

Genetica in de diagnose en behandeling van leukemie

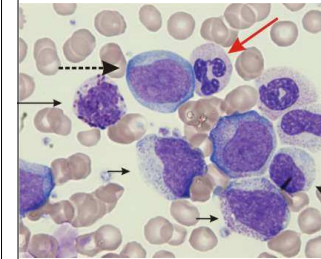
Peter Vandenberghe
Centrum Menselijke Erfelijkheid &
Afdeling Hematologie, UZ Leuven

Casus 1

GV, man, 45 j

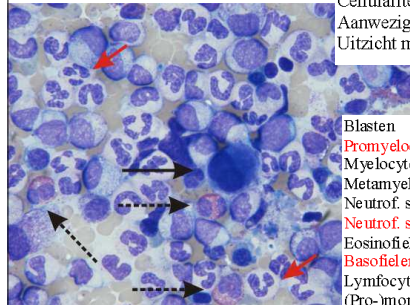
bloed		g/dL	
Hemoglobine	14.2		14.0 - 18.0
Hematocriet	0.425		0.400 - 0.540
RBC telling	4.64	10**12/L	4.50 - 6.00
WBC telling	44.2	10**9/L	4.0 - 10.0
Bloedplaatjes telling	174	10**9/L	150 - 450

Perifeer bloed



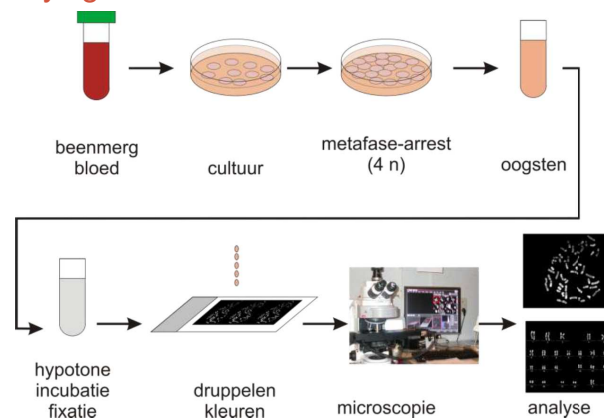
WBC differentiatie m microscopie			
Aantal gedifferentieerde WBC 119			
Promyelocyten %	2	%	≤ 0
Promyelocyten aantal	0.8	10**9/L	
Myelocyten %	8	%	≤ 0
Myelocyten aantal	3.7	10**9/L	
Metamyelocyten %	9	%	≤ 0
Metamyelocyten aantal	4.1	10**9/L	
Neutrofielen %	71	%	38 - 77
Neutrofielen aantal	31.5	10**9/L	2.5 - 7.8
Eosinofielen %	2	%	≤ 6
Eosinofielen aantal	0.8	10**9/L	≤ 0.4
Basofielen %	1	%	≤ 1
Basofielen aantal	0.4	10**9/L	≤ 0.1
Lymfocyten %	3	%	20 - 50
Lymfocyten aantal	1.5	10**9/L	1.2 - 3.6
Monocyten %	3	%	2 - 10
Monocyten aantal	1.5	10**9/L	0.2 - 0.8
Cell morfologie			Hypersegmentatie

Beenmergaspiraats

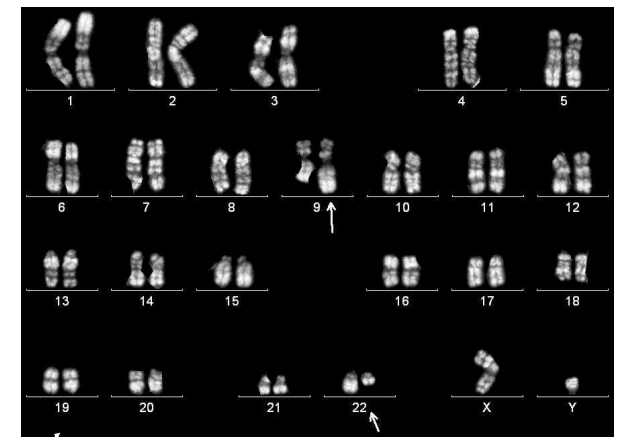


Kenmerken beenmergpreparaat			
Kwaliteit preparaat			goed
Cellulariteit preparaat			hypercellulair
Aanwezigheid mergbrokjes			meerdere
Uitzicht mergbrokjes			hypercellulair
Blasten	1.7 %	0.3 - 2.9	
Promyelocyten	13.0 %	1.7 - 8.4	
Myelocyten	10.7 %	3.3 - 12.0	
Metamyelocyten	7.3 %	3.1 - 10.7	
Neutrof. staafkernigen	5.0 %	5.4 - 16.2	
Neutrof. segmentkernigen	35.3 %	15.1 - 35.0	
Eosinofielen en voorlopers	1.7 %	1.0 - 6.3	
Basofielen en voorlopers	2.0 %	≤ 0.6	
Lymfocyten	3.7 %	6.9 - 24.5	
(Pro-)monocyten	2.3 %	0.4 - 5.2	
Plasmocyten	0.3 %	≤ 2.7	
Vroege erytroblasten	0.3 %	1.1 - 4.0	
Late erytroblasten	16.7 %	15.0 - 35.5	
Verhouding M/E	4.6	1.3 - 4.4	

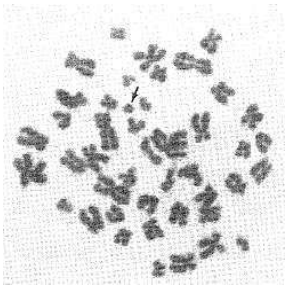
Cytogenetisch onderzoek



46,XY,t(9;22)(q34;q11)



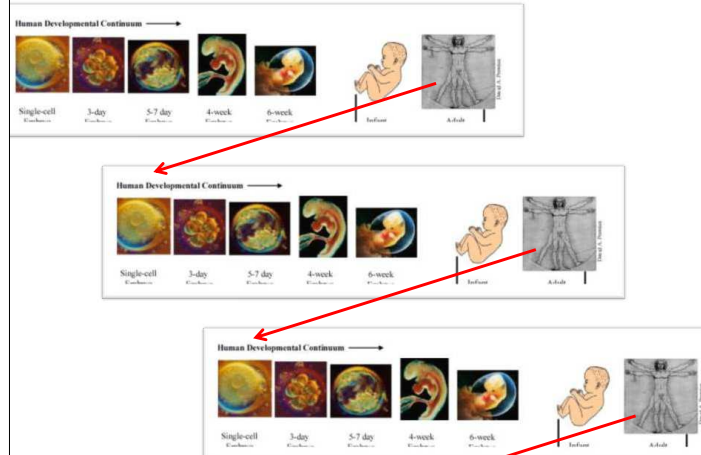
22q-, the *Ph1* chromosome



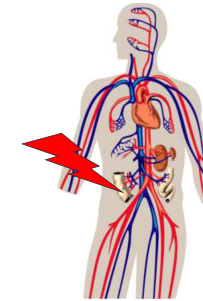
A Minute Chromosome in Human
Chronic Granulocytic Leukemia

Nowell and Hungerford, J Natl Canc Inst, 1960; Science 132, 1960, 1497.

Genetische mutaties in de kiemlijn



Kanker: verworven / somatische mutaties van het genetisch materiaal



Lichaamscellen
(‘somatische cellen’)

Alle dochtercellen van de
getroffen lichaamscel

Niet aangeboren
verworven

Veroudering
kanker

Oncogenen & tumorsuppressorgenen

Oncogenen

“pedalen”
bevorderen groei
overactief in tumoren

1 “hit” volstaat

Tumorsuppressorgenen

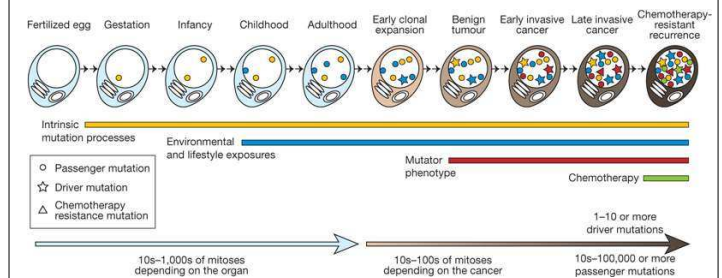
“remmen”
remmen groei
inactief in tumoren

2 “hits” nodig voor inactivering

Cellulair antwoord op DNA-schade

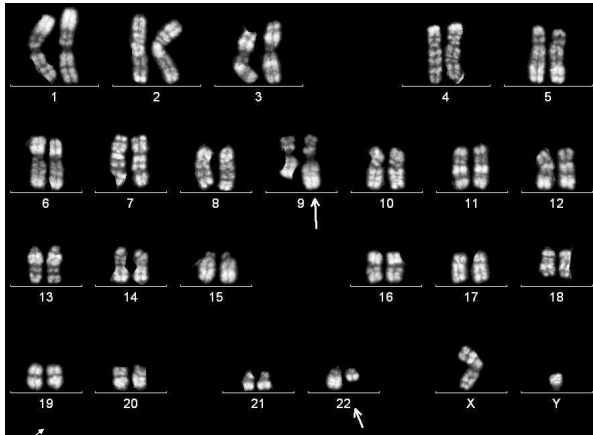


Kankercellen dragen verworven mutaties

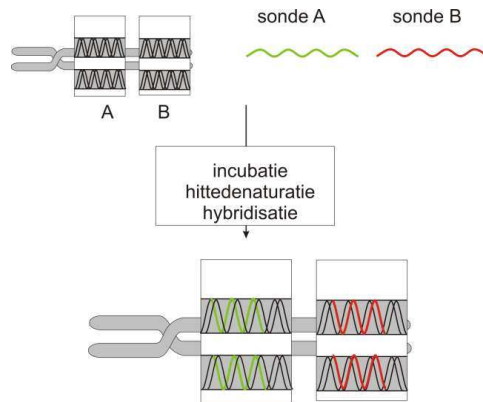


MR Stratton et al. Nature 458, 719-724 (2009)

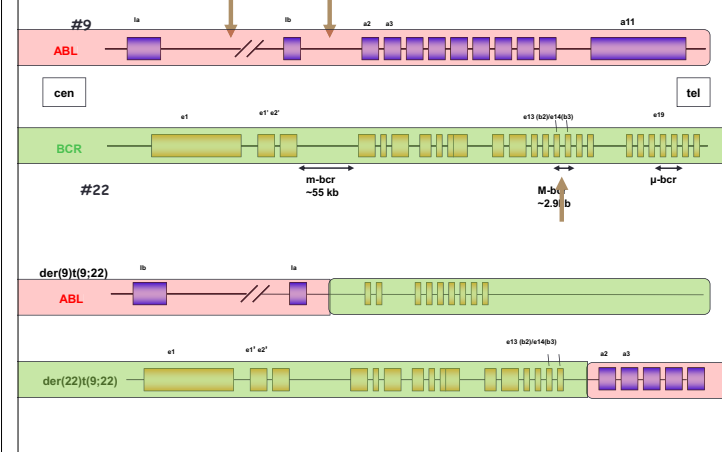
46,XY,t(9;22)(q34;q11)



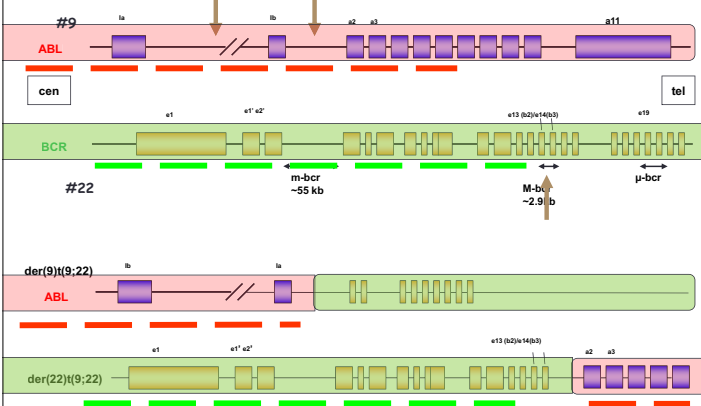
Fluorescence in situ hybridisatie (FISH)



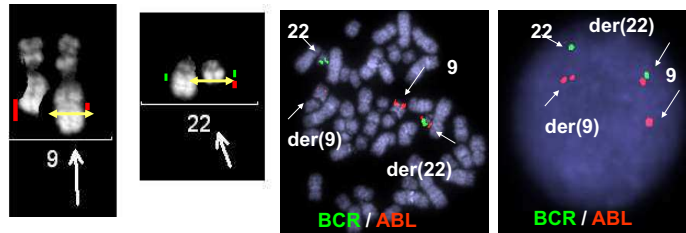
Genomische breukpunten



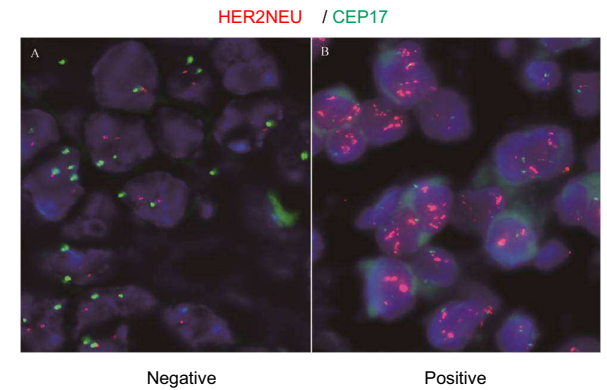
Genomische breukpunten



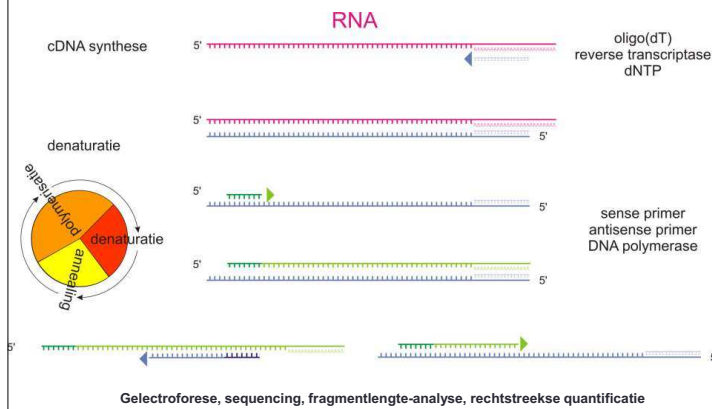
46,XY,t(9;22)(q34;q11)



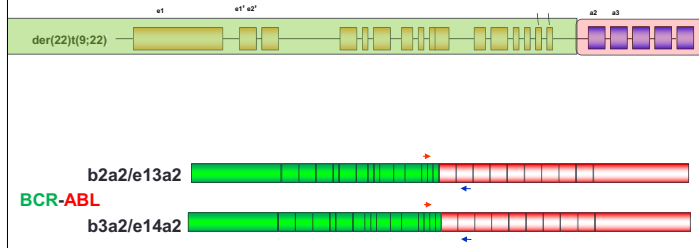
HER2NEU in borstkanker



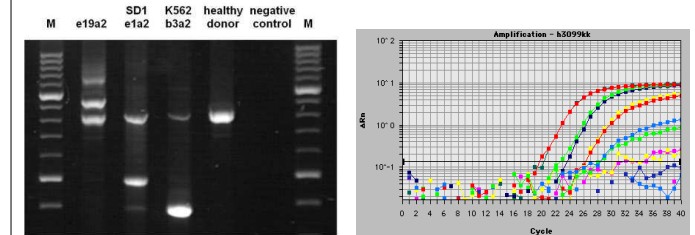
Polymerase chain reaction (PCR)



RT-PCR van fusietranscripten



PCR



Recurrente genetische afwijkingen in kanker :

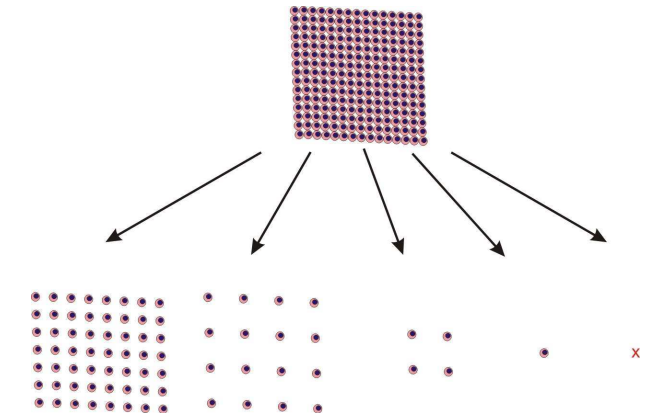
1. targets voor diagnostiek

- cytogenetische afwijking
- FISH
- RT-PCR, RQ-PCR, PCR, ...
- Plaats in
 - Diagnose van een maligne aandoening
 - Opvolging van een maligne aandoening

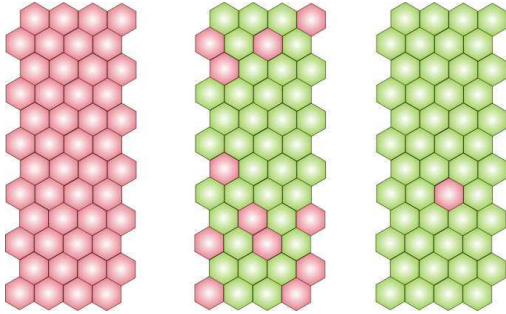
Recurrente genetische afwijkingen in kanker :

2.targets voor therapie

Behandeling van kanker



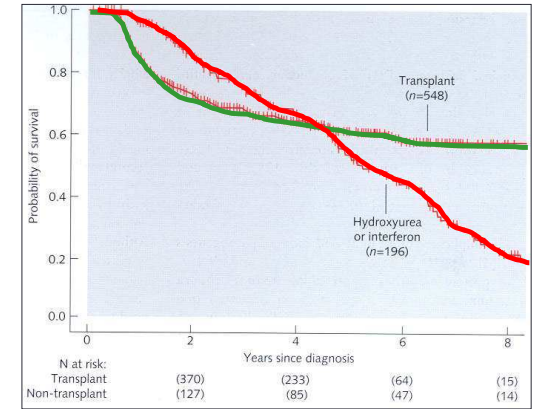
Kanker, mozaïek van kankercellen in achtergrond van normale cellen



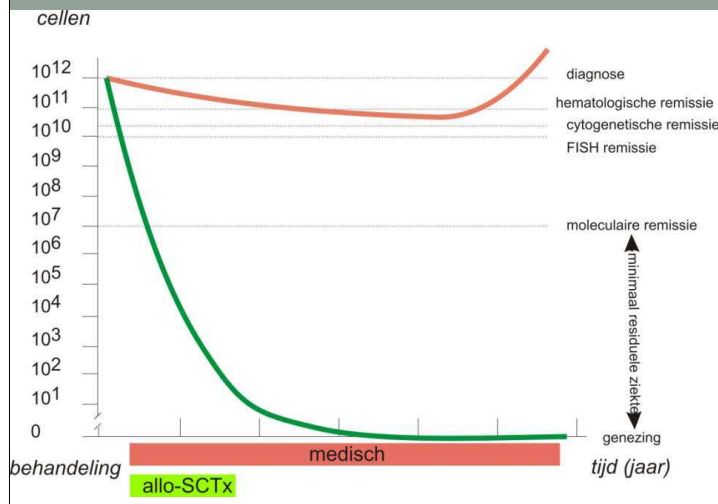
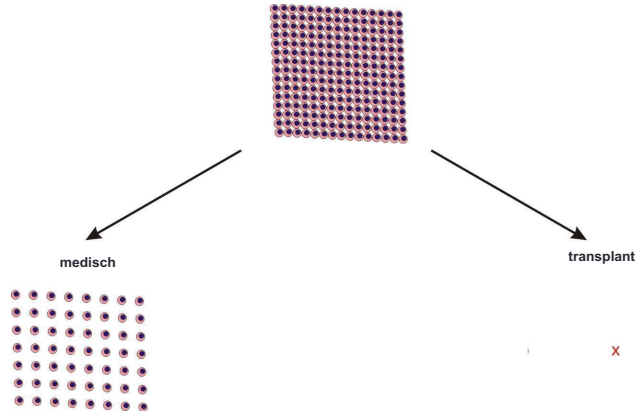
Genetische technieken : gevoeligheid, resolutie, scope

	gevoeligheid	resolutie	scope
Cytogenetica (20 mitosen)	~1-5 %	10 Mb	genoom
FISH (200 kernen)	~1 %	1 Mb	gericht
PCR	0,001 %	1b	gericht

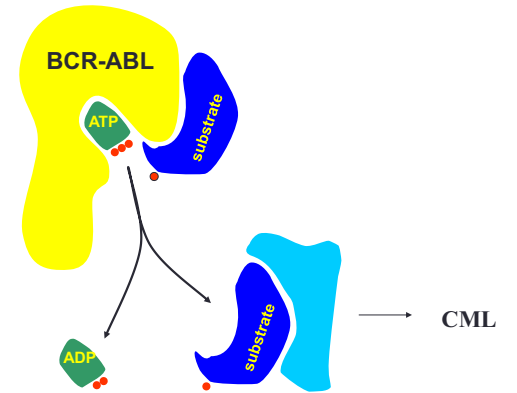
Behandeling van CML : < 2000



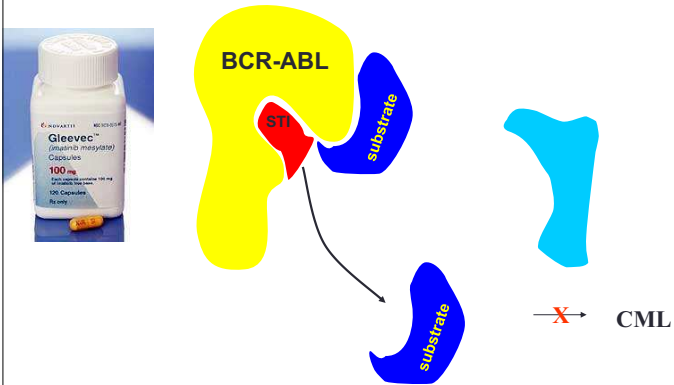
Behandeling van CML voor 2000



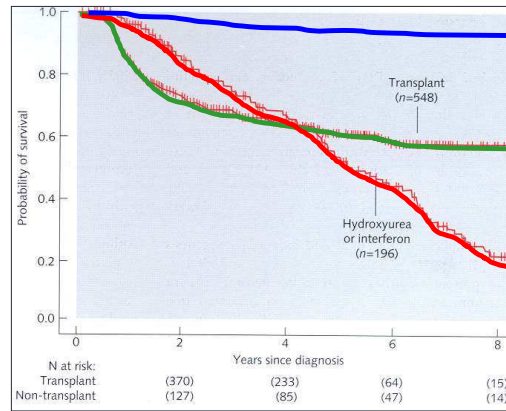
BCR-ABL1, de motor van CML



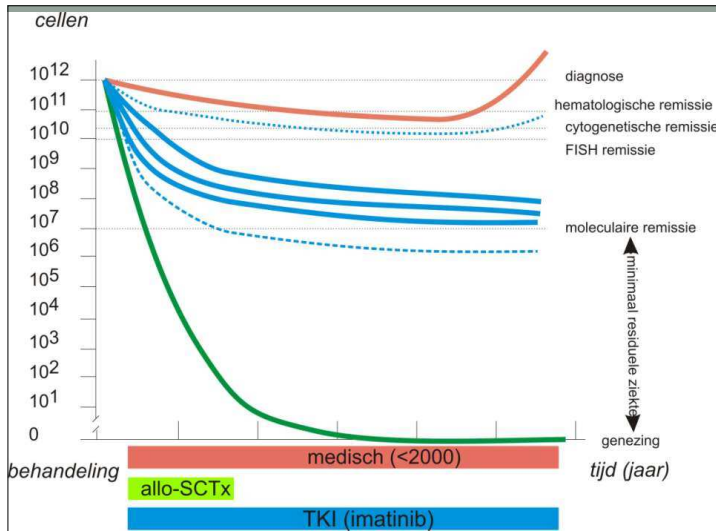
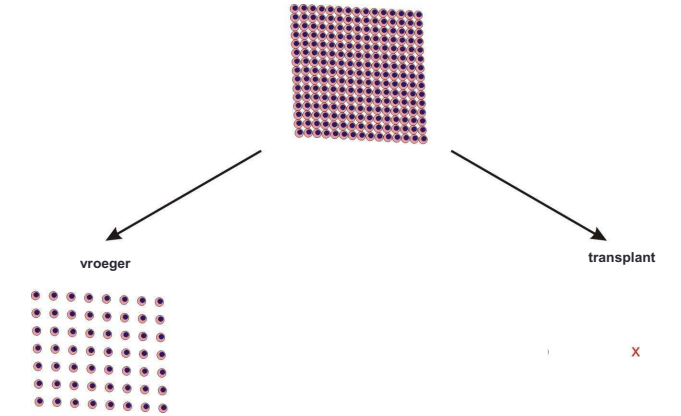
Doelgerichte behandeling van CML : > 2000



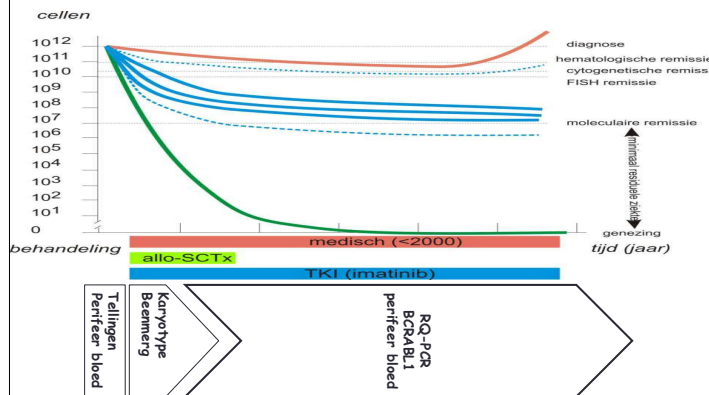
Behandeling van CML : > 2000



Behandeling van CML na 2000



De cytogenetische en moleculaire eenvoud van CML : opvolging



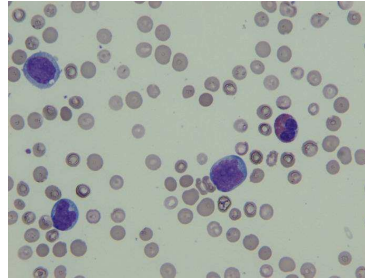
Chronische myeloïde leukemie



Casus 2. Man, 55 j

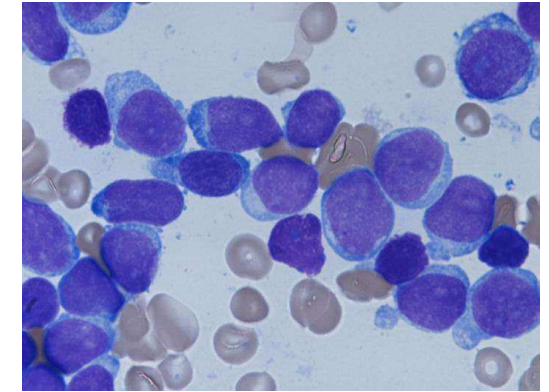
Hemoglobine	8.7	g/dL	13.0 - 16.0
Hematocriet	0.258		0.370 - 0.490
RBC telling	2.63	10**12/L	4.50 - 5.30
MCV	98.1	fL	78.0 - 100.0
MCH	33.1	pg	25.0 - 35.0
MCHC	33.7	g/dL	31.0 - 37.0
RDW (maat voor anisocytose)	17.3	%	11.7 - 14.5
Reticulocyten telling	33	10**9/L	20 - 100
Immature reticulocyten fractie	48.6	%	5.0 - 21.0
Erytroblasten telling	0.0	/ 100 WBC	0.0 - 0.7
Erytroblasten telling	0.0	10**9/L	0.0 - 0.1
Bloedplaatjes telling	106	10**9/L	150 - 450
MPV	10.3	fL	9.0 - 13.0
WBC telling	87.5	10**9/L	4.5 - 13.0

Casus 2 : differentiatie perifeer bloed



Blasten %	66	%
Blasten aantal	57.4	10**9/L
Myelocyten %	3	%
Myelocyten aantal	2.2	10**9/L
Metamyelocyten %	2	%
Metamyelocyten aantal	1.4	10**9/L
Neutrofielen %	6	%
Neutrofielen aantal	5.0	10**9/L
Lymfocyten %	19	%
Lymfocyten aantal	16.5	10**9/L
Monocyten %	6	%
Monocyten aantal	5.0	10**9/L

Casus 2 : beenmergonderzoek



Acute myeloïde leukemie

- Beenmergcytologie
- Beenmergimmunotypering
- Cytogenetisch onderzoek (voorkeur op BM)
- Moleculair onderzoek (BM of bloed)

Classificatie van acute myeloïde leukemie

Acute myeloid leukemia and related neoplasms
 Acute myeloid leukemia with recurrent genetic abnormalities
 AML with t(8;21)(q22;q22); *RUNX1-RUNX1T1*

AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22); *CBFB-MYH11*
 APL with t(15;17)(q22;q12); *PML-RARA*
 AML with t(9;11)(p22;q23); *MLL3-MLL*
 AML with t(6;9)(p23;q34); *DER-NUP214*
 AML with inv(3)(q21q26.2) or t(3;3)(q21;q26.2); *RPN1-EV11*
 AML (megakaryoblastic) with t(1;22)(p13;q13); *RBM15-MKL1*
 Provisional entity: AML with mutated *NPM1*
 Provisional entity: AML with mutated *CEBPA*

Acute myeloid leukemia with myelodysplasia-related changes
 Therapy-related myeloid neoplasms
 Acute myeloid leukemia, not otherwise specified

AML with minimal differentiation
 AML without maturation
 AML with maturation

Acute myelomonocytic leukemia
 Acute monoblastic/monocytic leukemia
 Acute erythroid leukemia
 Pure erythroid leukemia
 Erythroleukemia, erythroid/myeloid

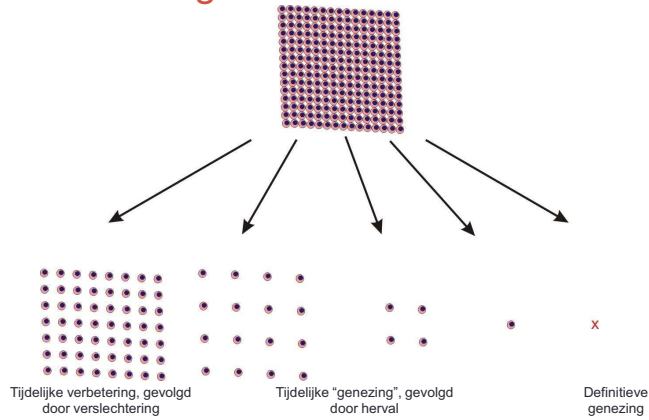
Acute megakaryoblastic leukemia
 Acute basophilic leukemia
 Acute panmyelosis with myelofibrosis
 Myeloid sarcoma
 Myeloid proliferations related to Down syndrome
 Transient abnormal myelopoiesis
 Myeloid leukemia associated with Down syndrome
 Blastic plasmacytoid dendritic cell neoplasm

Acute myeloïde leukemie

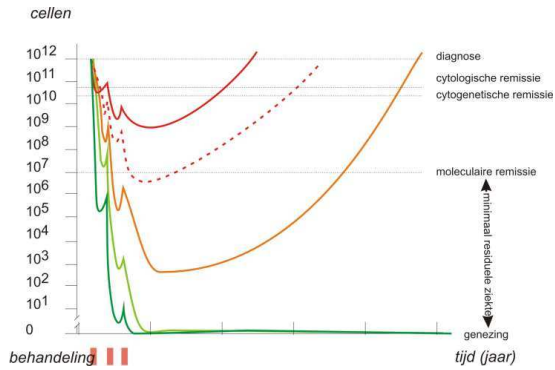
belang van cytogetisch/moleculair onderzoek

- Doelgerichte therapie : APL en vesanoid; Glivec en t(9;22)
- Bepalen van de prognose – therapieintensiteit “op maat”
 - Chemotherapie/ chemotherapie + allo-STx/ Experimenteel
- Merkers voor follow-up – moleculaire merkers zijn gevoeligst !

Behandeling van acute leukemie



AML met t(...;...) en een fusiegen



High-throughput sequencing Next generation sequencing



<http://www.uzleuven.be/diensten/cme/lab/aanvraagformulieren/>

Intern
Extern
nederlandstalig/franstaig
KRAS