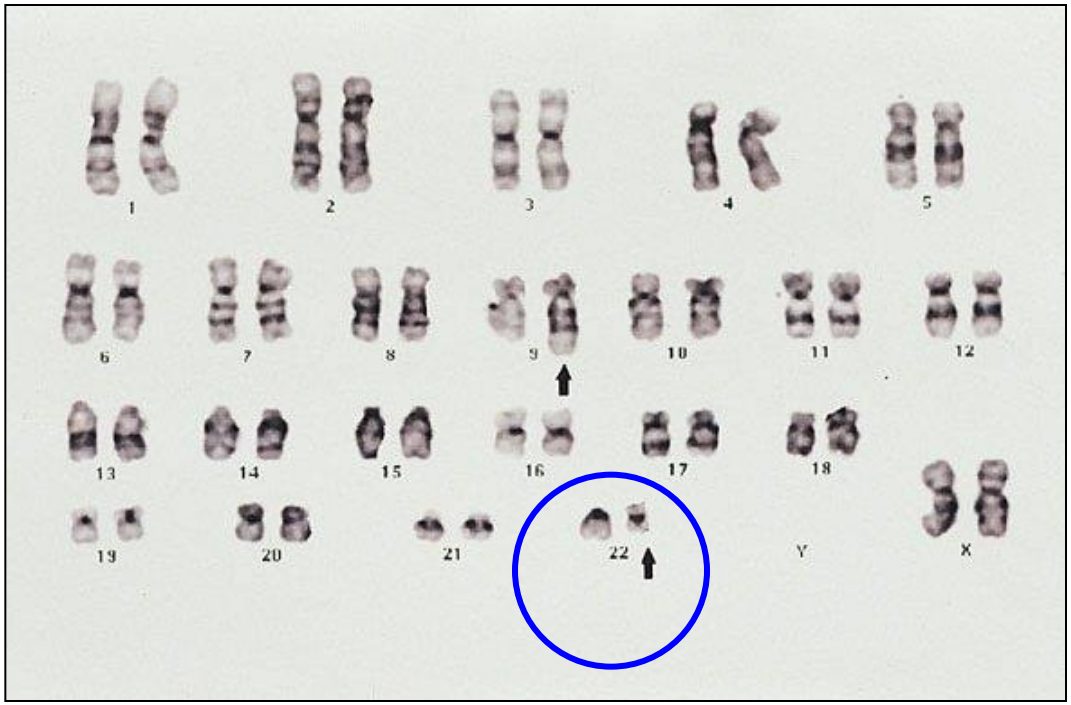


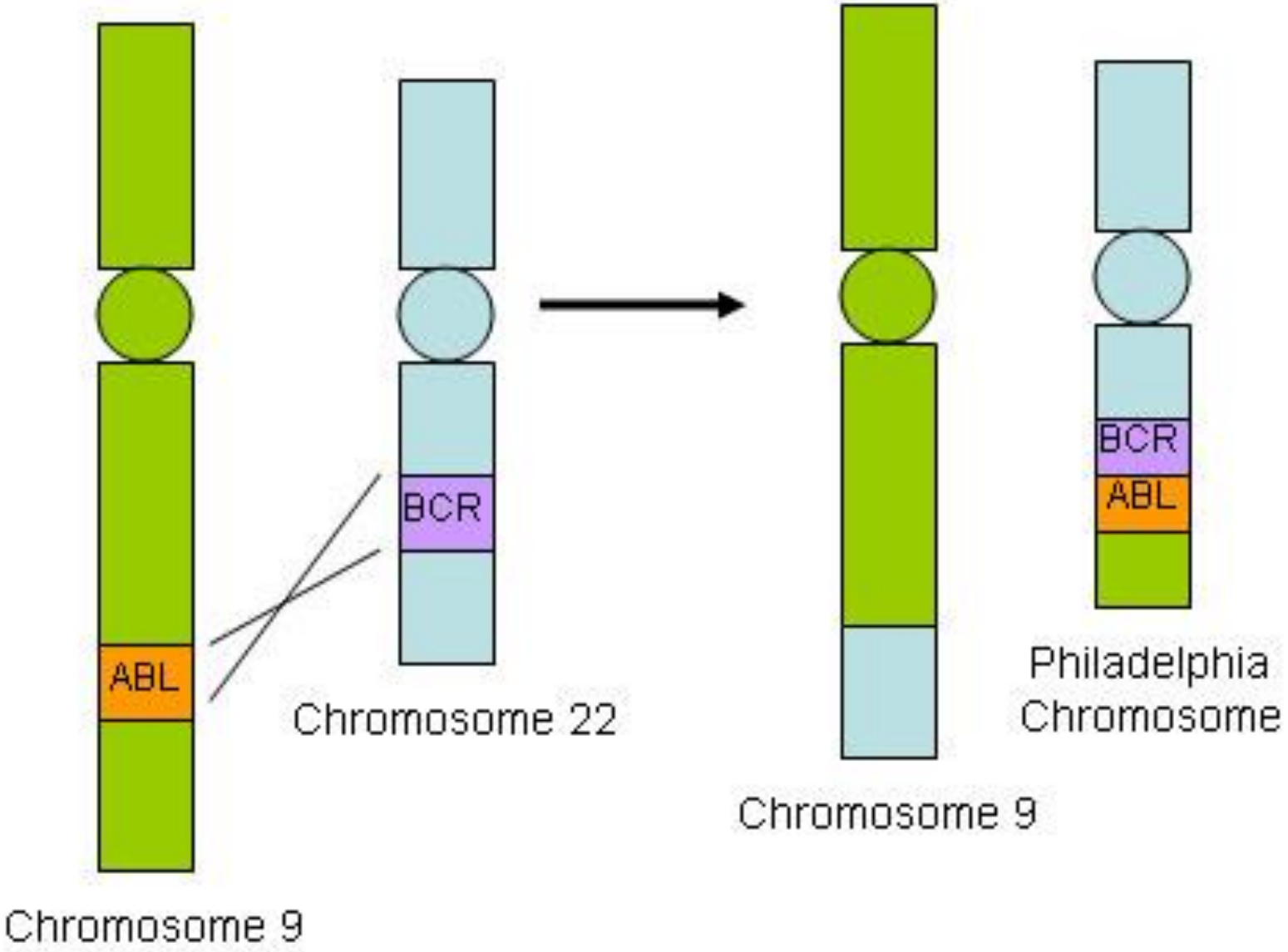
Polymerase ketting reactie
Polymerase chain reaction
PCR: 1:100.000-1.000.000 cellen!



CML: Ph chrom., t(9;22)(q34;q11.2): *BCR::BL1* fusiegen

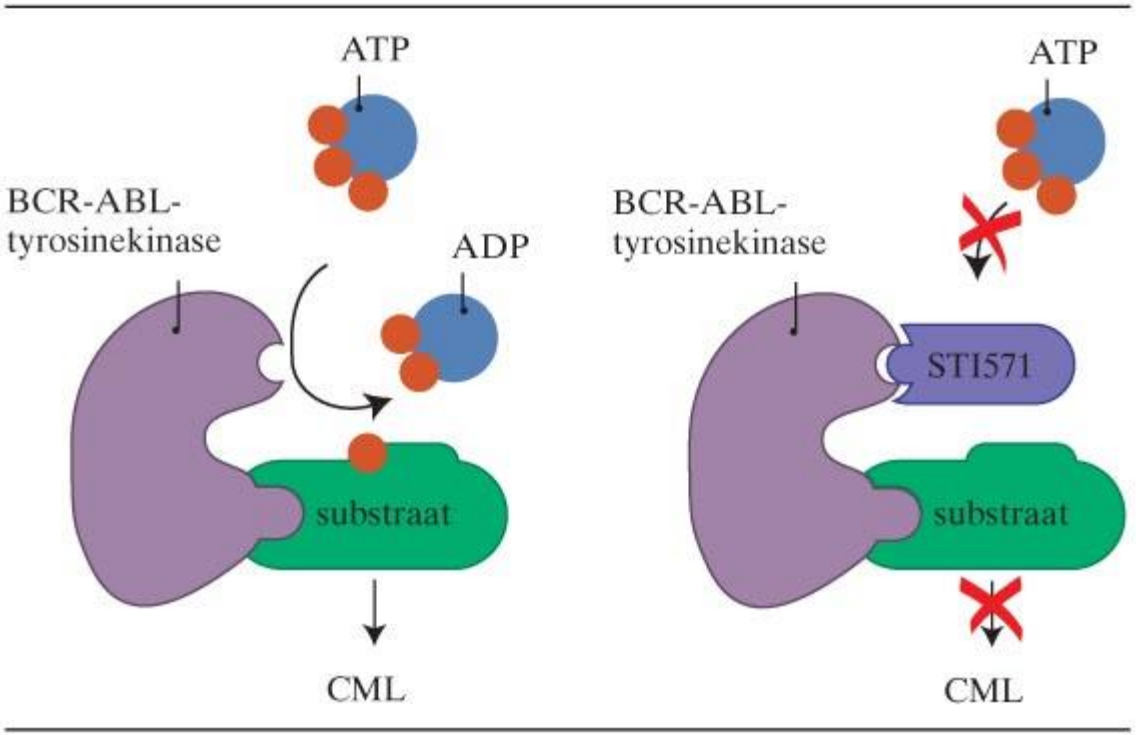
Nowell PC, Hungerford DA.
A minute chromosome in human chronic granulocytic leukemia.
Science 1960;132:1497, abstract.



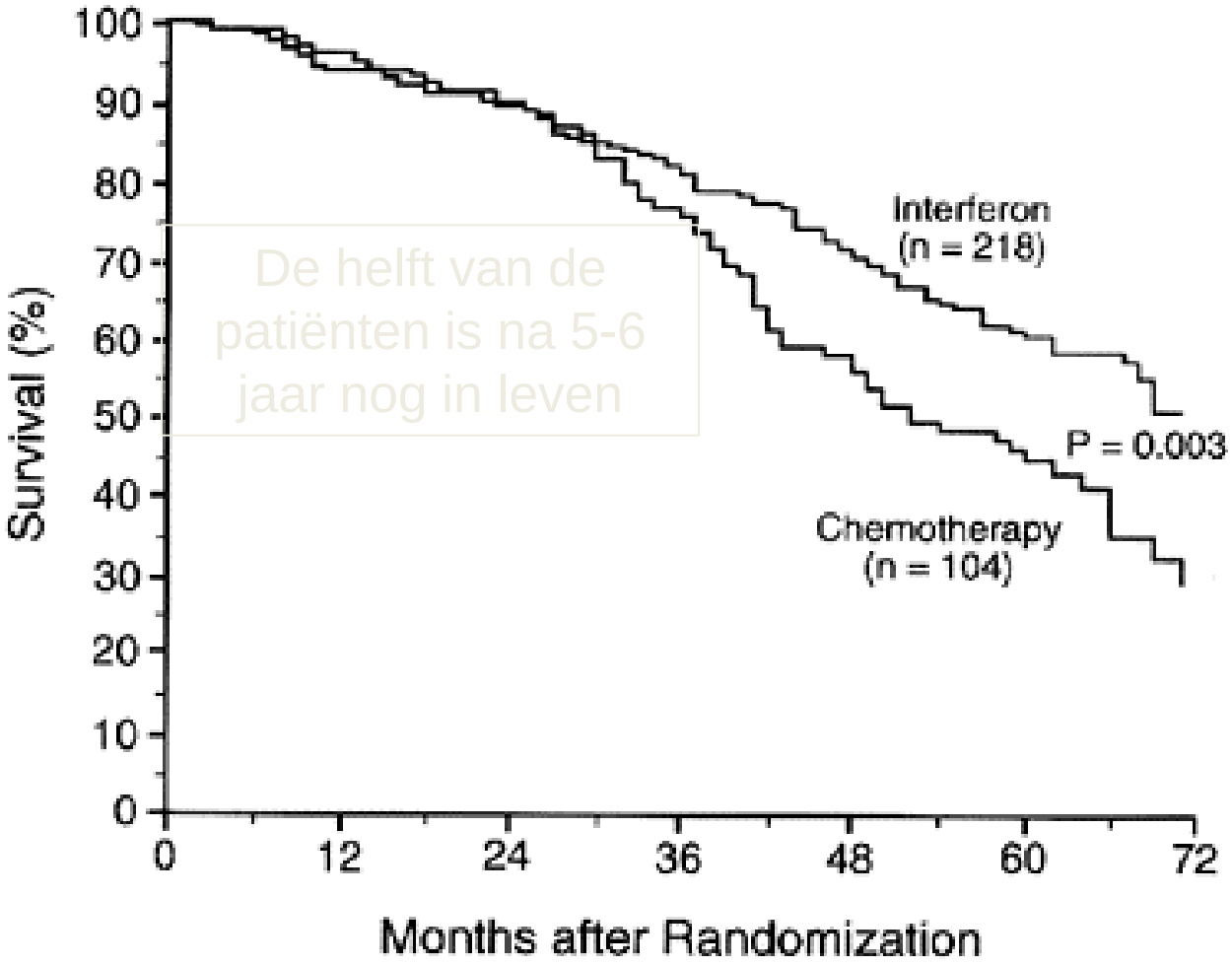


- **BCR::ABL1 fusiegen: leidt tot een Tyrosine kinase.**

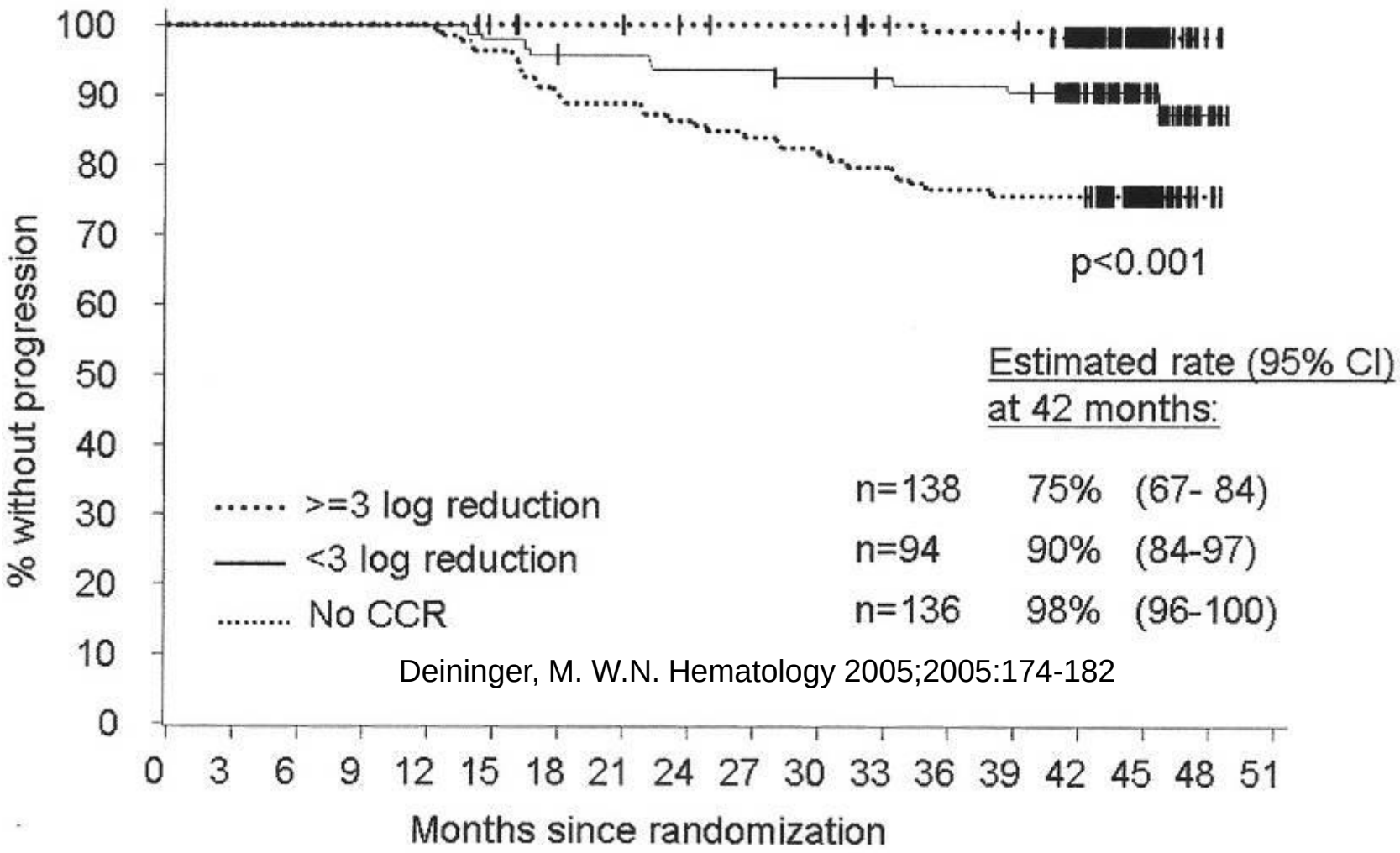
TKI: Imatinib (Glivec)



Overleving CML '**vroeger**' met twee verschillende behandelingen (1994)



Progressievrije overleving van CML-patiënten ‘nu’ behandeld met imatinib, afhankelijk van hun verbetering op 12 maanden



Deininger, M. W.N. Hematology 2005;2005:174-182

CML: BCR-ABL1 fusiegen

Diagnose: cytogenetica en PCR

- Breukpunt
- Concentratie
- Cryptic translocation: cytogenetica negatief
- Additionele mutaties

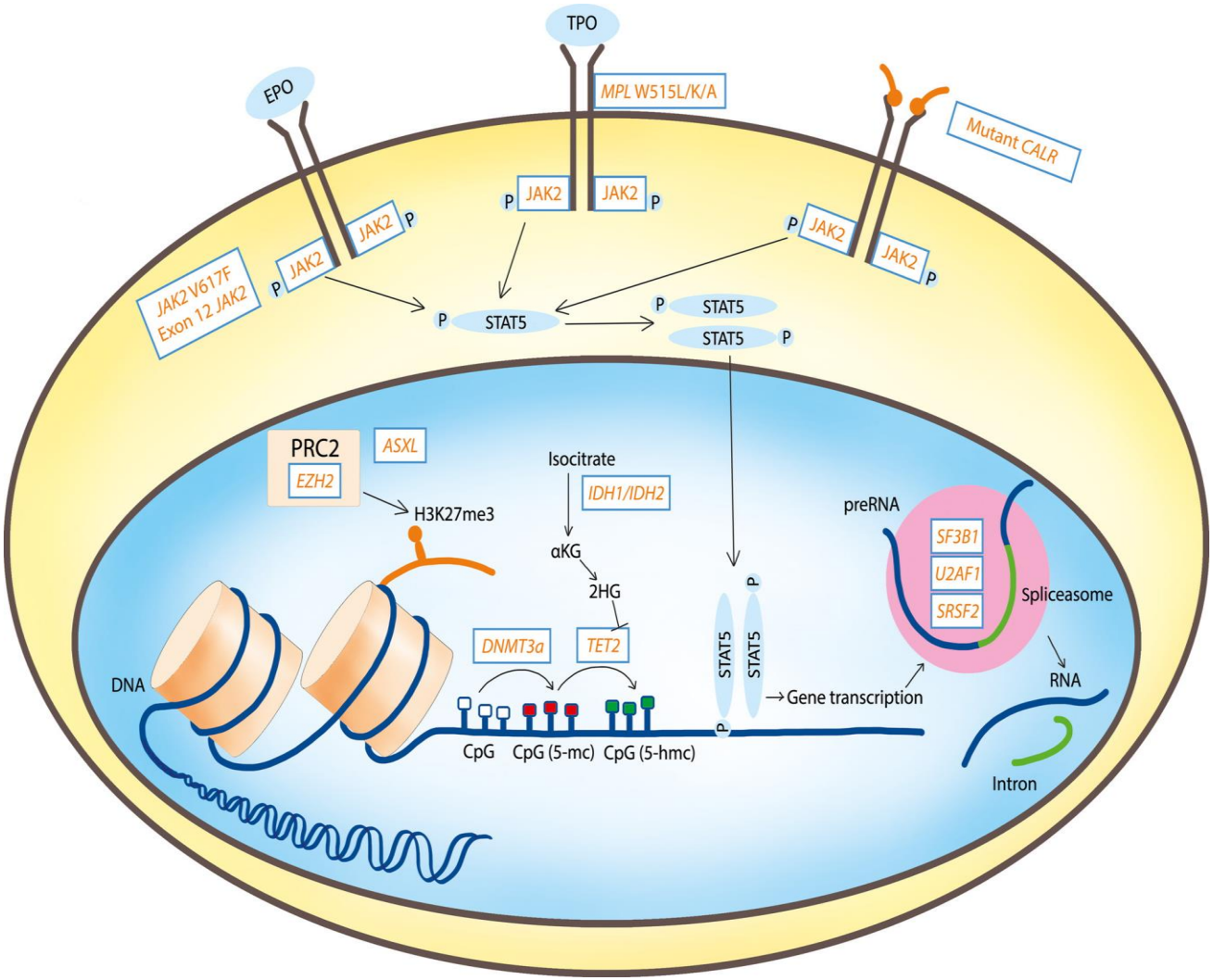
Follow-up: PCR en cytogenetica

- Concentratie
- Additionele mutaties

BCR-ABL1 fusiegen

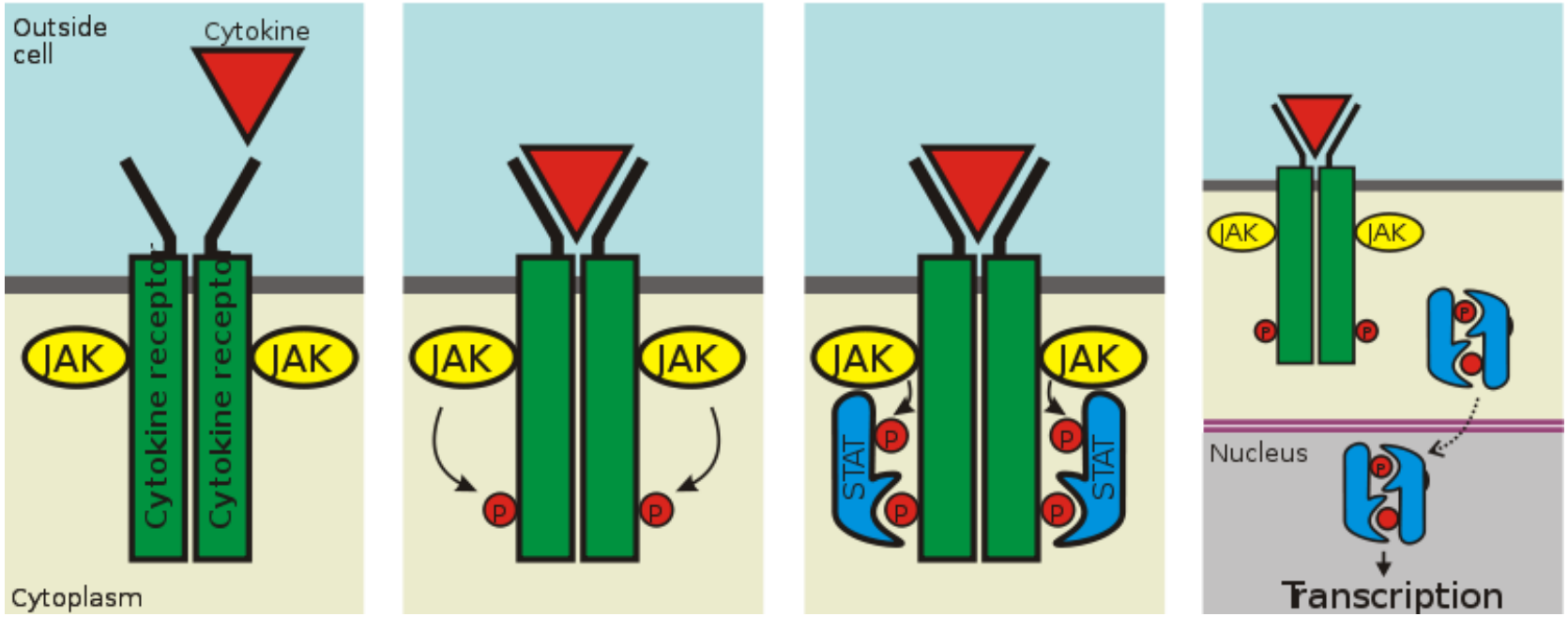
- Elke 3 maanden controleren
- MR: > 1 log toename: na 2 weken herhalen
- Mutatie-analyse BCR-ABL1 fusiegen:
  - Bijna alle additionele mutaties: over op 2e generatie tyrosine kinase remmer (Dasatinib)
  - Cave: T315I: ponatinib/allo-SCT

MPN

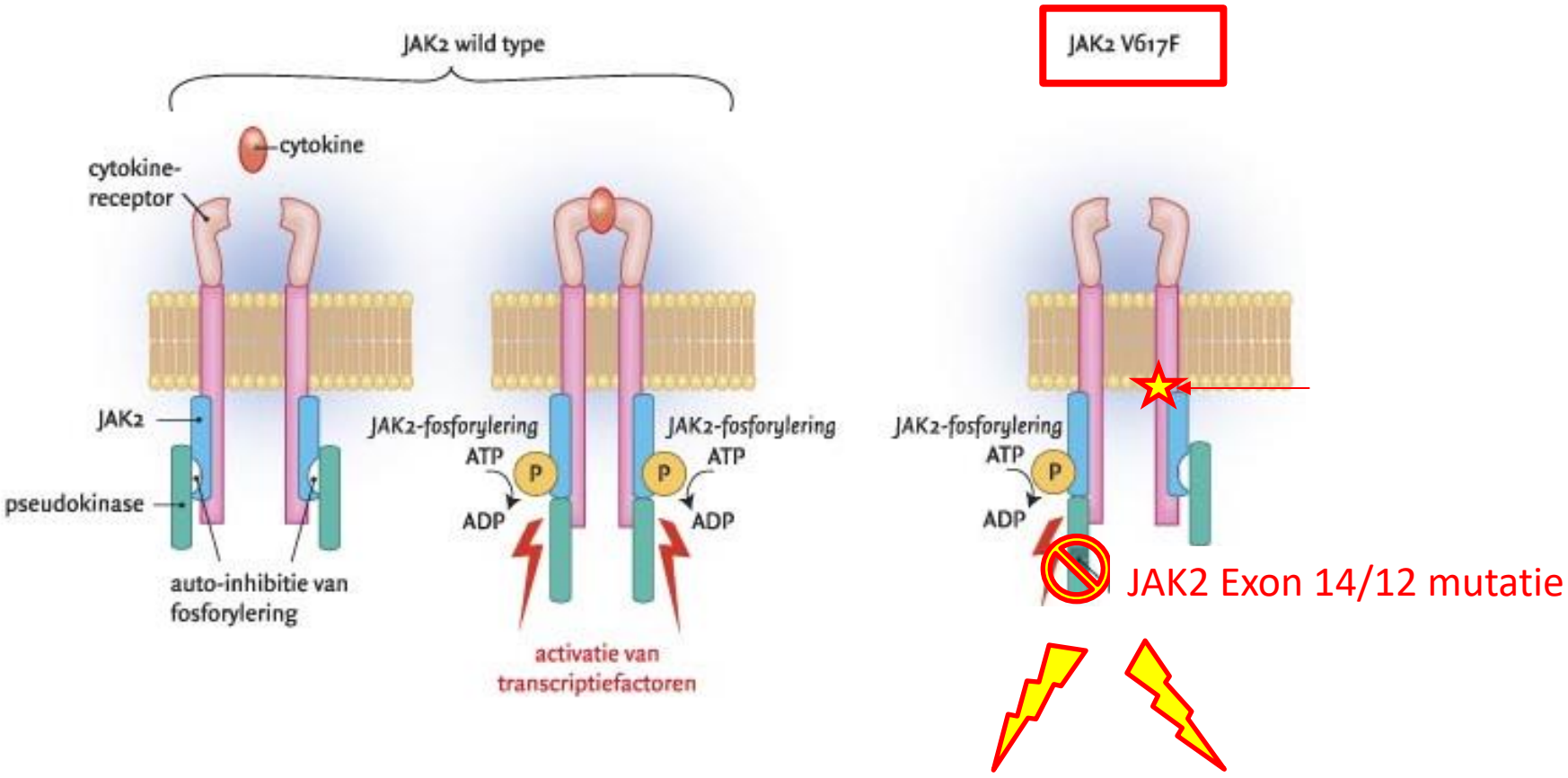

TPO
EPO
MPL W515L/K/A
Mutant CALR
JAK2 V617F
Exon 12 JAK2
JAK2
P
STAT5
PRC2
EZH2
ASXL
H3K27me3
Isocitrate
IDH1/IDH2
αKG
2HG
DNMT3a
TET2
DNA
CpG
CpG (5-mc)
CpG (5-hmc)
Gene transcription
preRNA
SF3B1
U2AF1
SRSF2
Spliceosome
RNA
Intron

JAK2

Janus Kinase 2: fosfo(P)-tyrosine kinase



Moleculaire oorzaak PV en ET



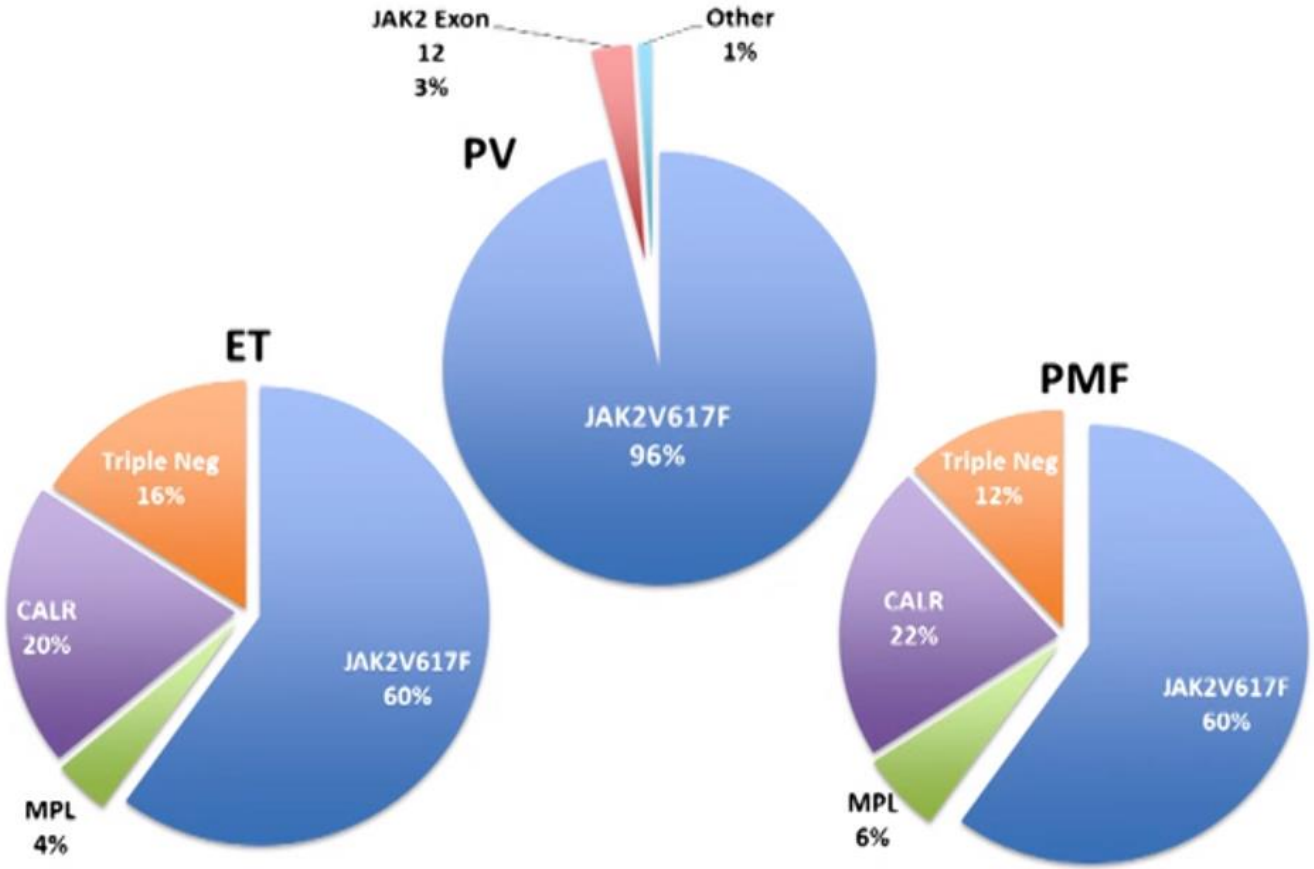
PV (**Epo-receptor**):
- erytrocytose
- Trombocytose
- Leukocytose

ET/MF (**TPO-receptor**):
- Trombocytose
- dysmegakaryopoiese

JAK2 V617F

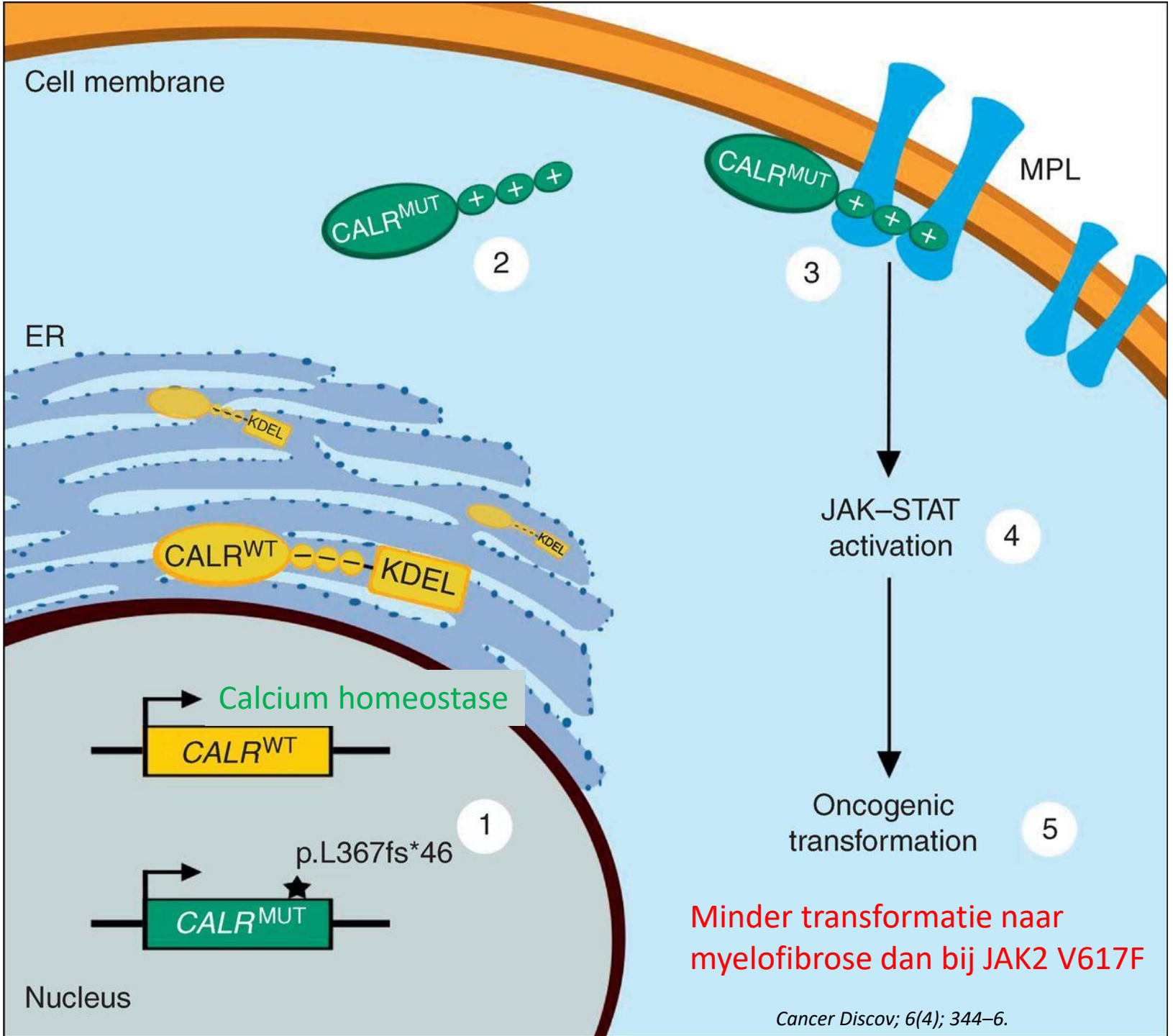
- Janus Kinase 2 eiwit: (Just another kinase)
  - Gen: bestaat uit 24 exonen op 9p24
  - Meestvoorkomende mutatie:
    - **Puntmutatie in exon 14**
    - **G**uanine vervangen door **T**hymine
    - 617^{e} aminozuur Valine vervangen door Fenylalanine
    - ***JAK2* V617F mutatie**

Prevalence of driver mutations in MPN patients



Unpublished data from Prof. J-J Kiladjian

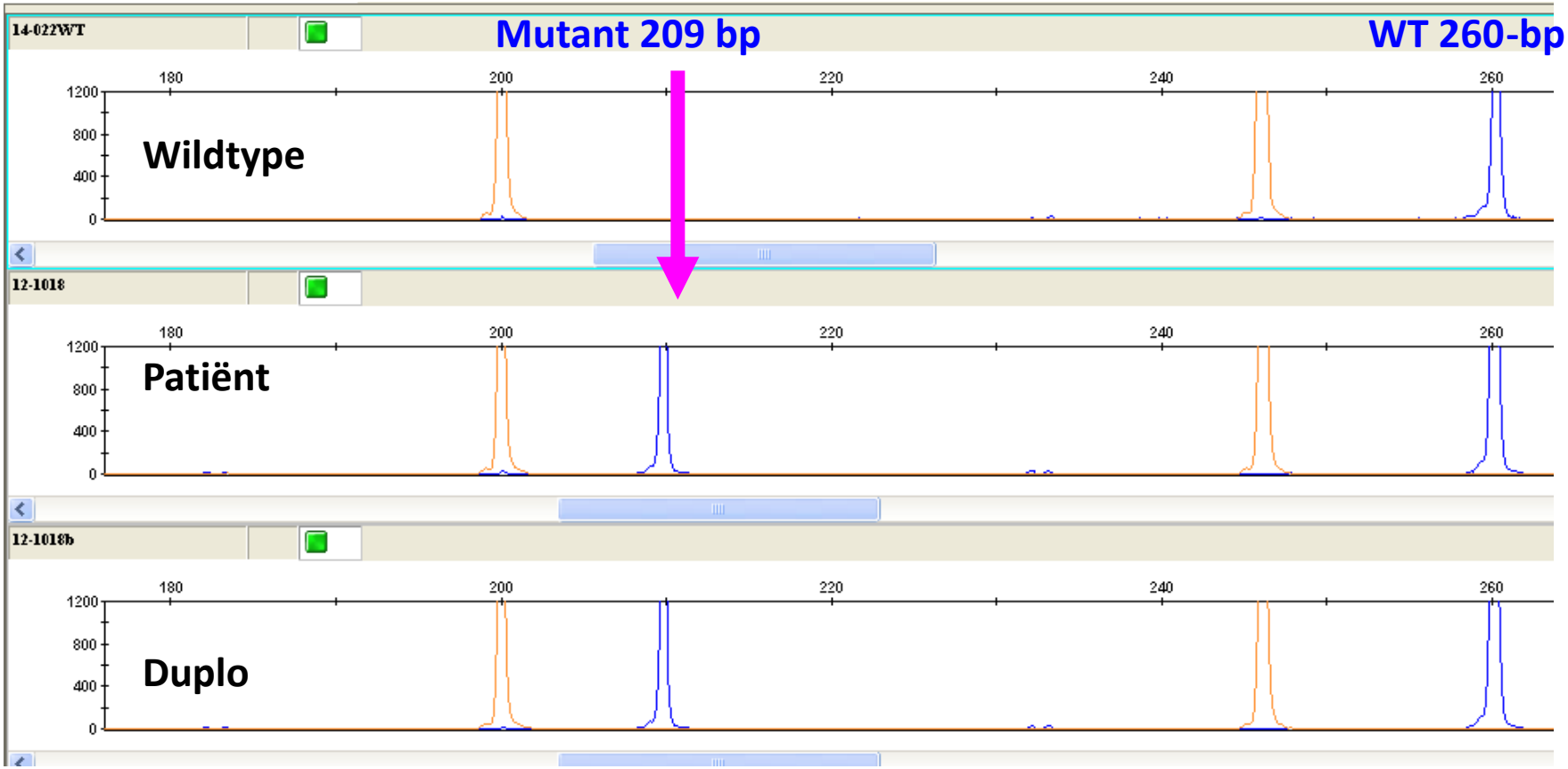
MPN: Myeloproliferative neoplasm; PMF: Primary myelofibrosis; PV: Polycythaemia vera; ET: Essential thrombocythaemia



Minder transformatie naar myelofibrose dan bij JAK2 V617F

Cancer Discov; 6(4); 344–6.

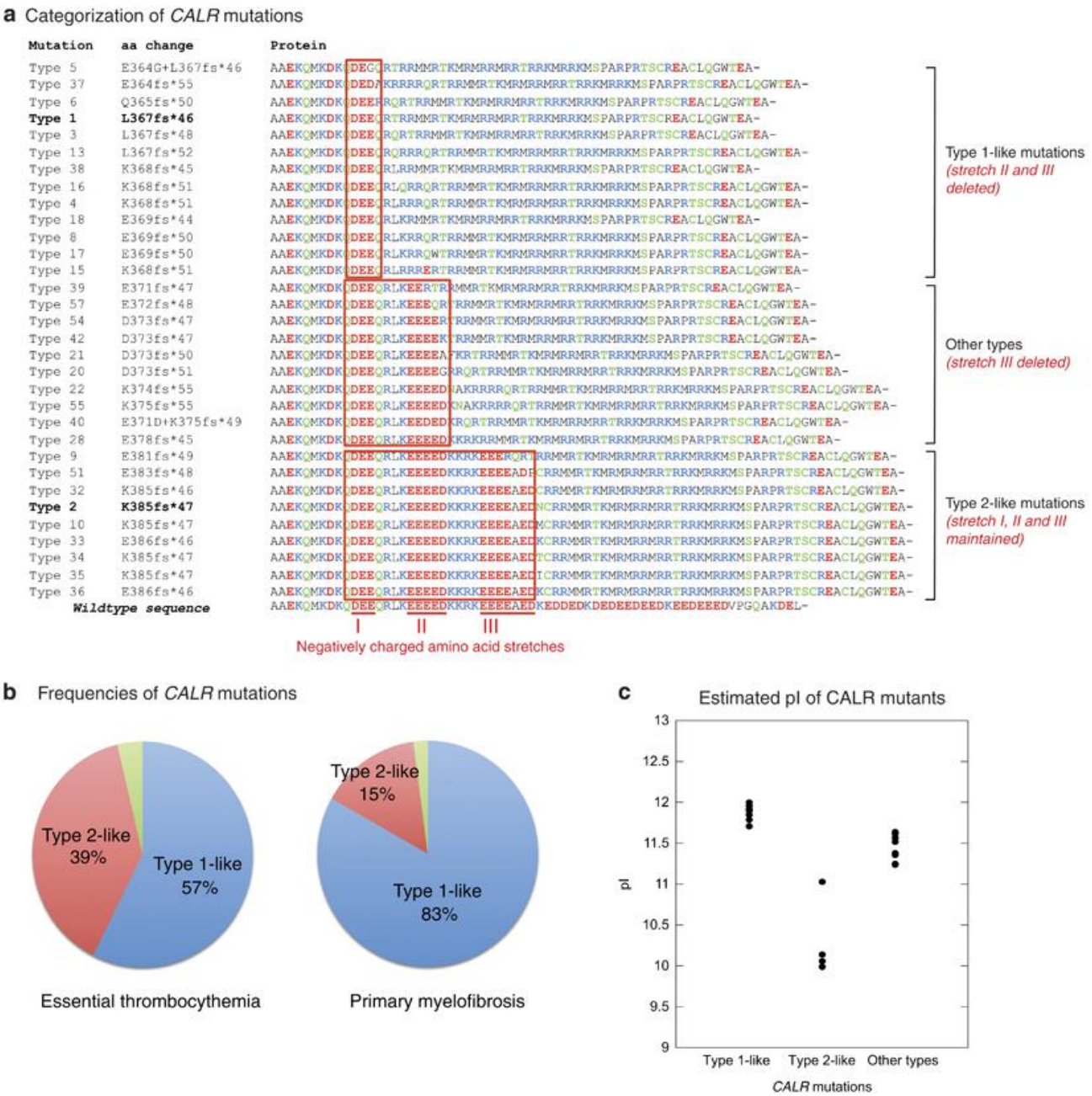
Resultaten: *CALR-mutaties*



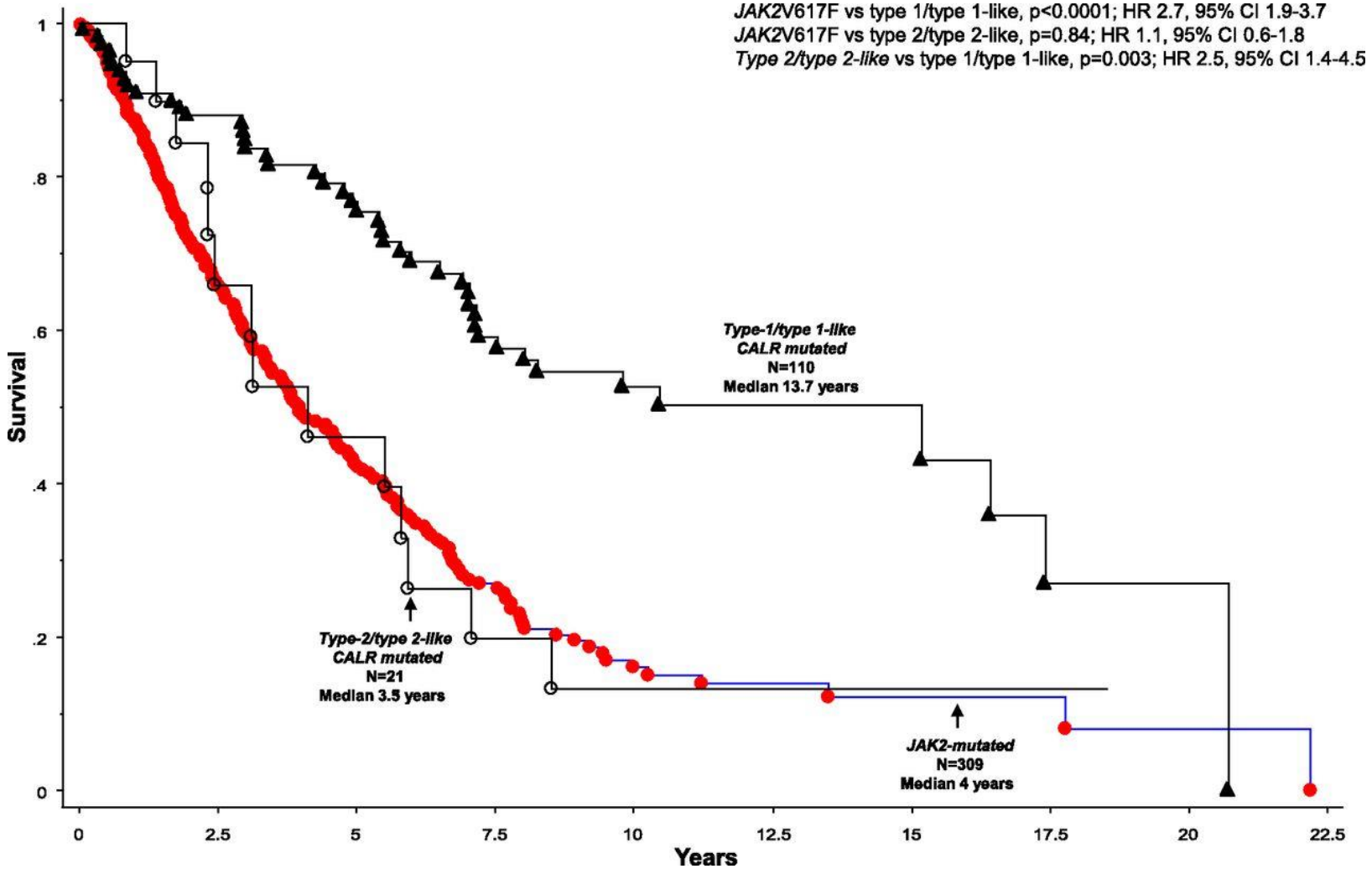
- Type 1 mutatie (52-bp deletie).

CALR-mutaties

- Type 2 mutatie (5-bp insertie).


a Categorization of CALR mutations
Mutation
aa change
Protein
Type 5 E364G+L367fs*46
Type 37 E364fs*55
Type 6 Q365fs*50
Type 1 L367fs*46
Type 3 L367fs*48
Type 13 L367fs*52
Type 38 K368fs*45
Type 16 K368fs*51
Type 4 K368fs*51
Type 18 E369fs*44
Type 8 E369fs*50
Type 17 E369fs*50
Type 15 K368fs*51
Type 39 E371fs*47
Type 57 E372fs*48
Type 54 D373fs*47
Type 42 D373fs*47
Type 21 D373fs*50
Type 20 D373fs*51
Type 22 K374fs*55
Type 55 K375fs*55
Type 40 E371D+K375fs*49
Type 28 E378fs*45
Type 9 E381fs*49
Type 51 E383fs*48
Type 32 K385fs*46
Type 2 K385fs*47
Type 10 K385fs*47
Type 33 E386fs*46
Type 34 K385fs*47
Type 35 K385fs*47
Type 36 E386fs*46
Wildtype sequence
I
II
III
Negatively charged amino acid stretches
Type 1-like mutations
(stretch II and III deleted)
Other types
(stretch III deleted)
Type 2-like mutations
(stretch I, II and III maintained)
b Frequencies of CALR mutations
Type 2-like 39%
Type 1-like 57%
Essential thrombocythemia
Type 2-like 15%
Type 1-like 83%
Primary myelofibrosis
c Estimated pI of CALR mutants
pI
13
12.5
12
11.5
11
10.5
10
9.5
9
Type 1-like
Type 2-like
Other types
CALR mutations

Survival data on 440 patients with PMF stratified by their JAK2 and CALR mutational status.



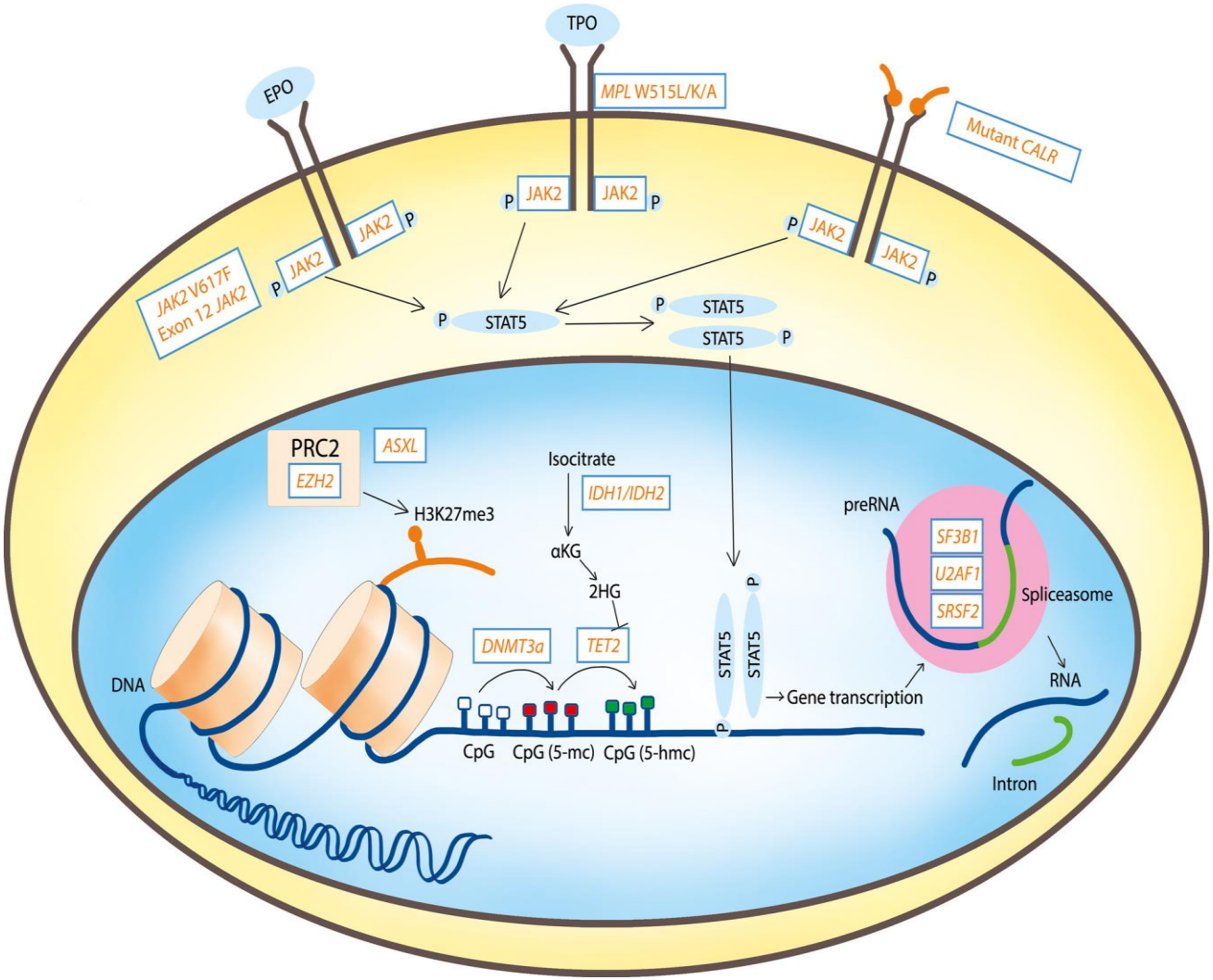
Ayalew Tefferi et al. Blood 2014;124:2465-2466

Mutaties bij PV/ET/PMF

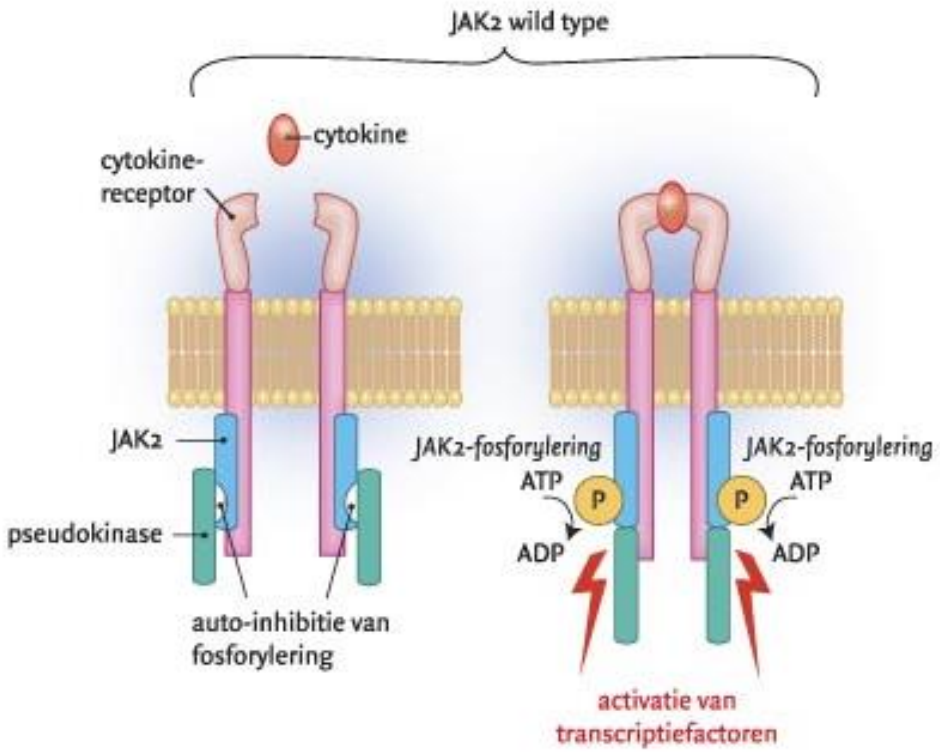
- **JAK en CALR mutaties zijn vrijwel “mutually exclusive”**

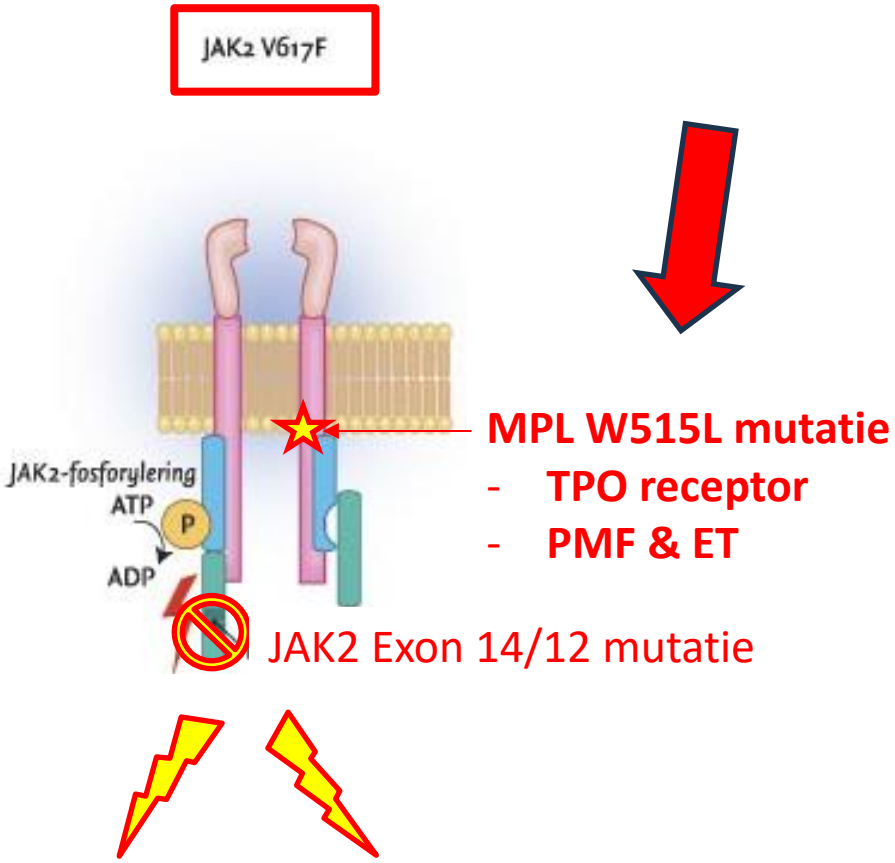
- **Kunnen toch beide mutaties bij een patient aanwezig zijn??**

MPN


TPO
EPO
MPL W515L/K/A
Mutant CALR
JAK2 V617F
Exon 12 JAK2
JAK2
P
STAT5
PRC2
EZH2
ASXL
H3K27me3
Isocitrate
IDH1/IDH2
αKG
2HG
DNMT3a
TET2
DNA
CpG
CpG (5-mc)
CpG (5-hmc)
Gene transcription
preRNA
SF3B1
U2AF1
SRSF2
Spliceosome
RNA
Intron

Moleculaire oorzaak PV en ET



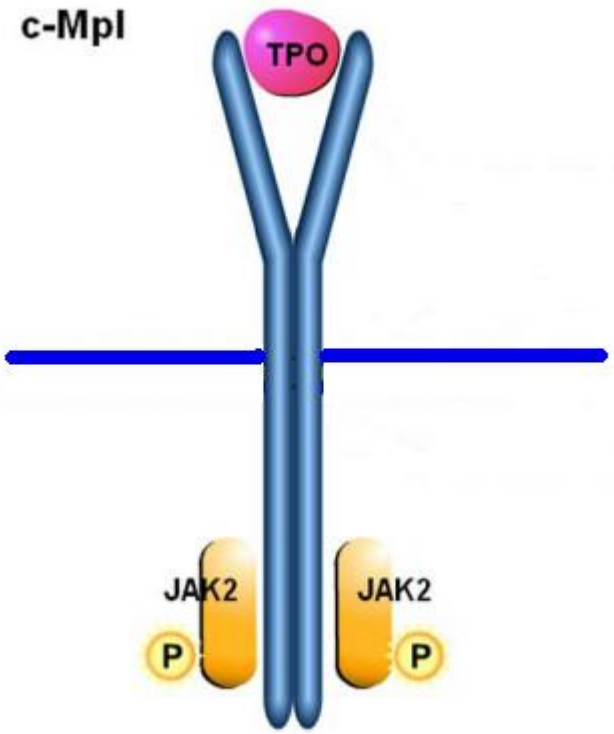


PV (**Epo-receptor**):
- erytrocytose
- Trombocytose
- Leukocytose

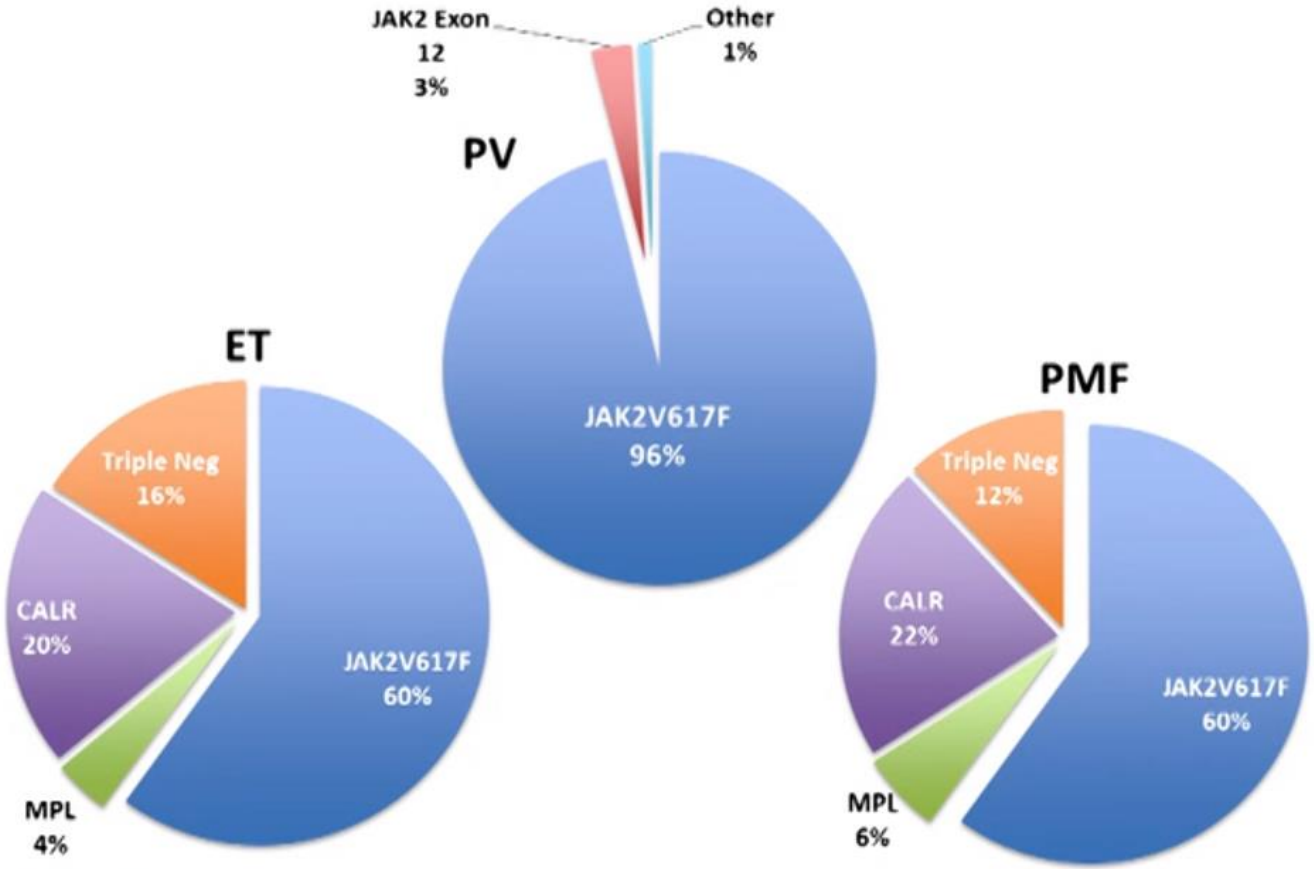
ET/MF (**TPO-receptor**):
- Trombocytose
- dysmegakaryopoiese

Het *MPL*-gen

- **Myeloproliferatief leukemie virus oncogen**
- Codeert voor de Trombopoïetine (TPO) receptor
    - Groeifactorreceptor voor de megakaryopoïese
    - Essentieel voor de productie van trombocyten
- Korte arm chromosoom 1 (1p34)
- Bestaat uit 12 exonen



Prevalence of driver mutations in MPN patients

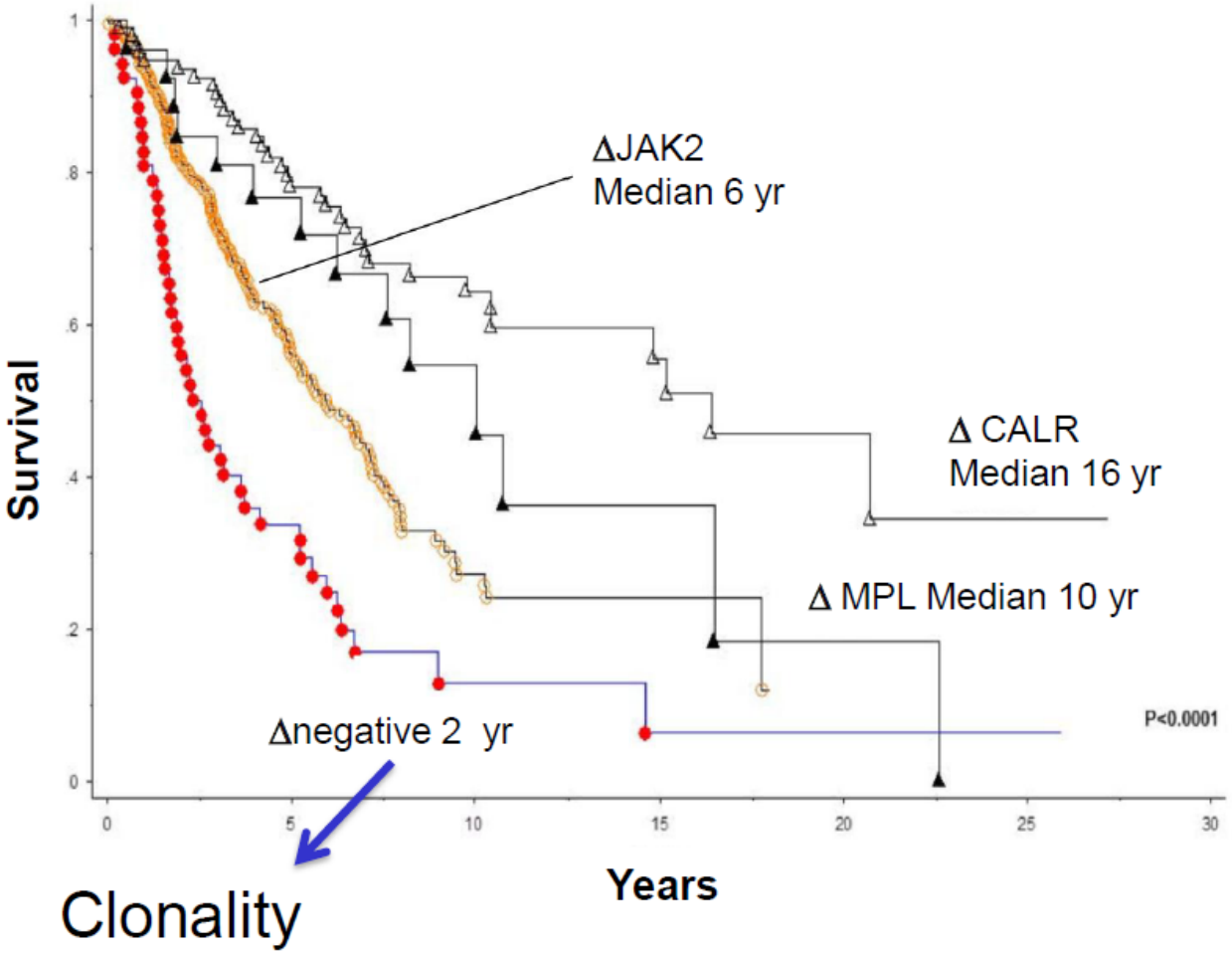


Unpublished data from Prof. J-J Kiladjian

MPN: Myeloproliferative neoplasm; PMF: Primary myelofibrosis; PV: Polycythaemia vera; ET: Essential thrombocythaemia

MODHEM

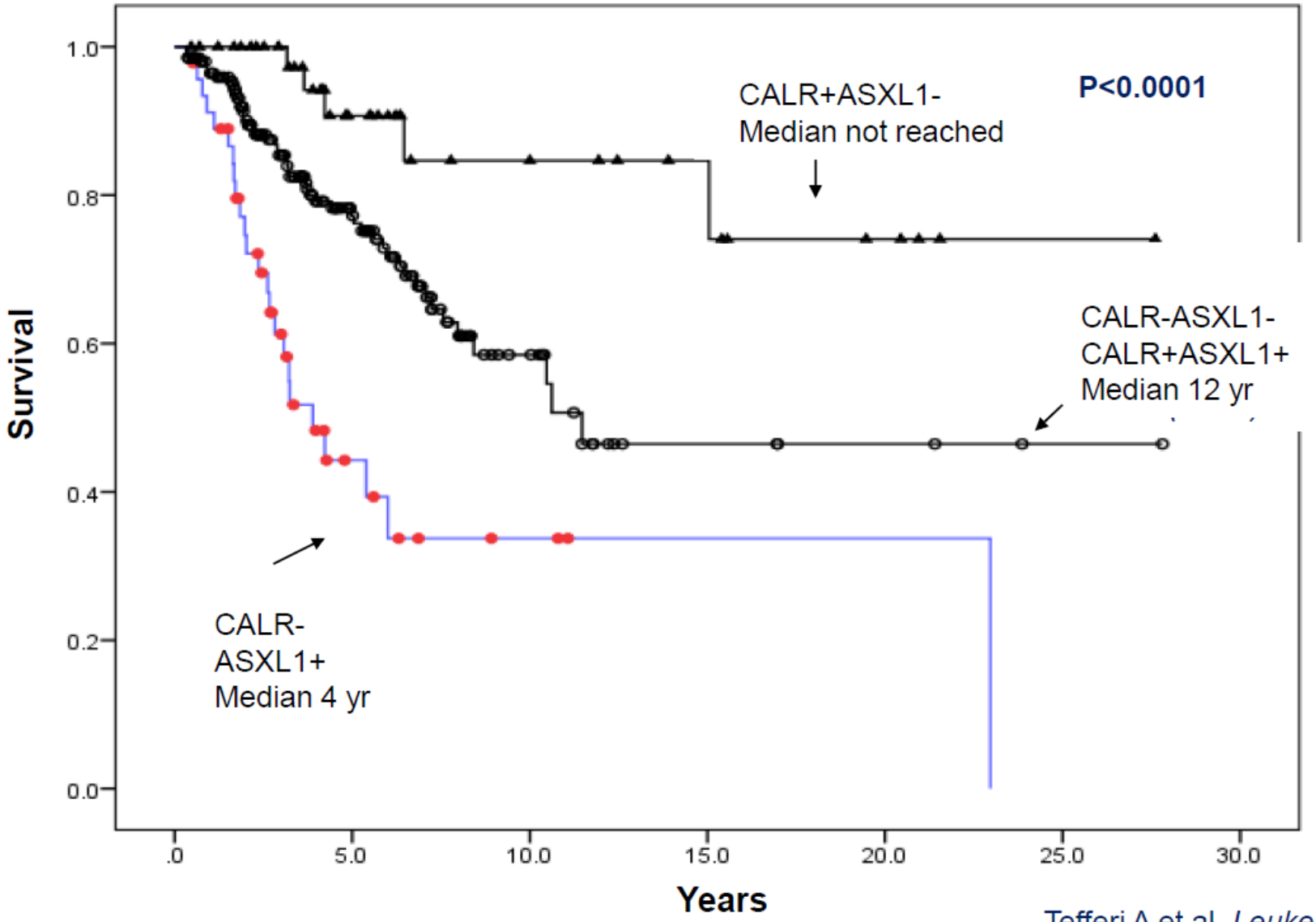
CALR best survival PMF



Clonality

skml

ASXL1 mutations: poor survival PMF



Tefferi A et al. *Leukemia* 2014

Mutation and karyotype-enhanced International Prognostic Scoring System **for transplant-age** patients ≤70 year (**MIPSS70**)

- **3 genetische variabelen:**
  - Afwezigheid van CALR type-1/like mutatie (**1**)
  - Aanwezigheid 1 hoog risico mutatie; ***ASXL1, SRSF2, EZH2, IDH1* or *IDH2* *(1)***
  - *Aanwezigheid van 2 of meer hoog risico mutaties (**2**)*
- **6 klinische variabelen:**
  - Hb < 10g/dL (1)
  - WBC > 25 x 10E9/L (2)
  - PLT < 100 x 10E9/L (2)
  - PB blasten > 1% (1)
  - BM fibrose > graad (1)
  - Constitutionele symptomen (1)

- MIPSS70 Low: ≤ 1 punt: median survival ranges of **27.7 years—'not reached**
- MIPSS70 intermediate: 2-4 punten: **6.3-7.1 years**
- MIPSS70 high: ≥ 5 punten: **2.3-3.1 years**

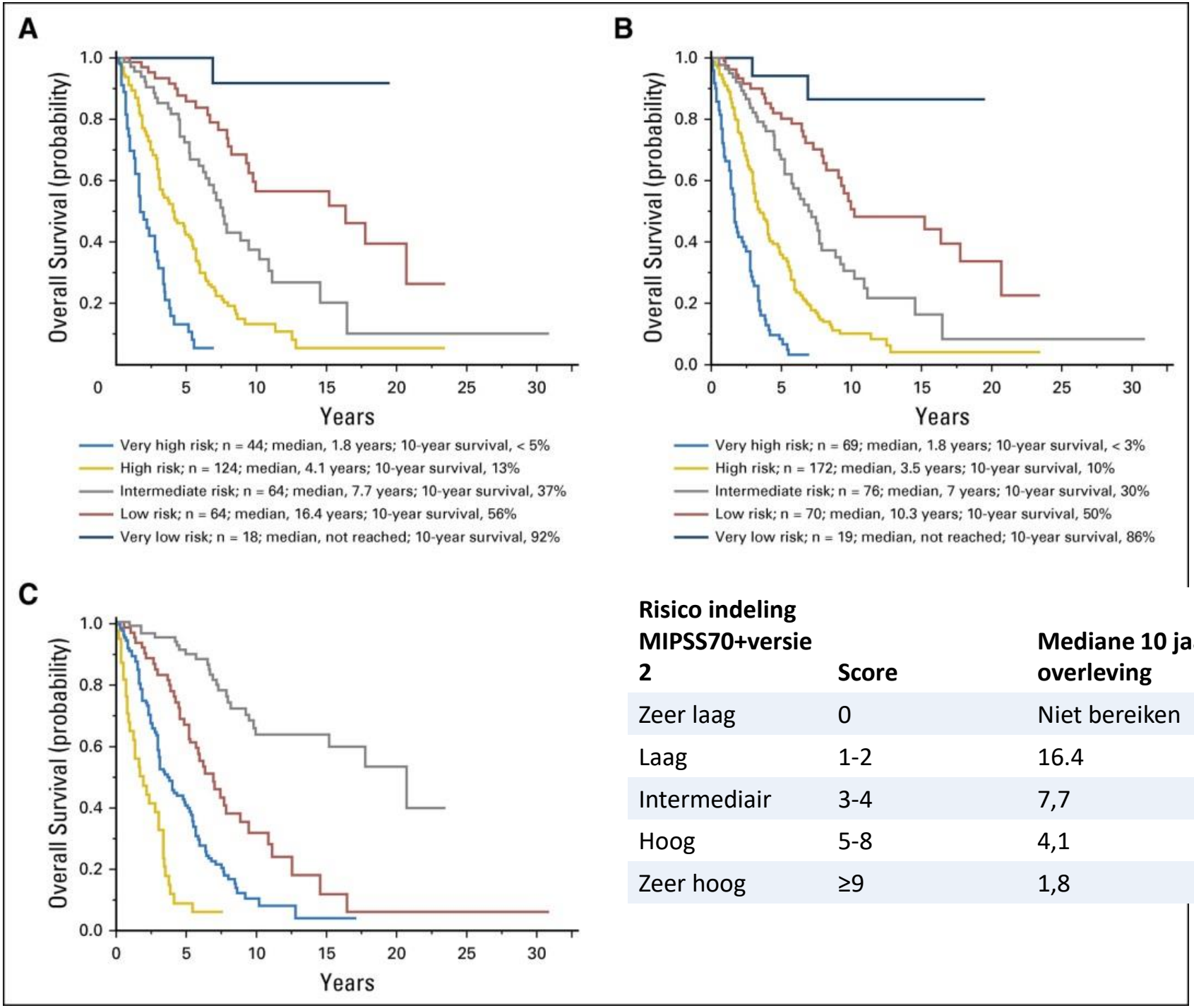
J Clin Oncol. 2018;36:310

Mutation and karyotype-enhanced International Prognostic Scoring System **for transplant-age** patients ≤70 year (**MIPSS70+, versie 2**)

MIPSS70+, versie 2:

- MIPSS70
- ***extra 5 cytogenetische risico variabelen***
- ***U2AF1* Q157**
- sex- and severity-adjusted hemoglobin thresholds
- 5 risico groepen

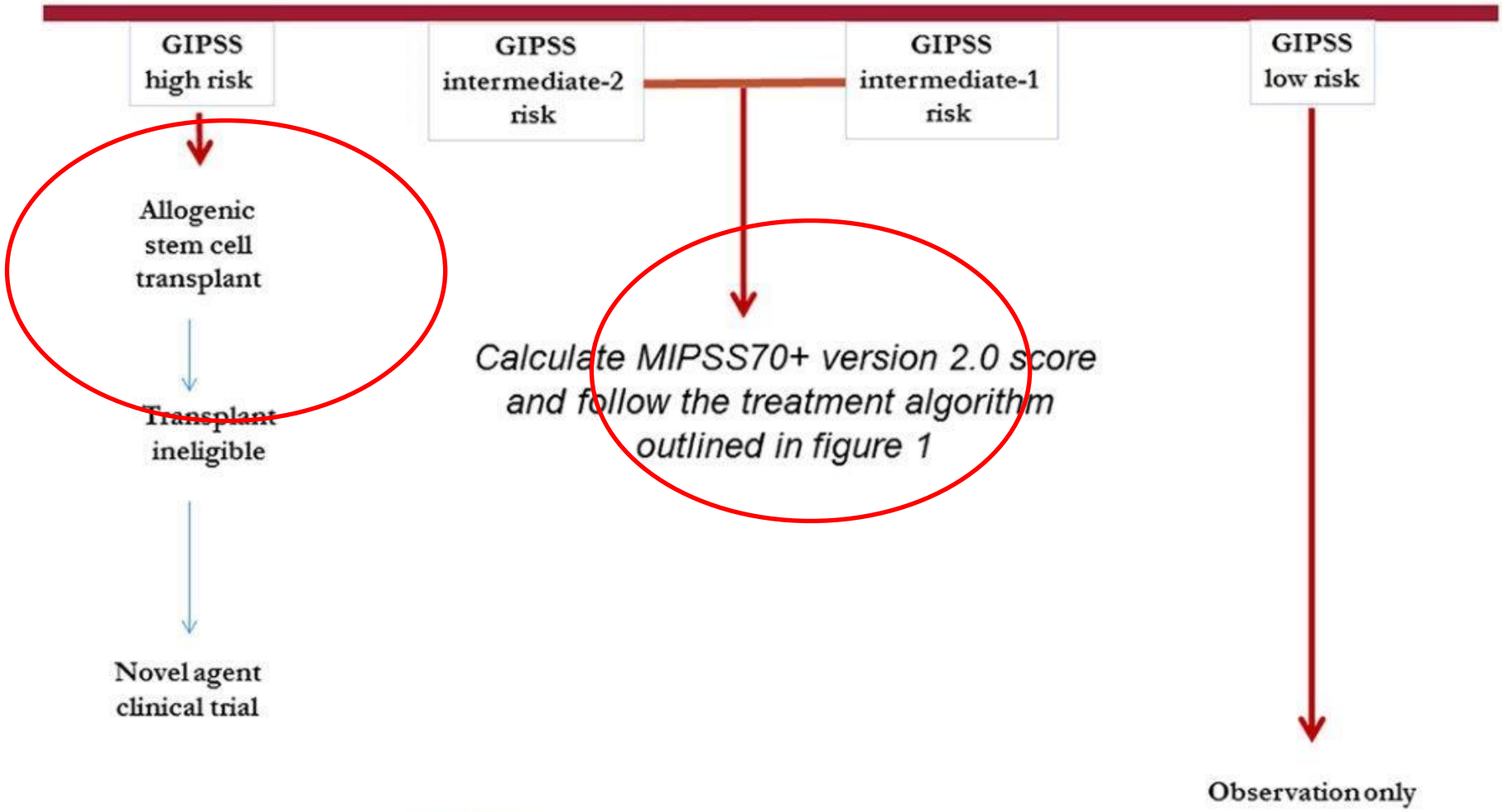
J Clin Oncol. 2018. doi:10.1200/JCO.2018.78.9867)



Risico indeling MIPSS70+versie 2	Score	Mediane 10 jaar overleving
Zeer laag	0	Niet bereiken
Laag	1-2	16.4
Intermediair	3-4	7,7
Hoog	5-8	4,1
Zeer hoog	≥9	1,8

Treatment Algorithm in Myelofibrosis

based on GIPSS (genetically-inspired international prognostic scoring system)



(Leukemia. 2018 Mar 23. doi: 10.1038/s41375-018-0107-z)

http://www.mipss70score.it/

WHO 2022

Acute myeloid leukaemia with defining genetic abnormalities
Acute promyelocytic leukaemia with *PML*::*RARA* fusion
Acute myeloid leukaemia with *RUNX1*::*RUNX1T1* fusion
Acute myeloid leukaemia with *CBFB*::*MYH11* fusion
Acute myeloid leukaemia with *DEK*::*NUP214* fusion
Acute myeloid leukaemia with *RBM15*::*MRTFA* fusion
Acute myeloid leukaemia with *BCR*::*ABL1* fusion
Acute myeloid leukaemia with *KMT2A* rearrangement
Acute myeloid leukaemia with *MECOM* rearrangement
Acute myeloid leukaemia with *NUP98* rearrangement
Acute myeloid leukaemia with *NPM1* mutation
Acute myeloid leukaemia with *CEBPA* mutation
Acute myeloid leukaemia, myelodysplasia-related
Acute myeloid leukaemia with other defined genetic alterations
Acute myeloid leukaemia, defined by differentiation
Acute myeloid leukaemia with minimal differentiation
Acute myeloid leukaemia without maturation
Acute myeloid leukaemia with maturation
Acute basophilic leukaemia
Acute myelomonocytic leukaemia
Acute monocytic leukaemia
Acute erythroid leukaemia
Acute megakaryoblastic leukaemia

ICC-MLN 2022

AML and related neoplasms
AML with recurrent genetic abnormalities (requiring ≥10% blasts in BM or PB)[a]
• APL with t(15;17)(q24.1;q21.2)/*PML*::*RARA*[b]
• AML with t(8;21)(q22;q22.1)/*RUNX1*::*RUNX1T1*
• AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22)/*CBFB*::*MYH11*
• AML with t(9;11)(p21.3;q23.3)/*MLLT3*::*KMT2A*[c]
• AML with t(6;9)(p22.3;q34.1)/*DEK*::*NUP214*
• AML with inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2)/*GATA2*, *MECOM*(*EVI1*)[d]
• AML with other rare recurring translocations[e]
• AML with mutated *NPM1*
• AML with in-frame bZIP mutated *CEBPA*[f]
• AML with t(9;22)(q34.1;q11.2)/*BCR*::*ABL1*[a]
Categories designated AML (if ≥20% blasts in BM or PB) or MDS/AML (if 10-19% blasts in BM or PB)
• AML with mutated *TP53*[g]
• AML with myelodysplasia-related gene mutations Defined by mutations in *ASXL1*, *BCOR*, *EZH2*, *RUNX1*, *SF3B1*, *SRSF2*, *STAG2*, *U2AF1*, or *ZRSR2*
• AML with myelodysplasia-related cytogenetic abnormalities[h]
• AML not otherwise specified (NOS)

Veranderingen in ELN risico classificatie AML

ELN 2017

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 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 genetic lesions)
	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*#

ELN 2022: ELN 2017 + 7 genen!

Risk Category[b]	Genetic Abnormality
Favorable	• t(8;21)(q22;q22.1)/*RUNX1*::*RUNX1T1*[b,c] • inv(16)(p13.1q22) or t(16;16)(p13.1;q22)/*CBFB*::*MYH11*[b,c] • Mutated *NPM1*[b,d] without *FLT3*-ITD • bZIP in-frame mutated *CEBPA*[e]
Intermediate	• Mutated *NPM1*[b,d] with *FLT3*-ITD • Wild-type *NPM1* with *FLT3*-ITD • t(9;11)(p21.3;q23.3)/*MLLT3*::*KMT2A*[b,f] • Cytogenetic and/or molecular abnormalities not classified as favorable or adverse
Adverse	• t(6;9)(p23;q34.1)/*DEK*::*NUP214* • t(v;11q23.3)/*KMT2A*-rearranged[g] • t(9;22)(q34.1;q11.2)/*BCR*::*ABL1* • t(8;16)(p11;p13)/*KAT6A*::*CREBBP* • inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2)/*GATA2*, *MECOM(EVI1)* • t(3q26.2;v)/*MECOM(EVI1)*-rearranged • -5 or del(5q); -7; -17/abn(17p) • Complex karyotype,[h] monosomal karyotype[i] • Mutated *ASXL1*, *BCOR*, *EZH2*, *RUNX1*, *SF3B1*, *SRSF2*, *STAG2*, *U2AF1*, or *ZRSR2*[j] • Mutated *TP53*[k]

MDS in ICC

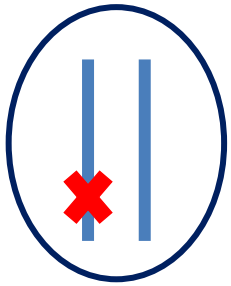
	Dysplastic lineages	Cytopenias	Cytoses*	BM and PB Blasts	Cytogenetics[b***]	Mutations
MDS with del(5q) [MDS del(5q)]	Typically ≥1[c]	≥1	Thrombocytosis allowed	<5% BM <2% PB[d]	del(5q), with up to 1 additional, except -7/del(7q)	Any, except multihit TP53
MDS with mutated SF3B1 (MDS-SF3B1)	Typically ≥1[c]	≥1	0	<5% BM <2% PB[d]	Any, except isolated del(5q), -7/del(7q), abn3q26.2, or complex	SF3B1 (**≥10% VAF**), without multi-hit TP53, or RUNX1
MDS with mutated TP53	Typically ≥1[c]	Any	0	0-9% BM, 0-9% PB	Complex karyotype often with loss of 17p when combined with TP53m (VAF>10%)	**Multi-hit TP53 mutation, or TP53 mutation (VAF >10%) and complex karyotype often with loss of 17p**
MDS, NOS – without dysplasia	**0**	≥1	0	<5% BM <2% PB[d]	-7/del(7q) or complex	**Any, except multihit TP53 or SF3B1 (≥10% VAF)**
MDS, NOS - with single lineage dysplasia	**1**	≥1	0	<5% BM <2% PB[d]	Any, except not meeting criteria for MDS-del(5q)	Any, except multihit TP53;not meeting criteria for MDS-SF3B1
MDS, NOS – with multilineage dysplasia	**≥2**	≥1	0	<5% BM <2% PB[d]	Any, except not meeting criteria for MDS-del(5q)	Any, except multihit TP53; not meeting criteria for MDS-SF3B1
MDS with excess blasts (MDS-EB)	Typically ≥1[c]	≥1	0	5-9% BM, 2-9% PB[d]	Any	Any, except multihit TP53
MDS/AML	Typically ≥1[c]	≥1	0	10-19% BM or Pb[e]	Any, except AML defining[f]	**Any, except NPM1, bZIP CEBPA or TP53**

NGS: Next Generation Sequencing

VAF: Variant allel frequentie

Veel genmutaties in principe **heterozygoot**

→ **1 mutant en 1 wild type allel**



$$VAF = \frac{\text{NGS reads mutatie}}{\text{NGS reads mutatie + NGS reads wild type}} \text{ X 100\% = VAF (\%)}$$

VAF 50% heterozygote mutatie in **alle 100%** cellen: kiembaan mutatie

VAF 8%: heterozygote mutatie in 16% van de cellen

Molecular International Prognostic Scoring System for Myelodysplastic Syndromes (IPSS-M)

- **Authors**: Elsa Bernard, Ph.D. bernare2@mskcc.org, Heinz Tuechler, Peter L. Greenberg, M.D., Robert P. Hasserjian, M.D., Juan E. Arango Ossa, M.S., Yasuhito Nannya, M.D., Ph.D., Sean M. Devlin, Ph.D., **+56**, and Elli Papaemmanuil, Ph.D.
- NEJM Evid 2022;1(7)

https://mds-risk-model.com **Risk Calculator**

- **16 prognostic genes:**
  ASXL1, *CBL*, *DNMT3A*, *ETV6*, *EZH2*, *FLT3*, *IDH2*, *KRAS*, *MLL*^{PTD}, *NPM1*,*NRAS*, *RUNX1*, *SF3B1*^{5q}, *SF3B1*$^{\alpha}$, *SRSF2*, *TP53*^{multihit}, and *U2AF1*

- **15 additional genes:**
  BCOR, *BCORL1*, *CEBPA*, *ETNK1*, *GATA2*, *GNB1*, *IDH1*, *NF1*, *PHF6*, *PPM1D*, *PRPF8*, *PTPN11*, *SETBP1*, *STAG2*, and *WT1*

- **TP53: 1 or ≥ 2**
- **Non-mutated/Mutated/Not assessed**

Moleculaire afwijkingen en clonale evolutie:

AML: 97.3% tenminste 1 mutatie; Patel et al. NEJM. 2012

MDS: 74% tenminste 1 mutatie; Papaemmanuil et al. Blood. 2013
89% tenminste 1 mutatie; Haferlach et al. Leukemia. 2013

Anemia of the elderly: moleculaire afwijkingen: 33%

Ouderen zonder afwijkingen in bloedbeeld: 70-100 jr: 10-20% **CHIP**

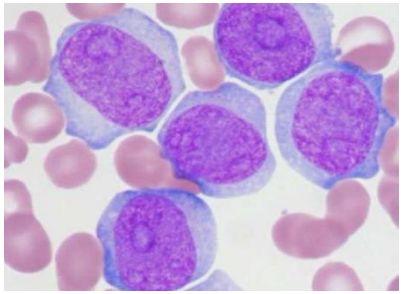
Pathogenesis: Clonal evolution to MDS/AML

Hematopoiesis

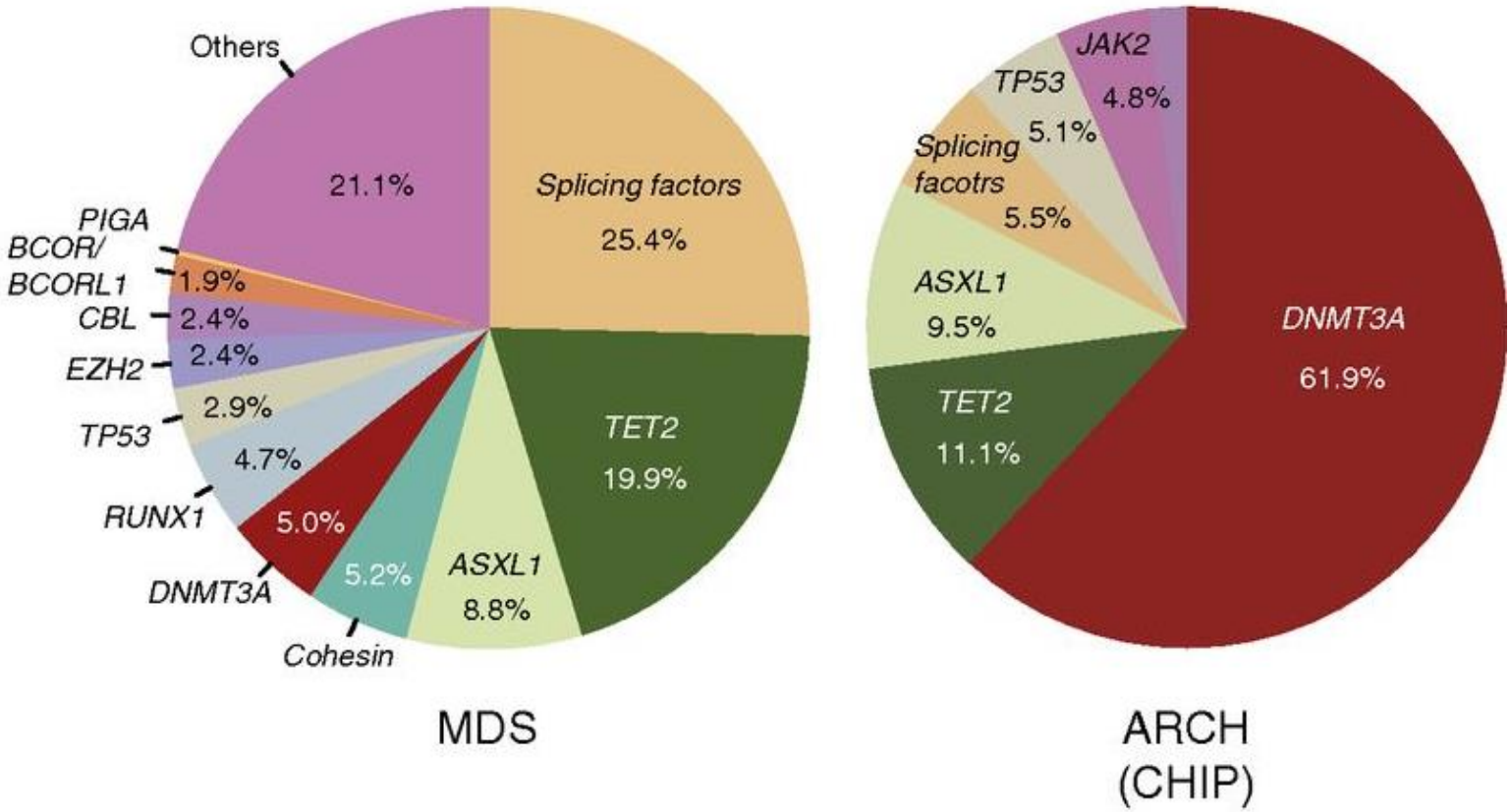
Clonal Hematopoiesis of indeterminate potential

CHIP

Risk about 1% per year



Lindsley et al. Blood 2015; Jaiswal et al. NEJM 2015; Genovese et al. NEJM 2015; Xie et al. Nature Medicine 2014



Seishi Ogawa Blood 2016;128:337-347

Features of CHIP, ICUS, CCUS, and MDS

	Cytopenia/dysplasia	VAF cutoff	Commonly mutated driver genes	Higher risk features
CHIP	**No/**Minimal (<10%)	≥2%/(≥4%)	DNMT3A, TET2, ASXL1, PPM1D, JAK2, ZBTB33, ZNF318, TP53, CBL, GNB1, SF3B1, SRSF2, loss of Y	Mutations in TP53, ASXL1, **JAK2**, SF3B1, SRSF2, U2AF1, or IDH1/IDH2; >1 driver mutations; VAF >10%
ICUS	Yes/Minimal (<10%)	None	None	None
CCUS	**Yes**/Minimal (<10%)	≥2%/(≥4%)	TET2, DNMT3A, ASXL1, SRSF2, ZRSR2, SF3B1, U2AF1, IDH1/2, RUNX1, EZH2, JAK2, CBL, KRAS, CUX1, TP53	Spliceosome gene mutations, DNMT3A, ASXL1, TET2 in comutational patterns (RUNX1, EZH2, CBL, BCOR, CUX1, TP53, or IDH1/IDH2 most specific), >1 driver mutation, VAF > 10%
MDS	Yes/Yes	None	See tekst	See text

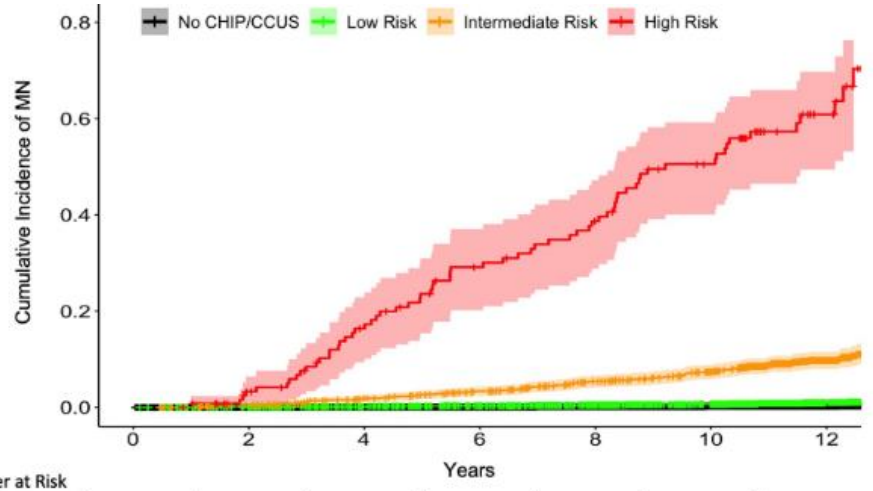
CHIP and development of malignancy

Prognostic variable	0.5	1	1.5	2	2.5
Single DNMT3a	+	-			
High risk mutation		-			+
Mutation number		1		≥2	
Variant allele fraction		<0.2		>0.2	
RDW		<15			≥15
MCV		<100			>100
Cytopenia		CHIP	CCUS		
Age		<65	≥65		

High risk mutations:

- splicing factor mutations: SF3B1, SRSF2, ZRSR2
- AML-like mutations: IDH1, IDH2, RUNX1, FLT-3
- JAK2
- TP53

Weeks L, et al. ASH 2022



Number at Risk

Time(years)	0	2	4	6	8	10	12
High-risk	128	114	94	75	63	46	16
Int-risk	1277	1251	1200	1156	1090	1031	372
Low-risk	9932	9876	9742	9620	9463	9275	3735
No CHIP/CCUS	182406	181674	180407	178734	176174	174455	72254

Risk	Score
High	≥ 12.5
Int	10-12
Low	≤ 9.5

Weeks L, et al. ASH 2022

Casus

- **Man, 1956**
  - **Verwijzer: MDL arts**
  - **VG: sinds 2013 linkszijdige colitus ulcerosa**
  - **2015: actuele klacht: jeuk na douchen**
    - **geen trombose**
    - **geen hoofdpijn**
    - **geen B-symptomen**
  - **LO: rood hoofd**
    - **geen palpabele milt en lever**
    - **geen tekenen van trombosebeen**
    - **ECHO: milt iets vergroot (13,9 cm)**

Casus

- **Lab:**
  - **Cytometrie/Diff:**
    - **Hb 12,5 mmol/L**
    - **MCV 91**
    - **WBC 7.5 x 10^9/L**
    - **PLT 252 x 10^9/L**
    - **Handdiff: normaal**
  - **Overig:**
    - **CRP <5**
    - **LDH normaal**
    - **EPO: 1,2 (4,5 – 19,6 U/L)**

Casus

Beenmergaspiraat:

- Hypercellulair, vlokrijk crista-aspiraat met een actieve, licht afwijkende erytropoiese, geen depotijzer en geen inbouw, normaal actieve, licht afwijkende myelopoiese, actieve, licht afwijkende megakaryopoiese en < 5% blasten.
- Conclusie: actief beenmerg, dd: reactief of beginnend MPN-beeld, mede gezien ijzergebrek is PV niet geheel uitgesloten.

Beenmergbiopt:

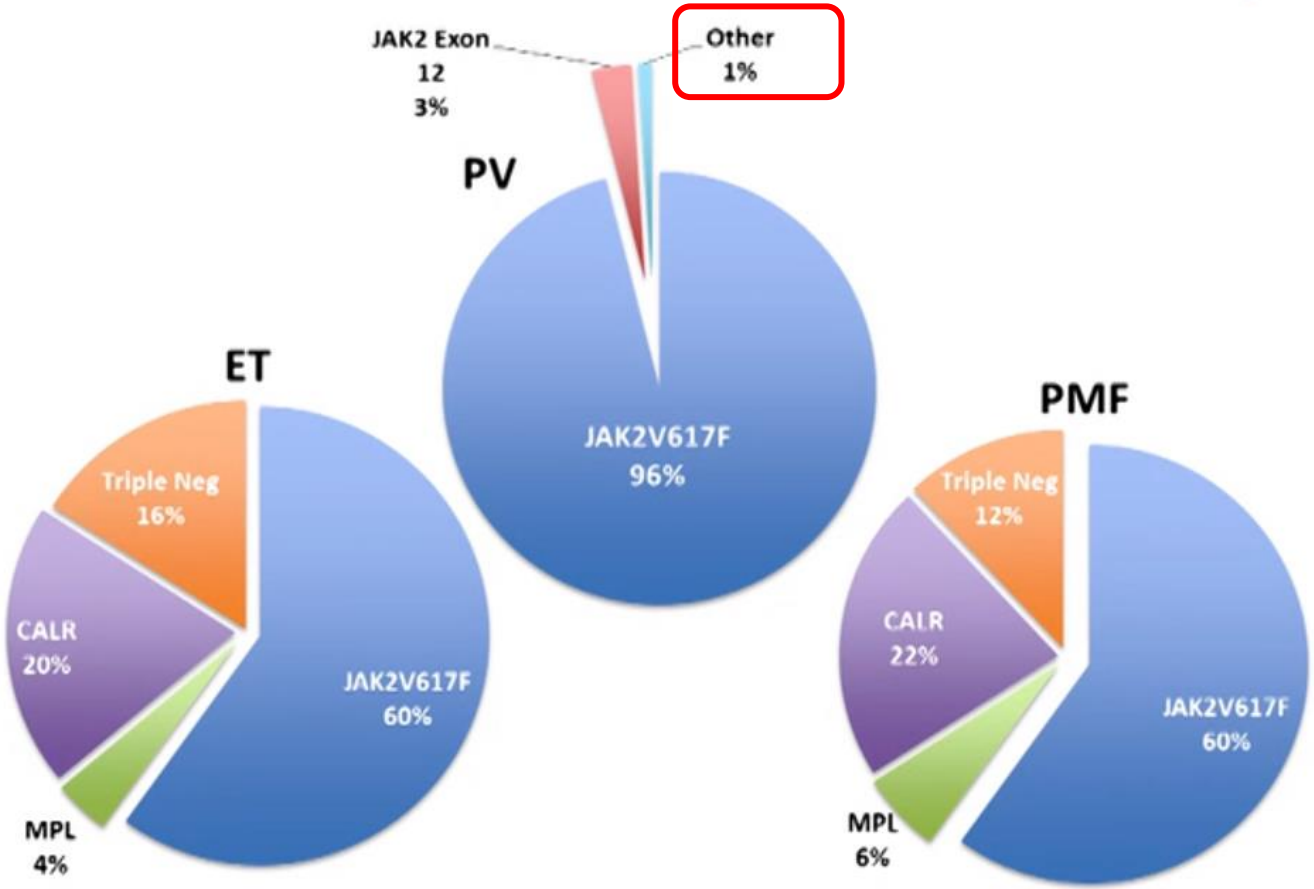
- Normocellulair (!!) beenmerg met toegenomen erytropoiese en megakaryopoiese. Megakaryocyten sporadisch iets groter dan normaal.
- Conclusie: dd: reactief of myeloproliferatief neoplasma

Casus

Aanvullend onderzoek:

- **Erytrocytenvolume: erytrocytenvolume relatief meer toegenomen dan plasmavolume. Beeld kan passen bij PV.**
- **Spontane groei van BFU-E waargenomen.**
- **Cytogenetica:**
  - **46, XY**
- **Moleculaire diagnostiek:**
  - **BCR-ABL niet aangetoond (detectiegrens 1%)**
  - **JAK2V617F niet aangetoond (detectiegrens 0,1%)**
  - **JAK2 exon 12 mutaties niet aangetoond (detectiegrens 10-20%)**
  - **CALR mutaties niet aangetoond (detectiegrens 10-20%)**

Prevalence of driver mutations in MPN patients



Unpublished data from Prof. J-J Kiladjian

MPN: Myeloproliferative neoplasm; PMF: Primary myelofibrosis; PV: Polycythaemia vera; ET: Essential thrombocythaemia

SOPHiA GENETICS®

Next generation Sequencing (NGS): 107 genen (sinds begin 2021)

Myeloid Extended Solution - 98 genes

ABL1
ANKRD26
ASXL1
ASXL2
ATM
ATRX
BCOR
BCORL1
BRAF
BRCC3
CALR
CBL
CBLB
CBLC
CCND2
CDKN2A
CEBPA
CHEK2
CREBBP
CSF3R
CSMD1
CSNK1A1
CTCF
CUX1
DDX41
DHX15
DNMT3A
ELANE
ETNK1
ETV6
EZH2
FANCA
FANCL
FLT3
GATA1
GATA2
GNAS
GNB1
HNRNPK
HRAS
IDH1
IDH2
IKZF1
JAK1
JAK2
JAK3
KDM6A
KIT
KMT2A
KMT2D
KRAS
LUC7L2
MECOM
MET
MPL
MYC
NF1
NOTCH1
NOTCH2
NPM1
NRAS
PAX5
PDGFRA
PHF6
PIGA
PML
PPM1D
PTPN11
RAD21
RAF1
RB1
RBBP6
RPS19
RTEL1
RUNX1
SAMD9
SAMD9L
SBDS
SETBP1
SF3B1
SH2B3
SMC1A
SMC3
SOS1
SRP72
SRSF2
STAG1
STAG2
STAT3
STAT5B
TERC
TERT
TET2
TP53
U2AF1
WT1
ZBTB7A
ZRSR2

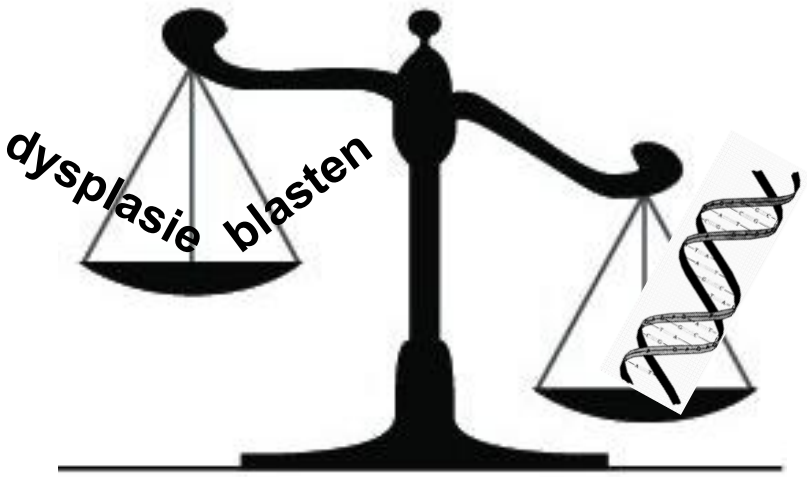
Extra 9 genen:
MYD88, FBXW7, PTEN, NFE2, BIRC3, IL2RG, SAMHD1, TCL1A, UBA1

Casus: PV?

Geen BCR-ABL, JAK2 V617F, CALR mutatie en MPL aangetoond

Gen	cNomen	pNomen	Allelfreq.
JAK2	c.1849G>T	p.V617F	37
JAK2	c.1852T>C	p.C618R	37
ASXL1	c.2344_2350delCATCCGG	p.H782Mfs*34	6
DNMT3A	c.875T>C	p.I292T	6

WHO/ELN/ICC-Myeloid 2022: only molecular characterization matters?



- CH
- CHIP
- CCUS
- CMUS
- CCMUS
- MDS/AML
- VAF%
- TP53