

ONKOHEMATOLÓGIA

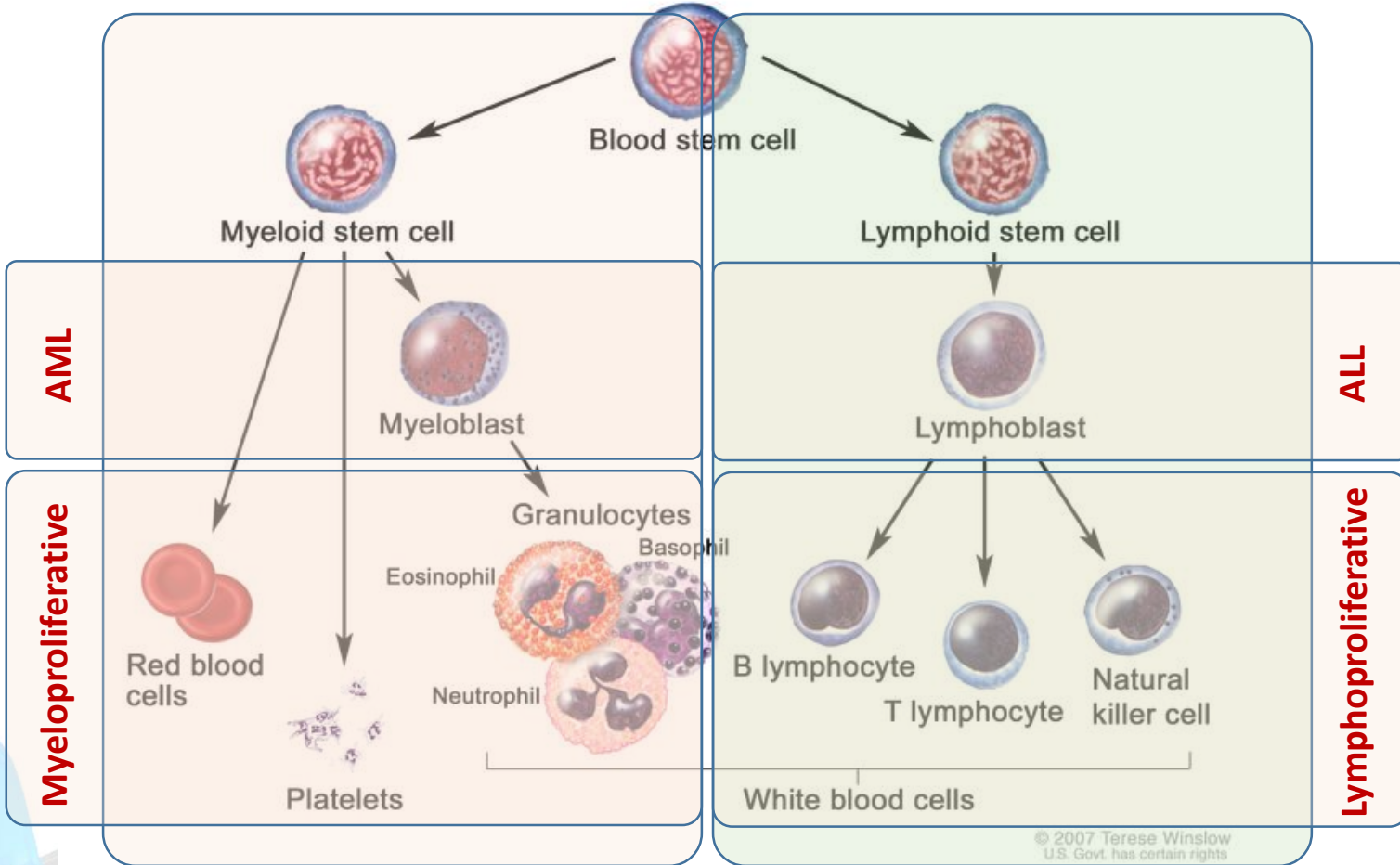
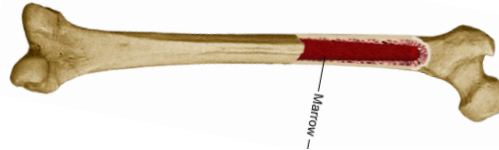
Klinikai laboratóriumi szempontok

Bödör Csaba



MTA-SE Lendület Molekuláris Onkohematológia Kutatócsoport,
I. sz. Patológiai és Kísérleti Rákkutató Intézet, Semmelweis Egyetem

Bevezetés- Hematológiai daganatok



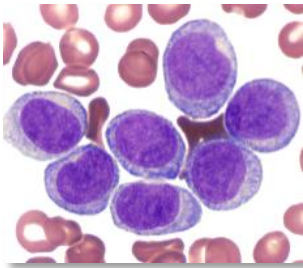
© 2007 Terese Winslow
U.S. Govt. has certain rights

MYELOID

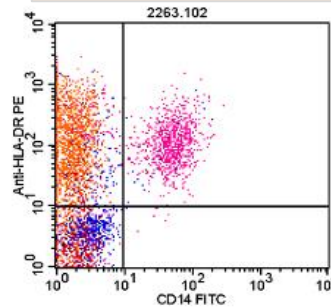
LYMPHOID

Onkohematológiai betegségek komplex diagnosztikája

Morfológia



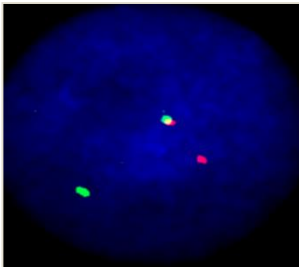
Flow citometria



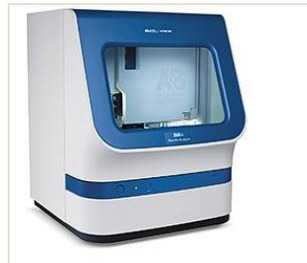
Citogenetika



FISH



Szekvenálás



Q-PCR



Mi a cél ??

- **Diagnózis**
- **Prognózis**
- **Terápiás hatás felmérése**
- **Terápiás célpont**

Alkalmazott metodikák

konvencionális PCR

allélspecifikus PCR

multiplex PCR

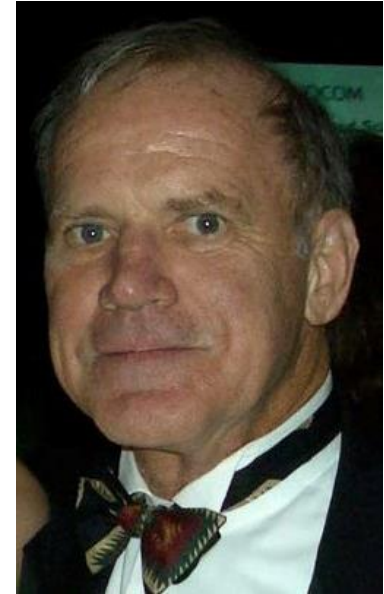
RQ-PCR

Sanger szekvenálás

NGS

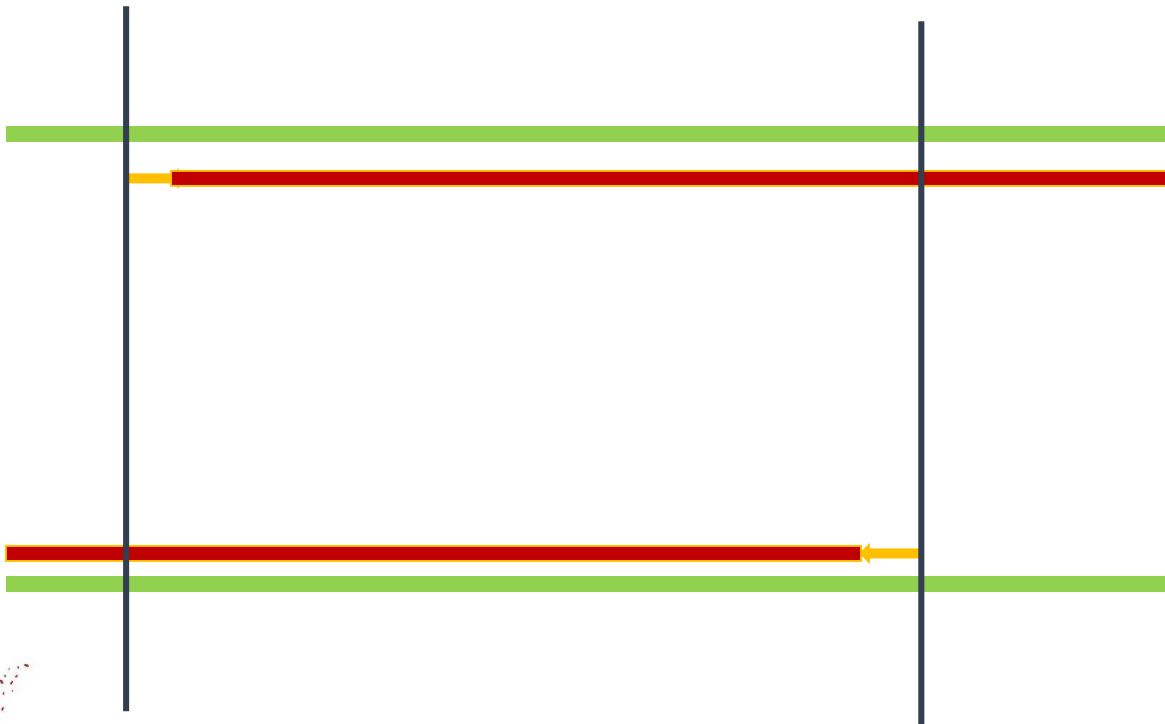
Polimeráz láncreakció (PCR)

- 1983, Kary Mullis: PCR
- 1993, **Nobel díj**
- Tetszőleges DNS szakasz „felsokszorozása”
- Denaturáció, Anelláció, Extenzió



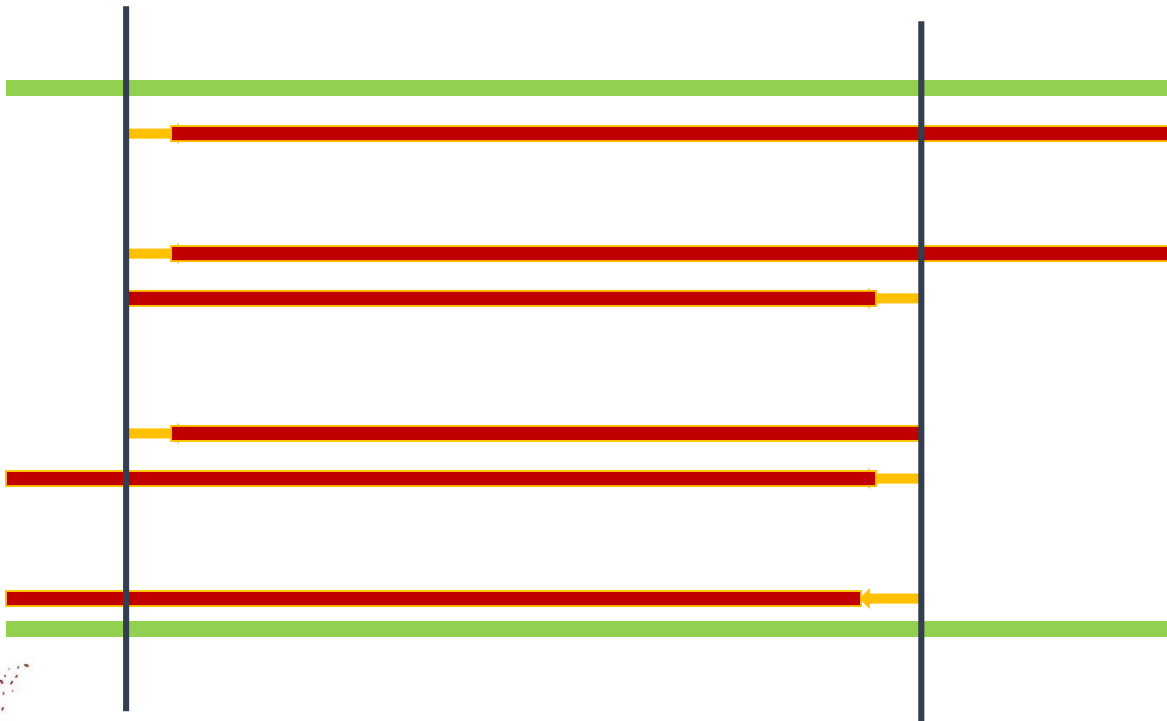
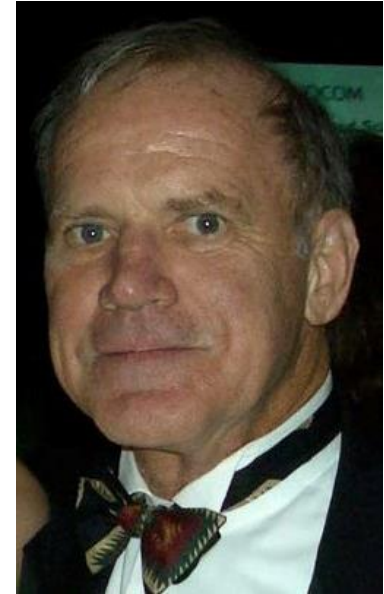
Polimeráz láncreakció (PCR)

- 1983, Kary Mullis: PCR
- 1993, **Nobel díj**
- Tetszőleges DNS szakasz „felsokszorozása”
- Denaturáció, Anelláció, Extenzió



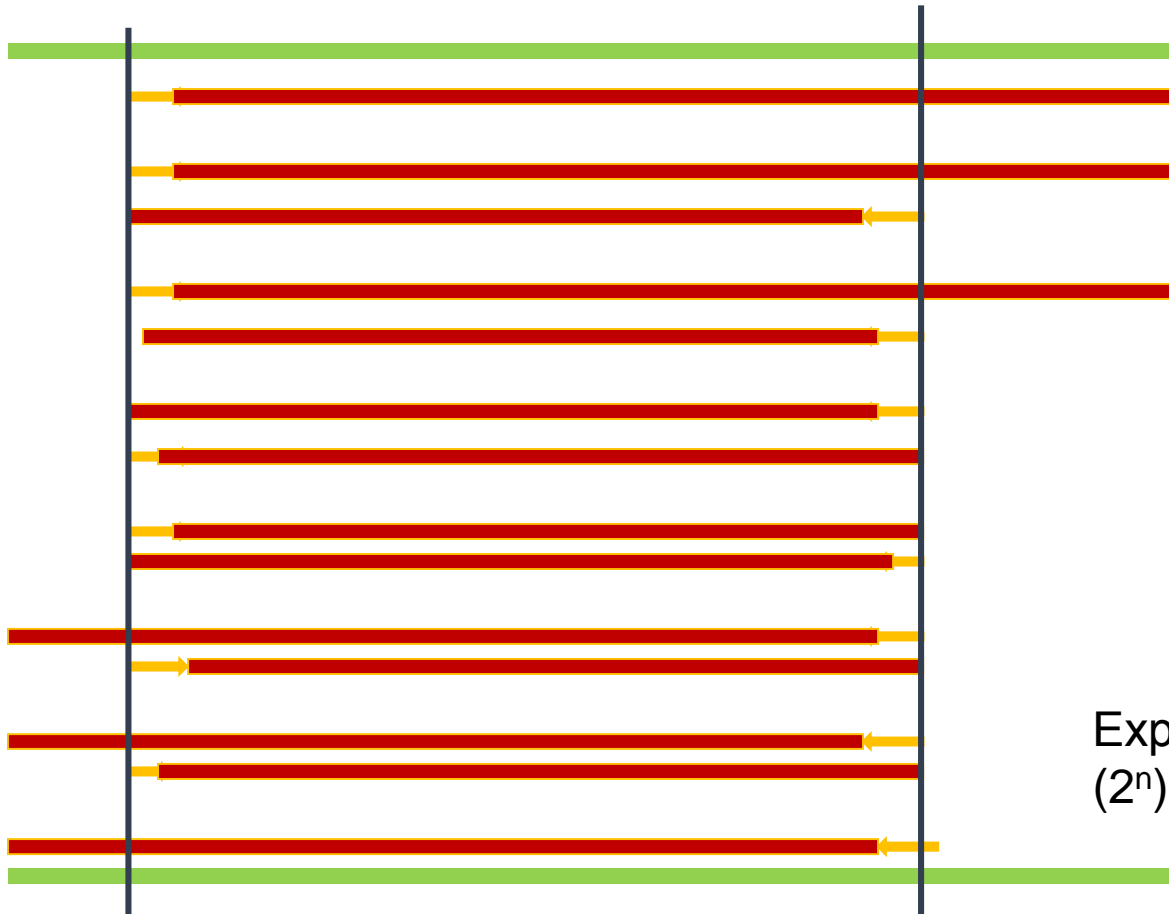
Polimeráz láncreakció (PCR)

- 1983, Kary Mullis: PCR
- 1993, **Nobel díj**
- Tetszőleges DNS szakasz „felsokszorozása”
- Denaturáció, Anelláció, Extenzió



Polimeráz láncreakció (PCR)

- 1983, Kary Mullis: PCR
- 1993, **Nobel díj**
- Tetszőleges DNS szakasz „felsokszorozása”
- Denaturáció, Anelláció, Extenzió



Exponenciális amplifikáció !
(2^n)

Polimeráz láncreakció (PCR) – A kezdetek



58°C



72°C

94°C



Copyright © 1999-2006 Artisan Scientific



Copyright © 1999-2006 Artisan Scientific

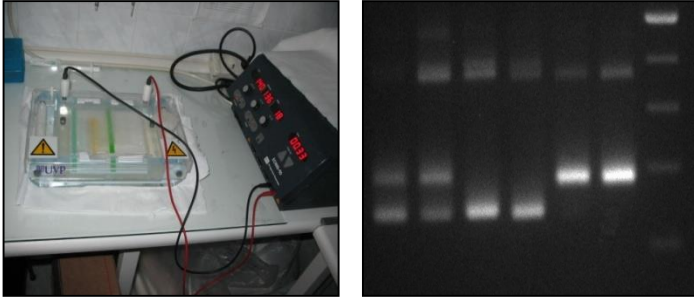


Copyright © 1999-2006 Artisan Scientific

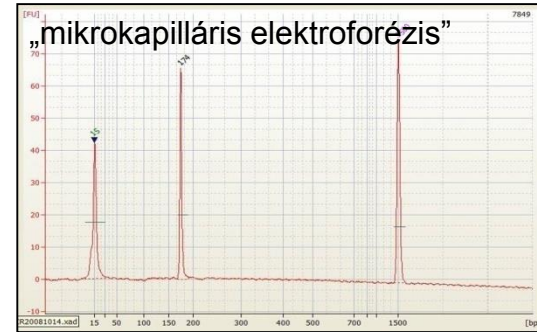
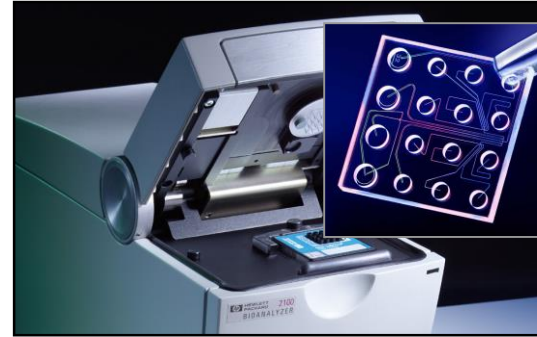


PCR- termékek detektálása

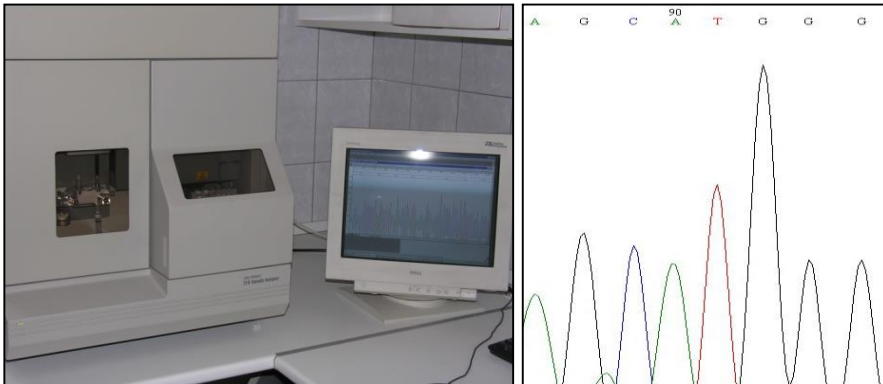
Klasszikus gél-elektroforézis



Lab-on-Chip rendszer

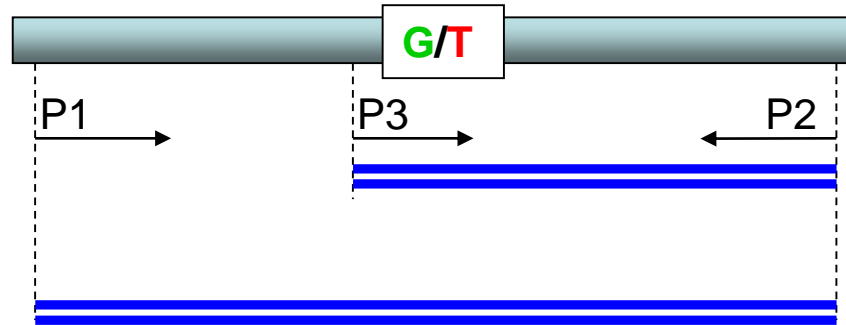


Kapilláris elektroforézis

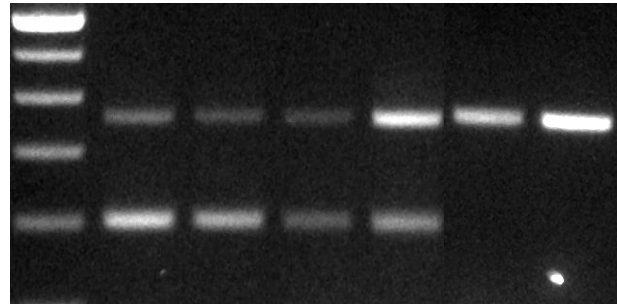
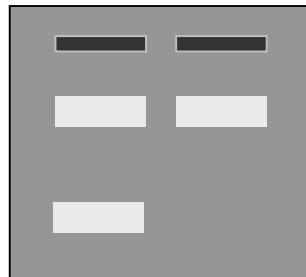


Allélspecifikus PCR

Allélspecifikus PCR

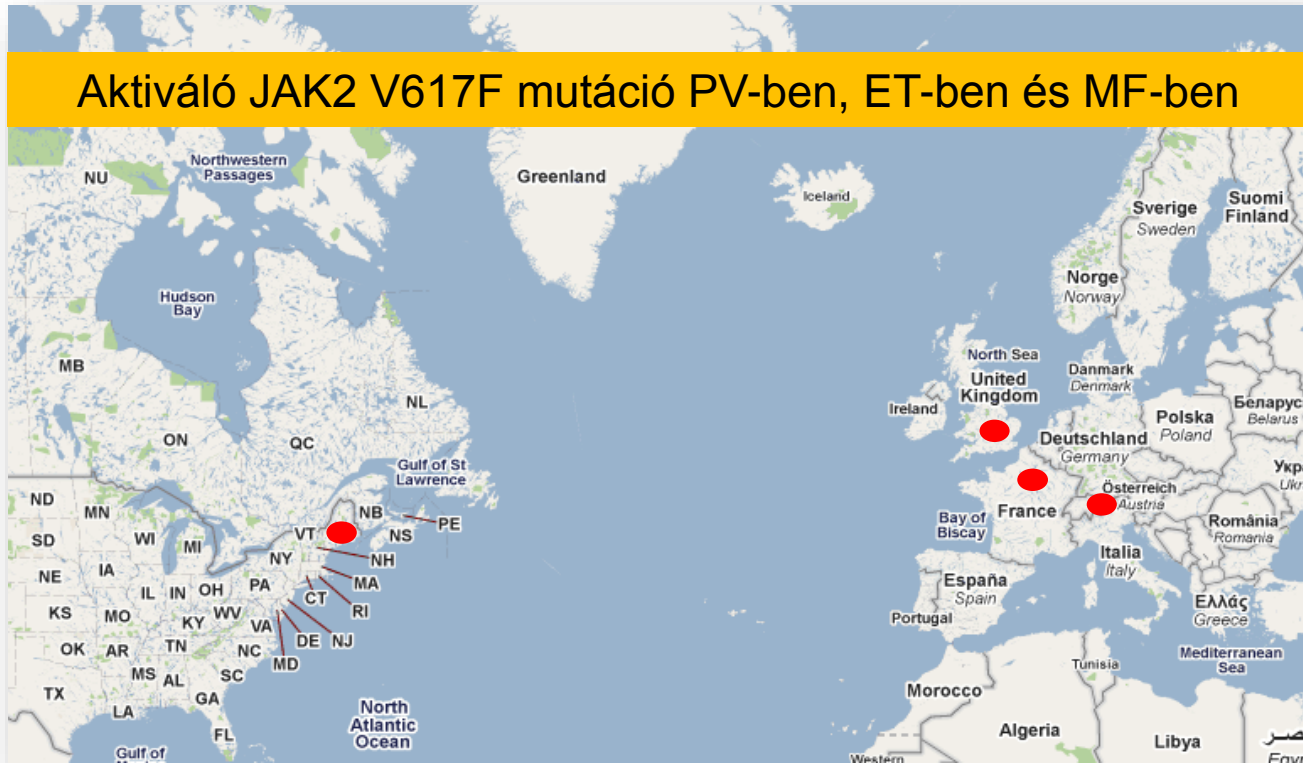


T **G**



A JAK2 story (PV, ET, MF)

2005: Párizs, Basel, Cambridge, Boston



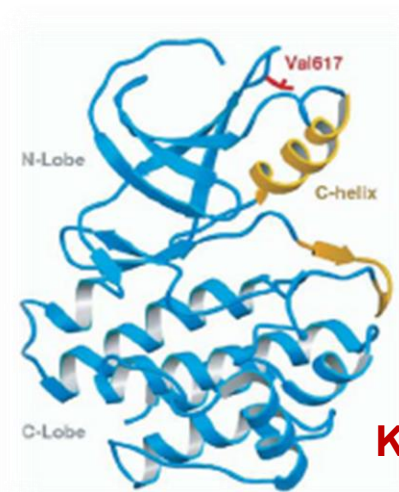
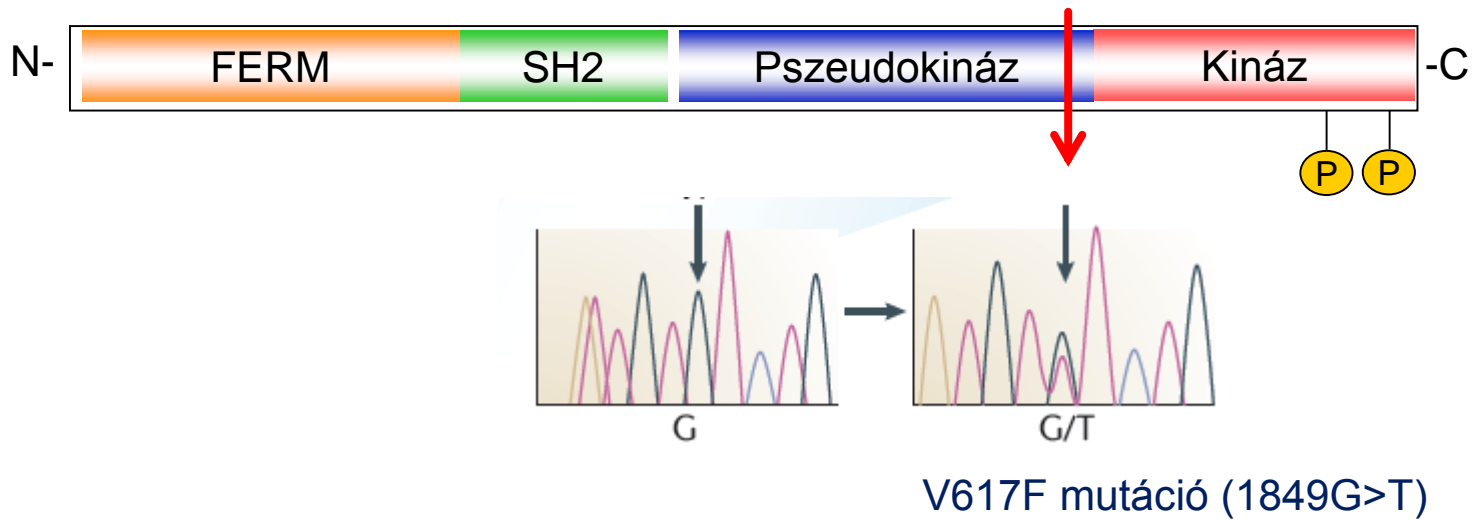
James C, et al. Nature. 2005; 434:1144.

Levine R, et al, Cancer Cell 2005, 7: 387.

Kralovics R, et al. N Engl J Med. 2005; 352: 1779.

Baxter EJ, et al. Lancet. 2005; 365: 1054.

Janus-2 kináz V617F mutációja



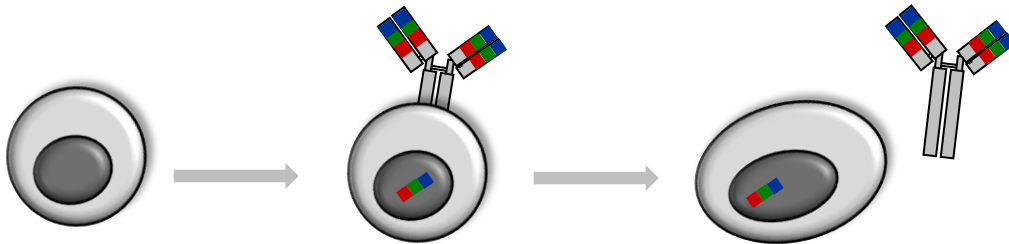
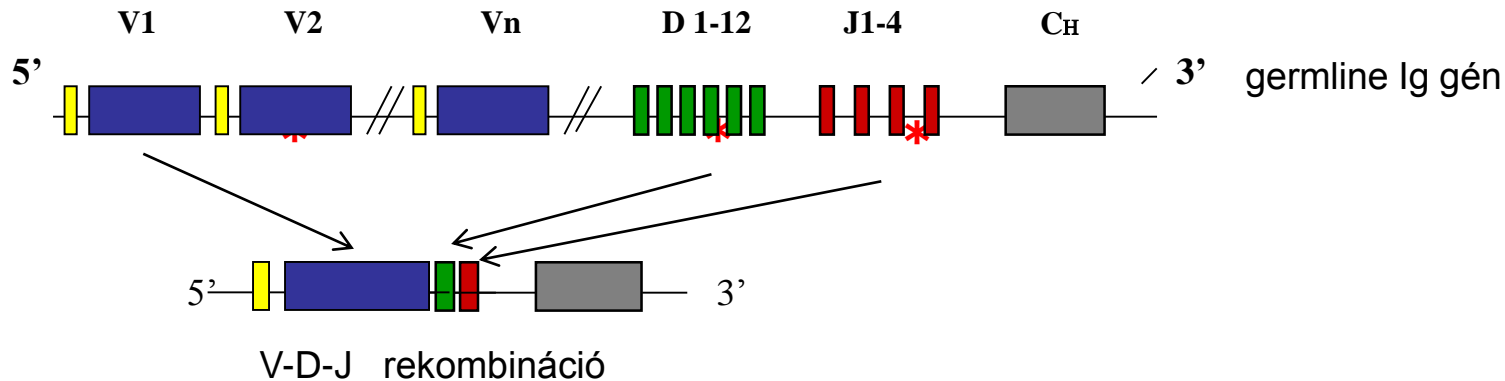
Konstitutív aktiváció

James C, Nature 2005; 434: 1144-1148.

A unique clonal JAK2 mutation leading to constitutive signaling causes polycythaemia vera

Multiplex PCR – Klonalitás meghatározása

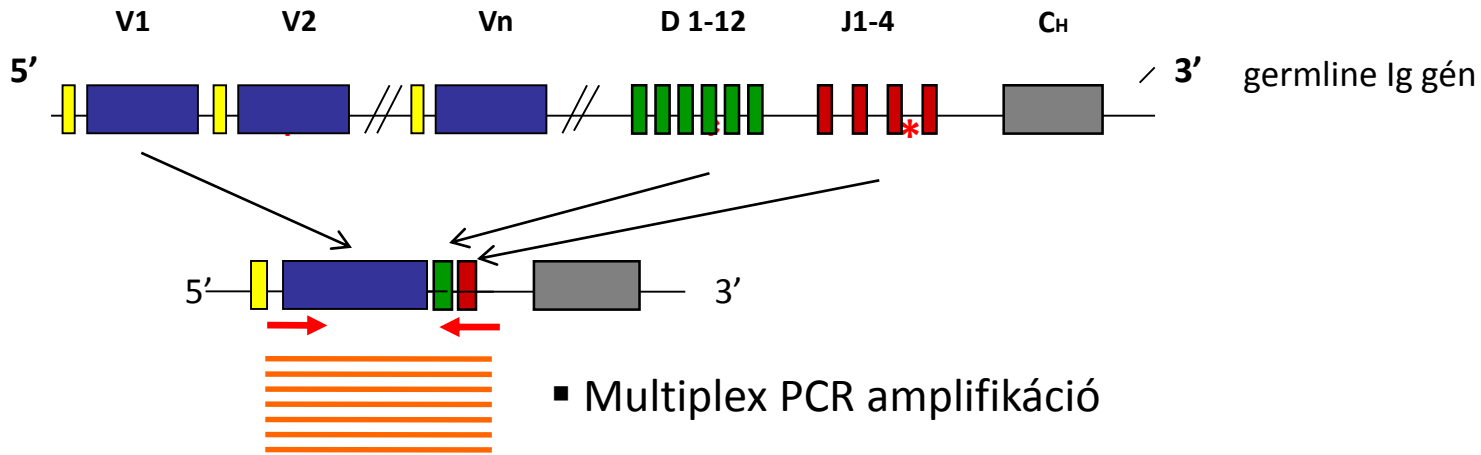
▪ Klonalitás meghatározás (B-sejtes, T-sejtes daganatok)



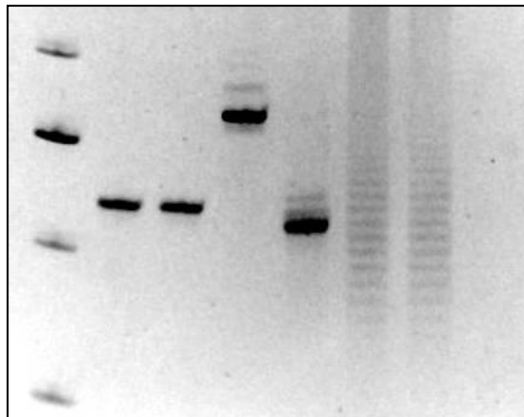
- Minden B-sejt egyedi génkonfigurációt hordoz
- Óriási variabilitás (2×10^6), antigénfelismerő készlet, +
- Monoklonalitás: minden sejt ugyanazt hodozza !

Multiplex PCR – Klonalitás meghatározása

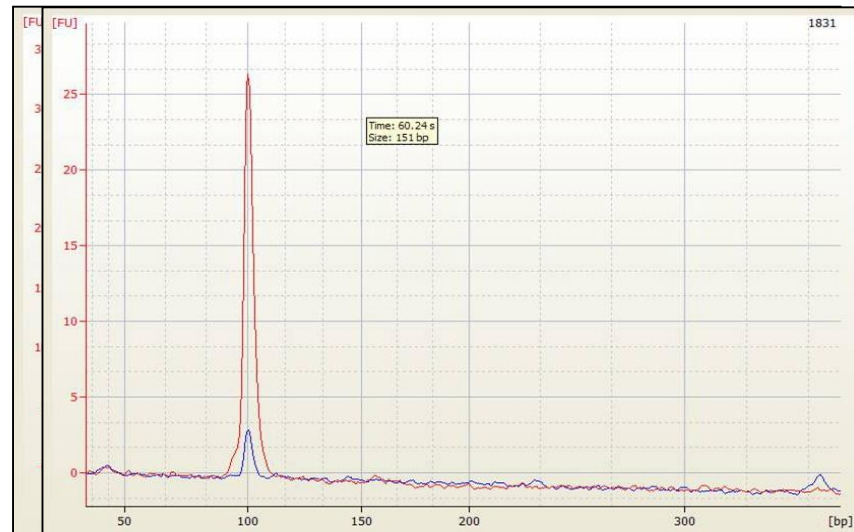
■ Immunglobulin génátrendeződés vizsgálata (IgH)



B-sejtes; IgV_H

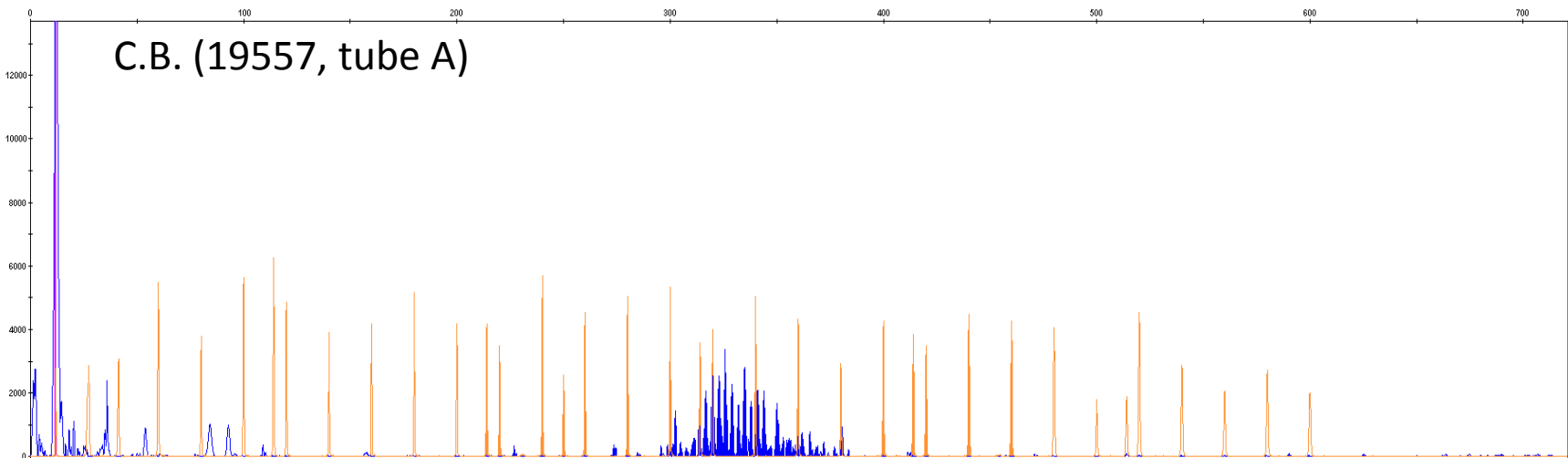
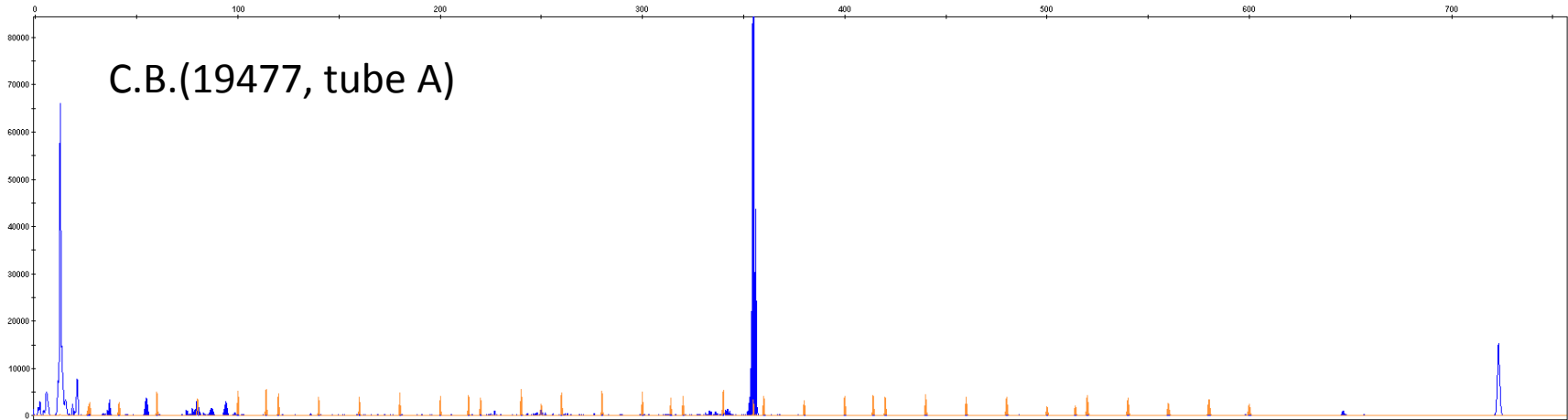


Marker Minta + - NTC



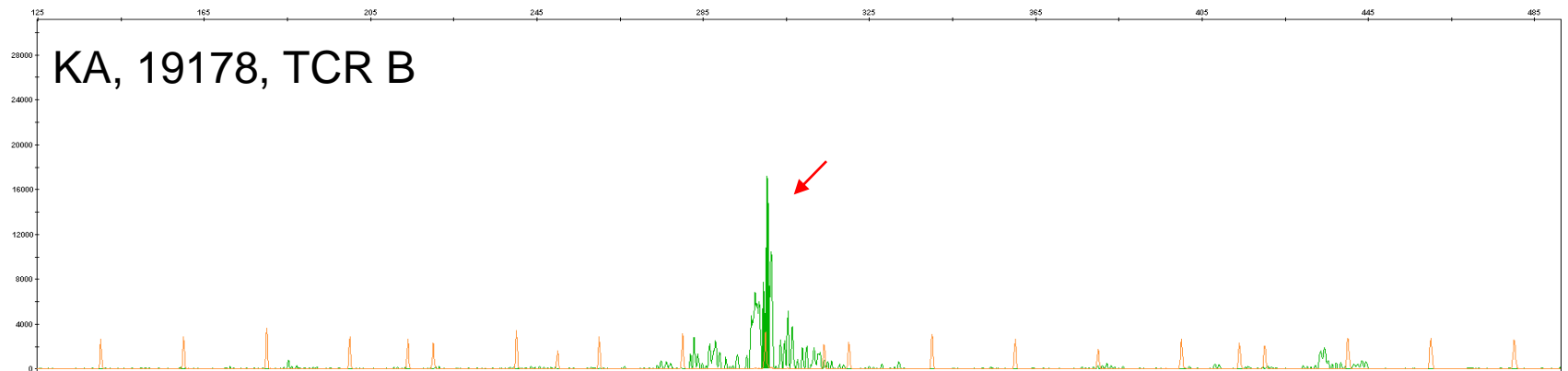
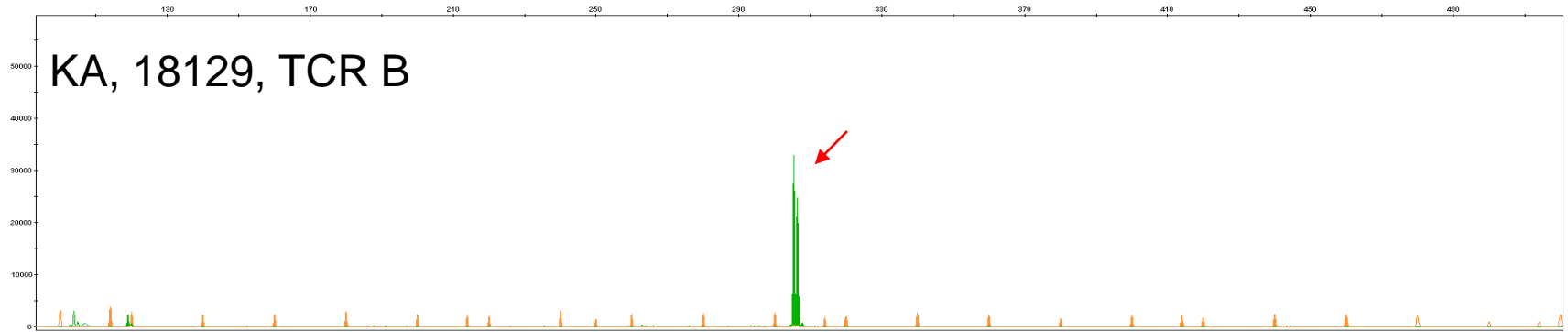
Multiplex PCR – Klonalitás meghatározása

■ Immunglobulin (IgH) génátrendeződés vizsgálata



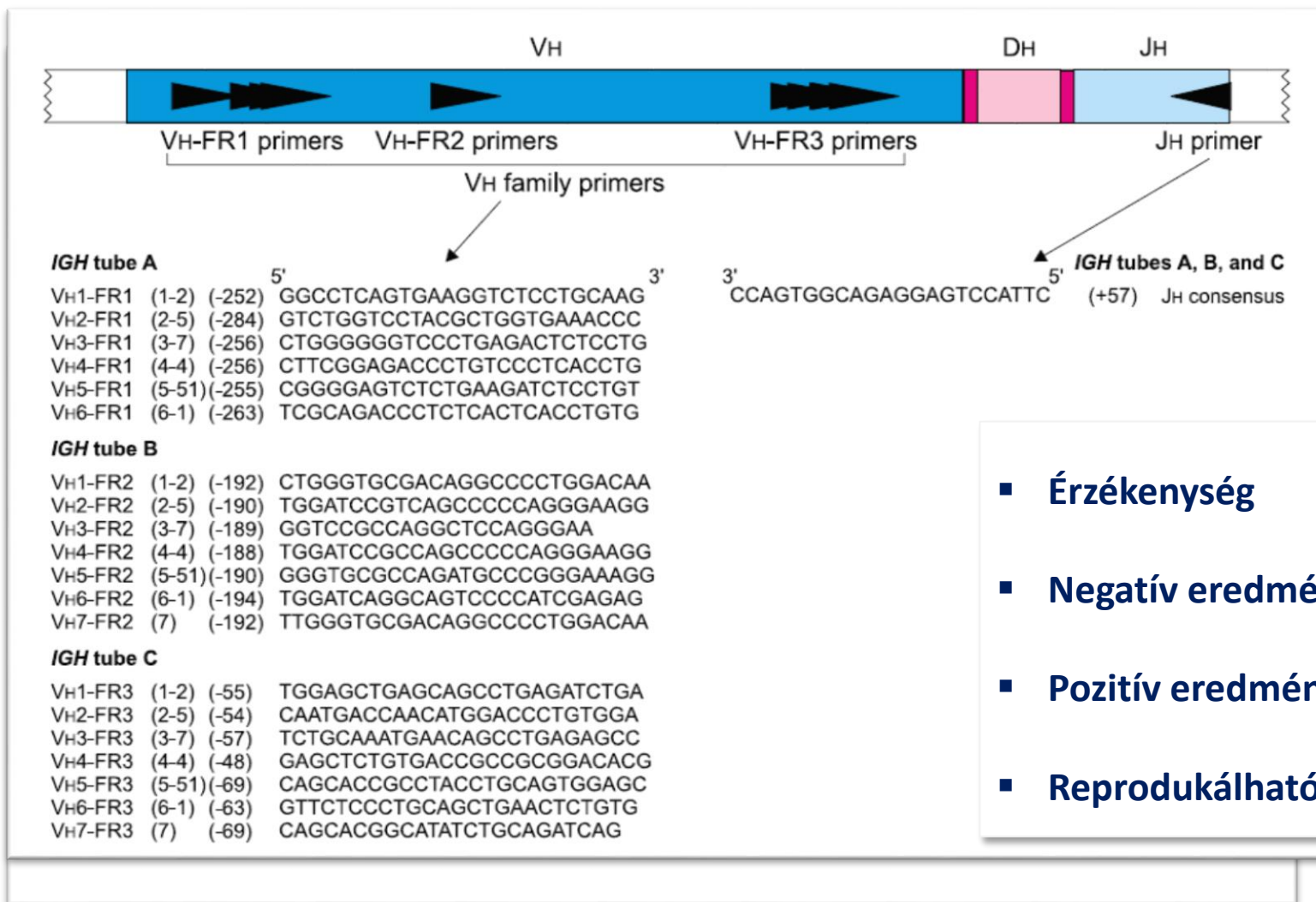
Multiplex PCR – Klonalitás meghatározása

▪ T-sejt receptor (TCR) génátrendeződés vizsgálat



Multiplex PCR – Klonalitás meghatározása

- ... Valójában sokkal bonyolultabb a helyzet ...



- Érzékenység
- Negatív eredmény
- Pozitív eredmény
- Reprodukálhatóság

Valós-idejű (real-time PCR) megjelenése

- **Konvencionális PCR**

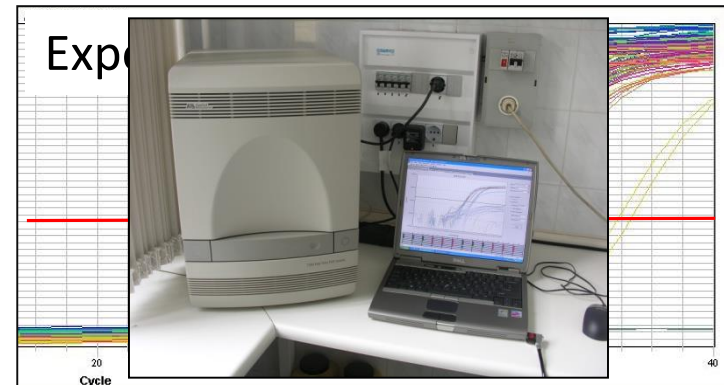


- 1996: **TaqMan alapú Valós idejű PCR**



Klasszikus PCR

Real-Time PCR



- **Előnyök: kvantitatív, szenzitivitás, specificitás, reprodukálhatóság**

Valós-idejű PCR alkalmazása

▪ Fúziós transzkriptumok mennyiségi kimutatása

Krónikus myeloid leukémia

- *BCR-ABL, t(9;22)*

Akut lymphoid leukémia

- *MLL-AF4, t(4;11)*
- *TEL-AML1, t(12;21)*
- *E2A-PBX-1, t(1;19)*

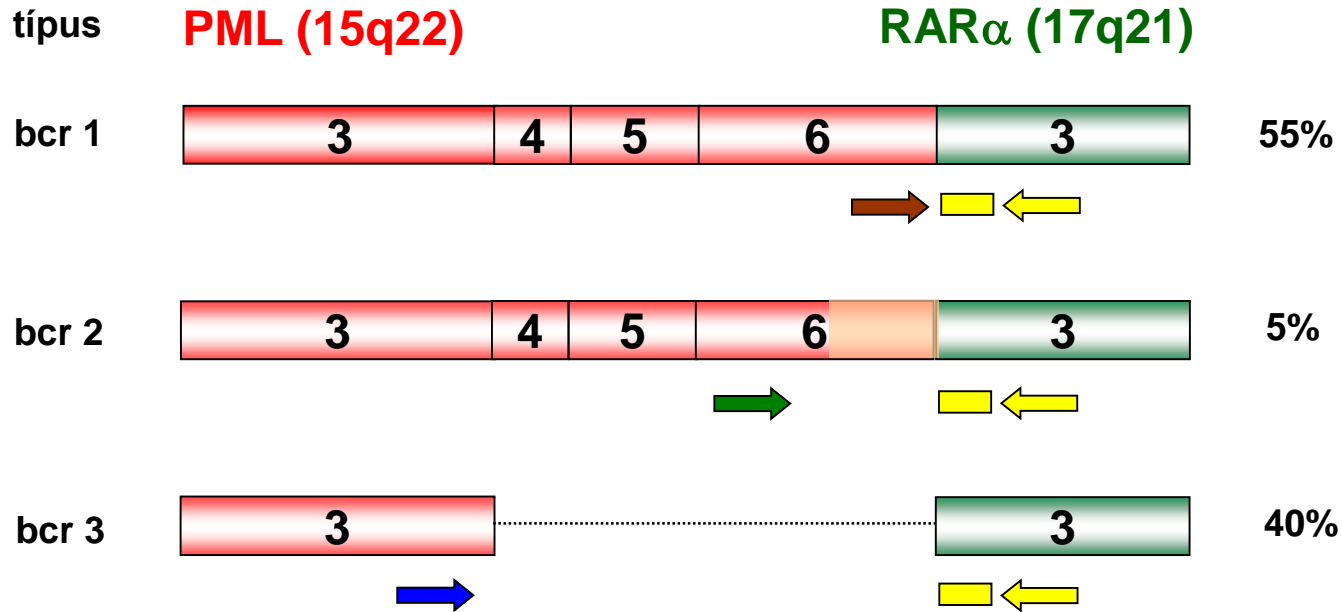
Akut myeloid leukémia

- *PML-RAR α , t(15;17)*
- *AML-ETO1, t(8;21)*
- *CBFB-MYH11 inv(16)*

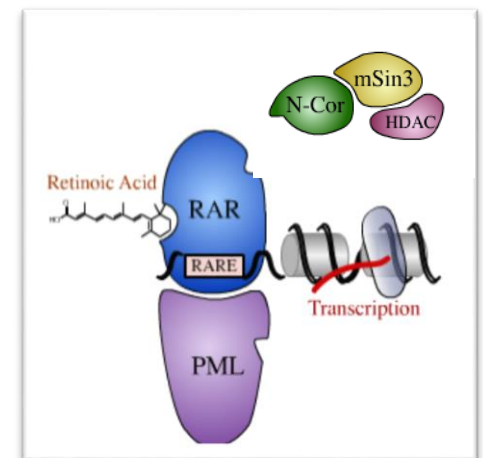
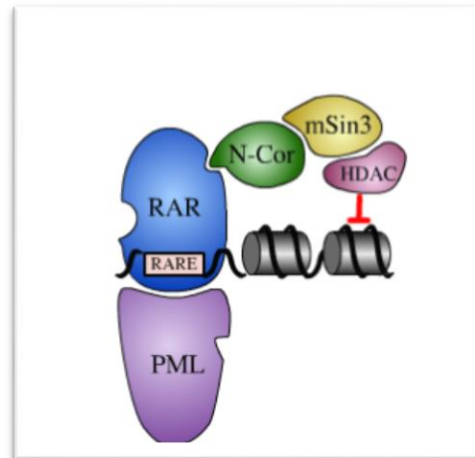
Gyerekkori T-ALL

- *SIL-TAL1, microdel 1p32*

PML-RAR α fúziós transzkriptum - t(15;17)(q22;21)



- APL többségében (M3 AML)
- Kedvező prognózis
- ATRA kezelés



KROMOSZÓMÁK VIZSGÁLATA (Citogenetika)



Citogenetika

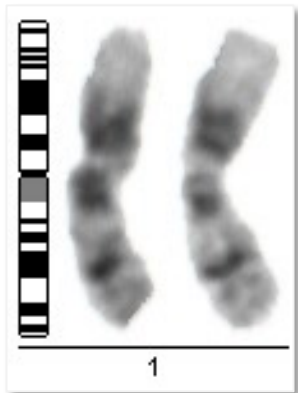
Kariotipizálás ("sávozás")

- Giemsa festés (G sávozás)
- Specifikus mintázat
- Kariotípus

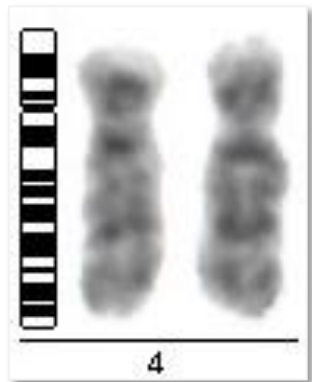


Citogenetika – A kromoszómák felépítése

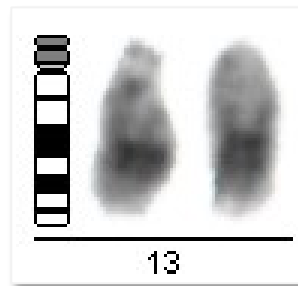
- $2n=46$, (46, XX; 46, XY)
- Centromer, telomerek
- Karok, régiók



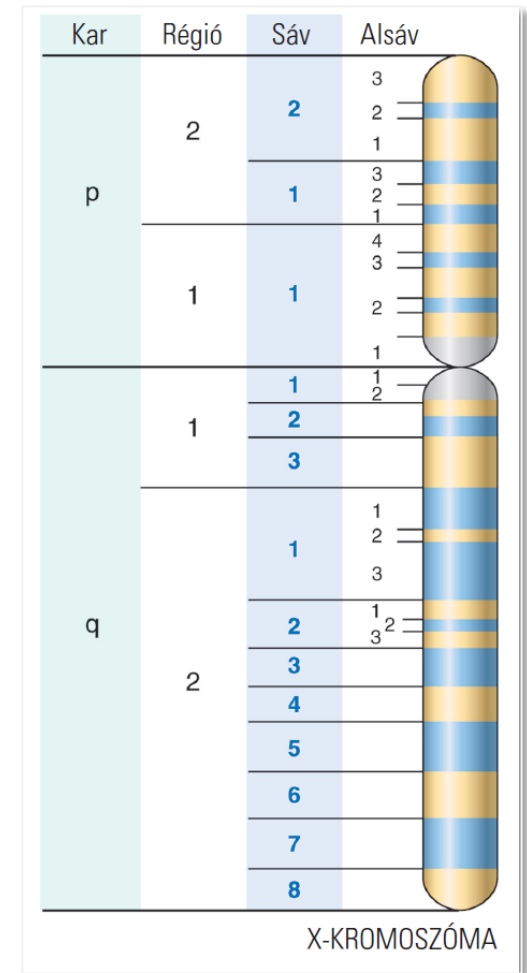
Metacentrikus



Szubmetacentrikus



Acrocentrikus



Kromoszóma eltérések

▪ Számbeli változások

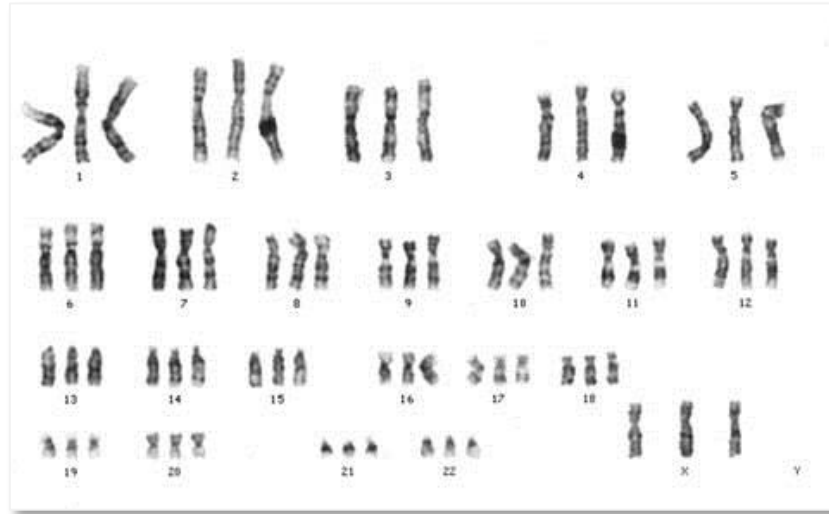
- Poliploidia
- Aneuploidia (monoszómia, triszómia)

▪ Szerkezeti változások

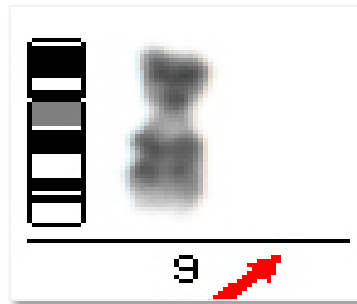
- Transzlokáció
- Deléció
- Amplifikáció
- Inverzió
- Izokromoszóma
- Gyűrűkromoszóma

Számbeli kromoszóma eltérések

- POLIPLOIDIA (3n, 4n, ...)



- ANEUPLOIDIA (Monoszómia, Triszómia)

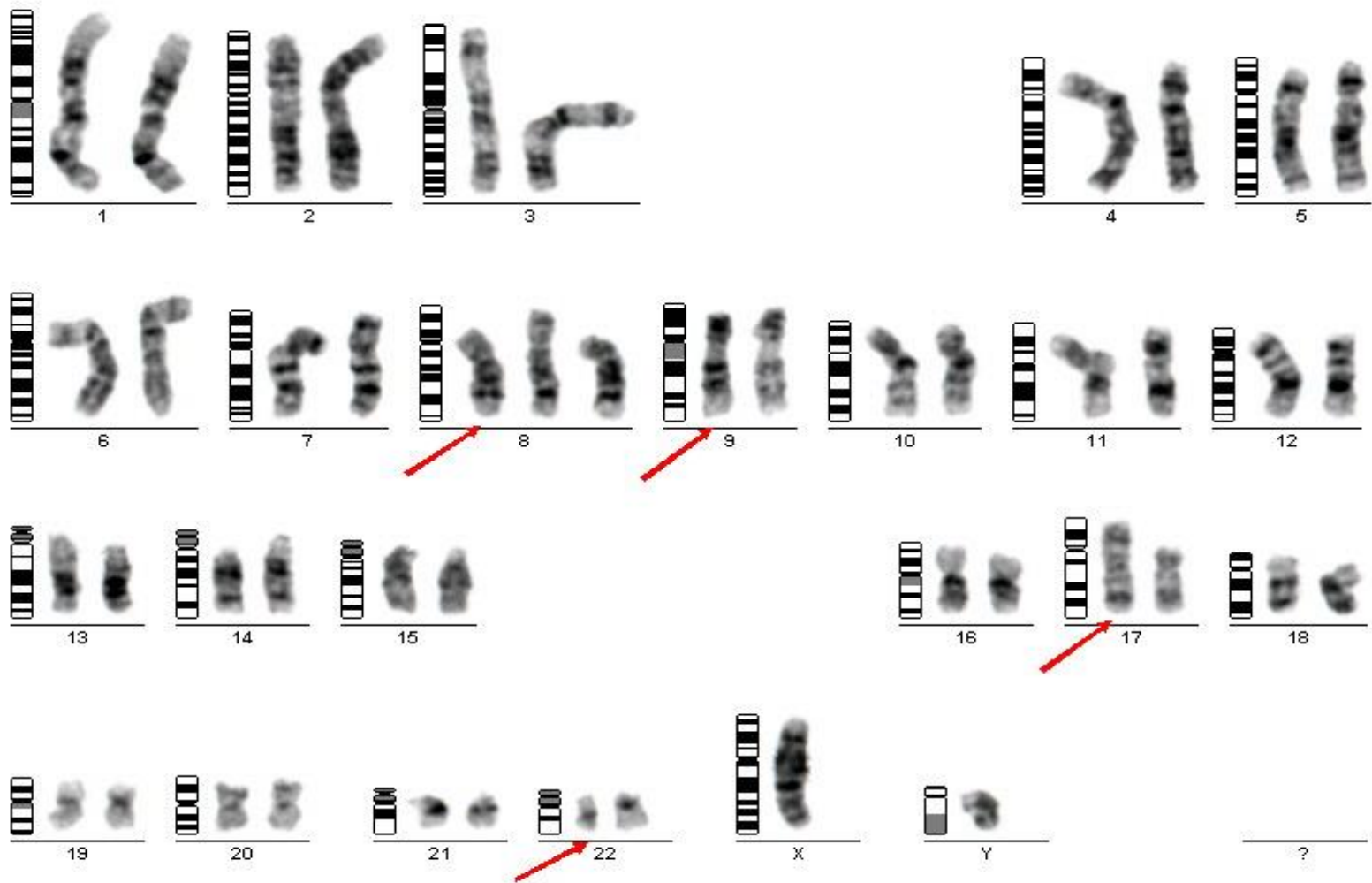


Monoszómia

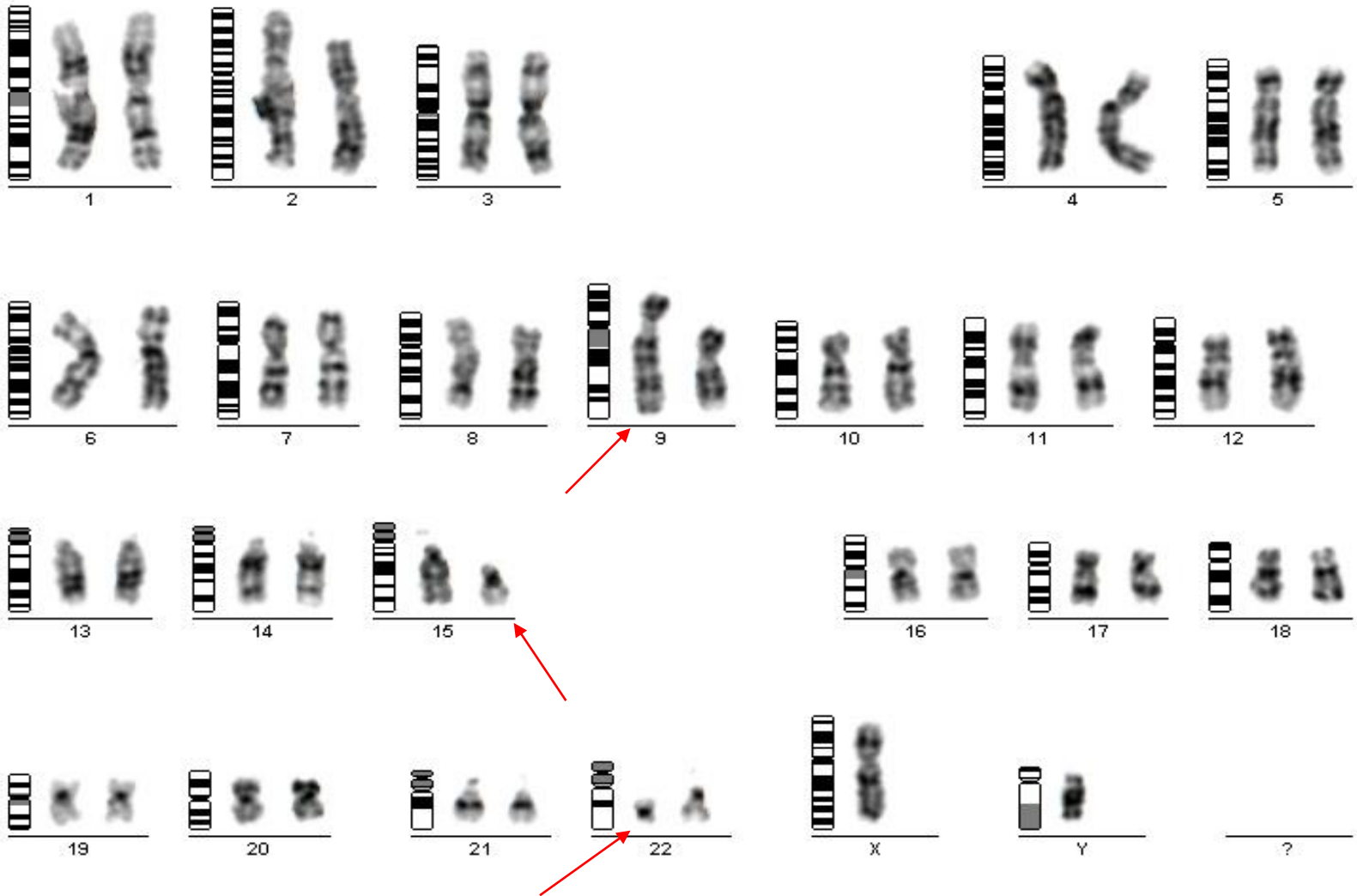


Triszómia

46,XY, +8, t(9;22), iso(17q)

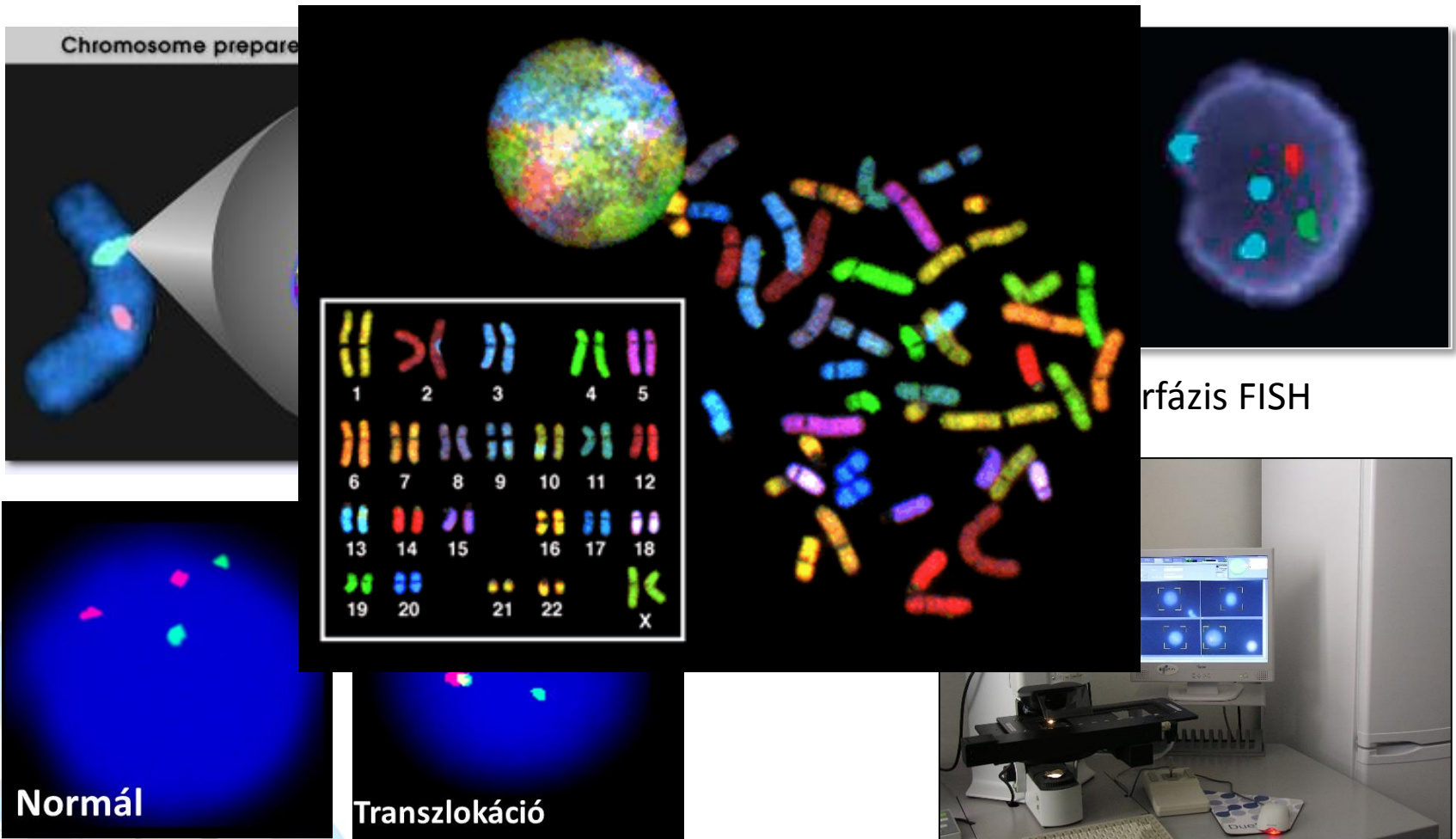


46,XX,t(9;15;22)



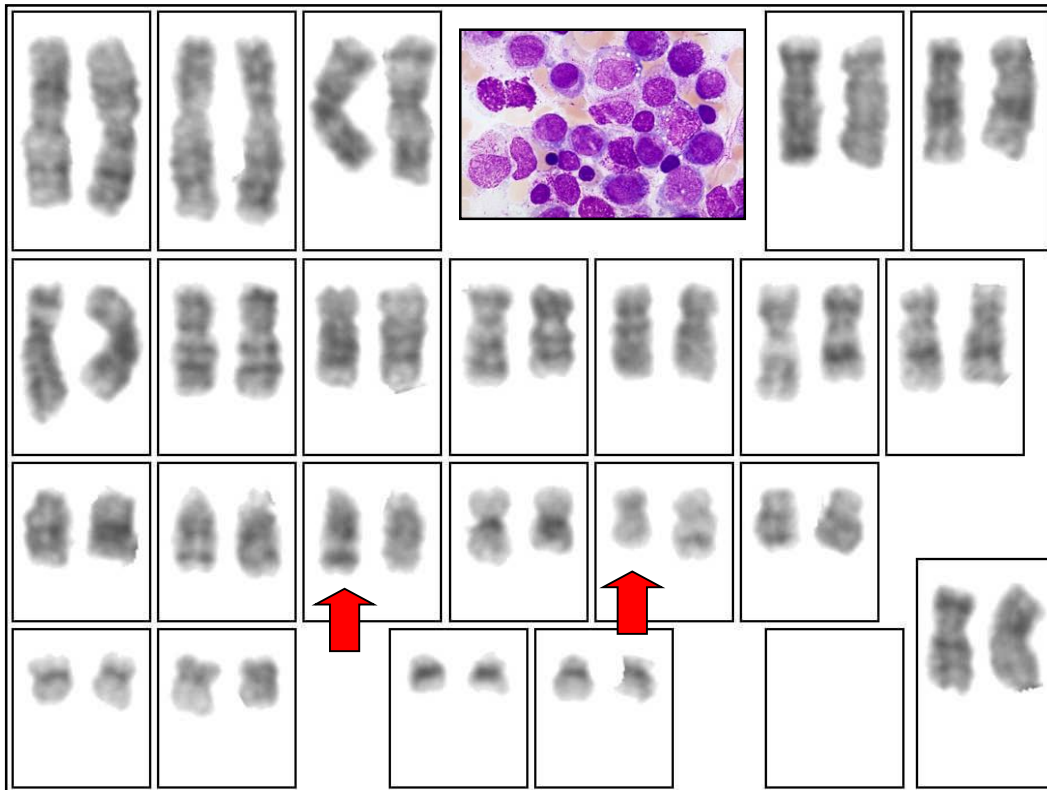
Citogenetika – FISH

- FISH (fluoreszcens in situ hibridizáció)
- Specifikus próbák
- Nem szükséges osztódó sejt

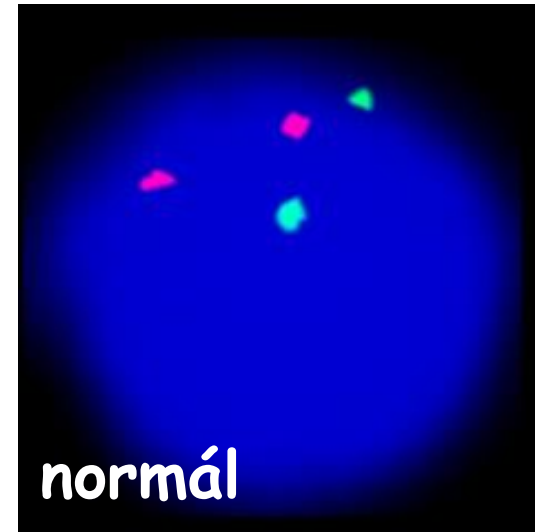


FISH - példák

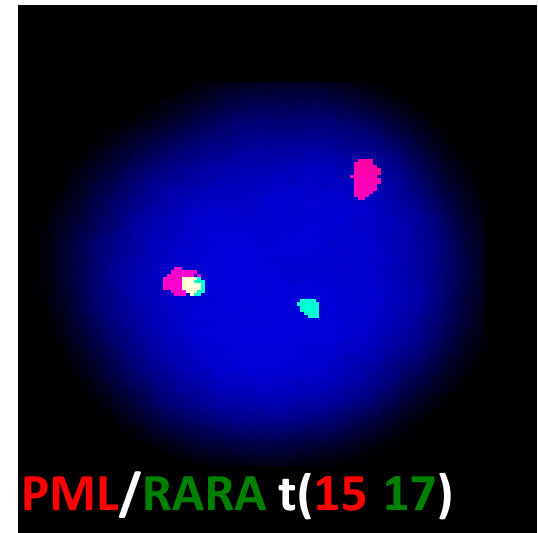
•t(15;17), PML-RARA fúziós gén



Fúziós próba



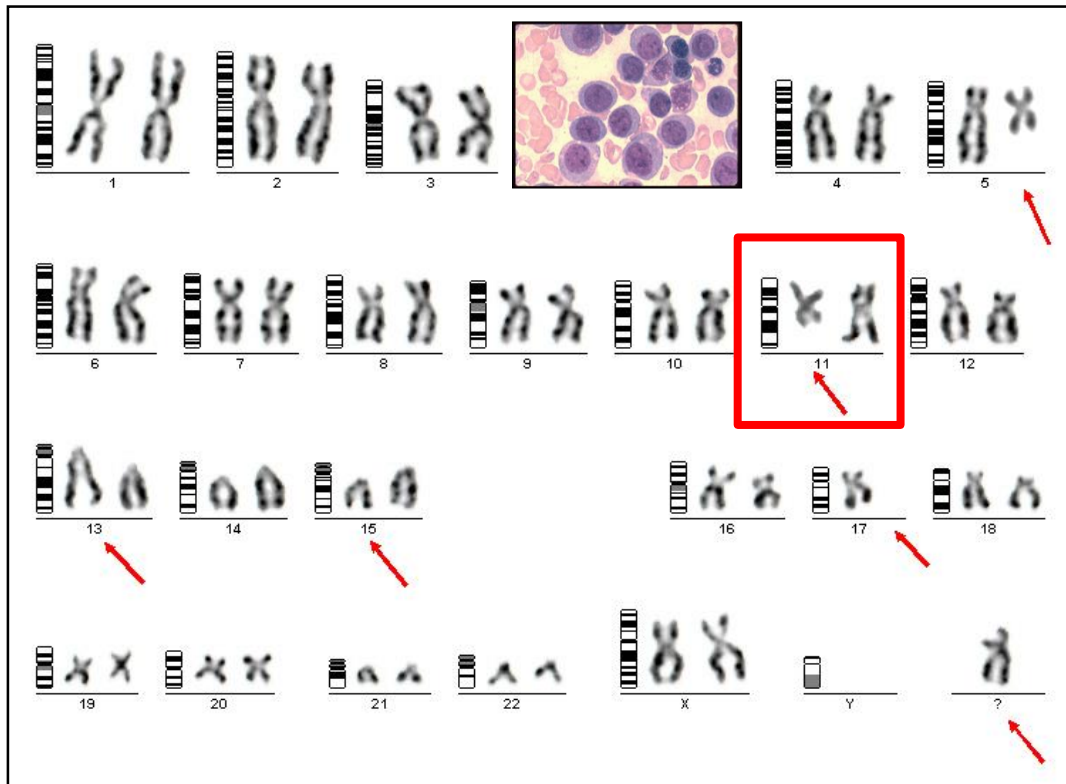
normál



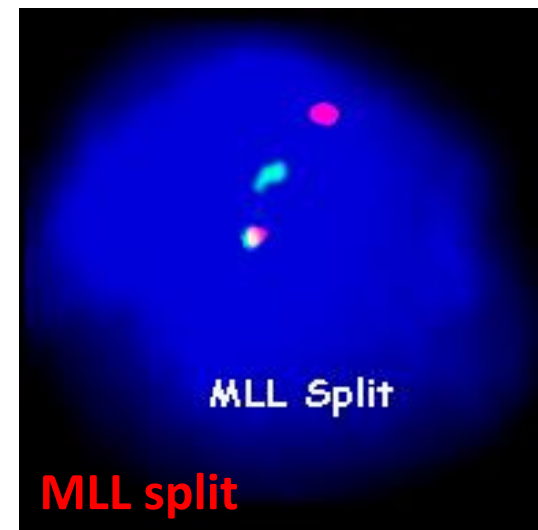
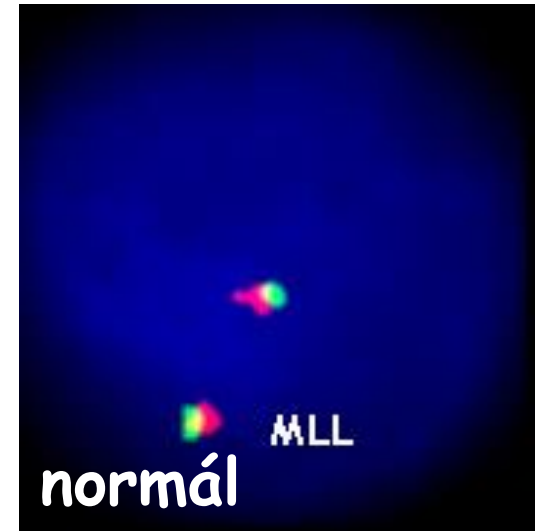
PML/RARA t(15 17)

FISH - példák

- MLL- ? Fúziós gén



Split próba



Acute myeloid leukaemia

Krónikus Myeloid Leukémia - Történeti Áttekintés

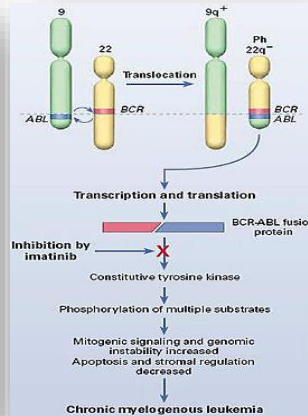
Philadelphia
kromoszóma



t(9;22)
transzlokáció



BCR-ABL1
fúziós gén



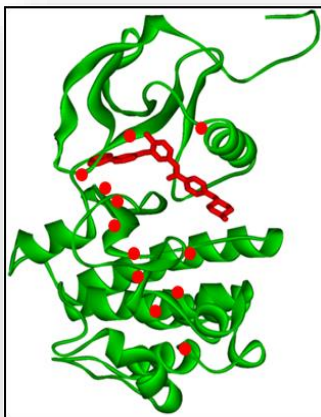
B. Druker
STI 517



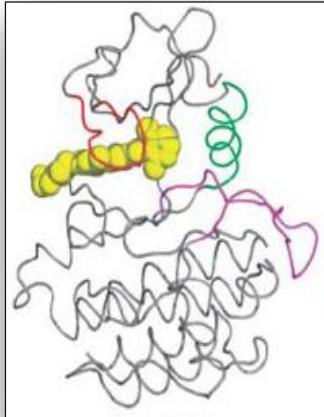
Imatinib
(Glivec)



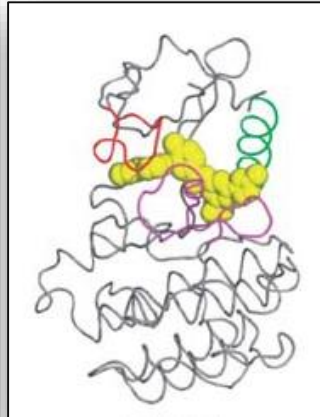
ABL1 KD
mutációk



Dasatinib
(Sprycel)



Nilotinib
(Tasigna)



3. generációs
szerek



2G-TKI
elsővonalban

- Követés
- Citogenetika
- Génexpresszió
- Mutációk
- TKI kezelés elhagyása
- NGS diagnosztika

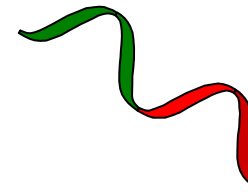
A CML MOLEKULÁRIS MONITOROZÁSA

Fúziós gén

Philadelphia
kromoszóma
t(9;22)



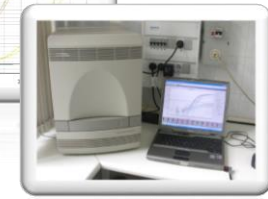
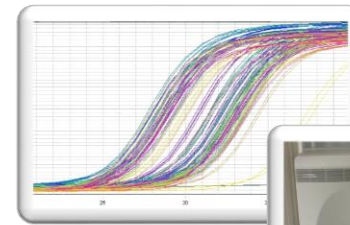
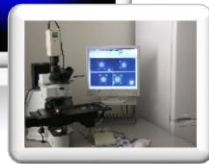
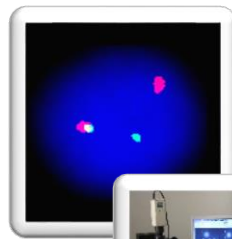
Fúziós transzkriptum



Citogenetika

FISH

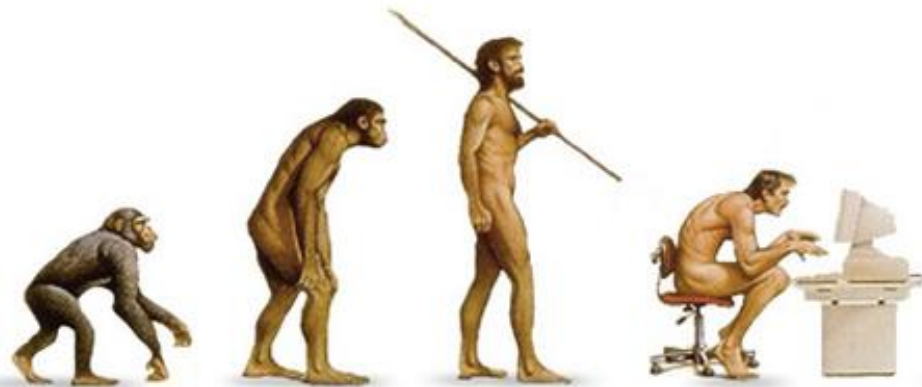
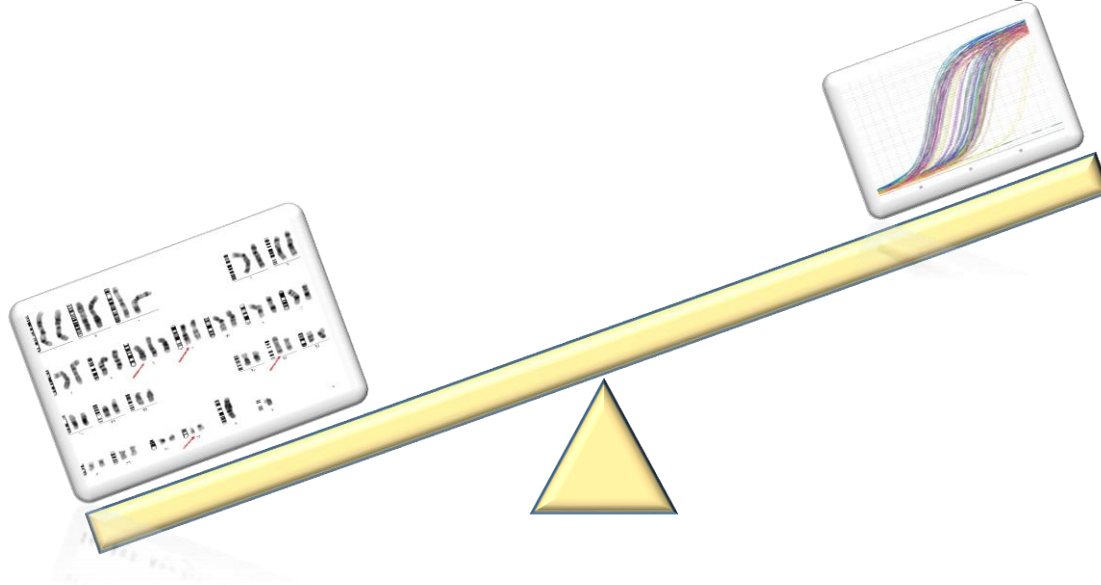
BCR-ABL1 RQ-PCR



A MOLEKULÁRIS MONITOROZÁS EVOLÚCIÓJA (2006-13)

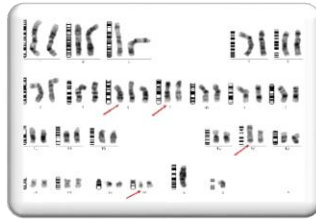
CITOGENETIKA

BCR-ABL1 RQ-PCR

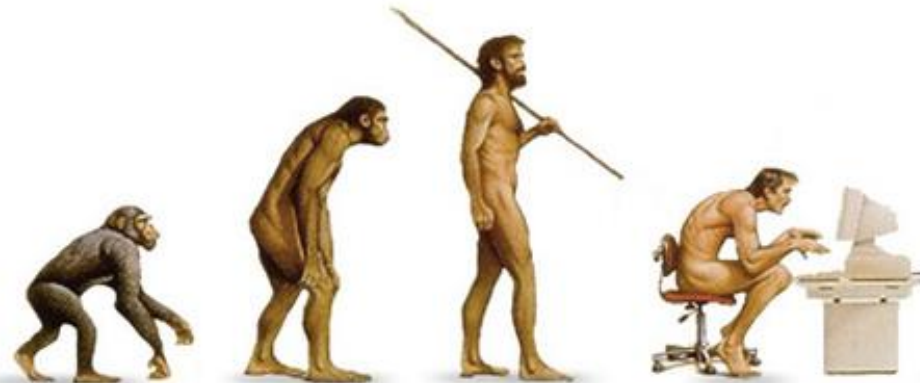
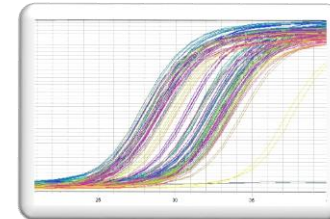


A MOLEKULÁRIS MONITOROZÁS EVOLÚCIÓJA (2006-13)

CITOGENETIKA



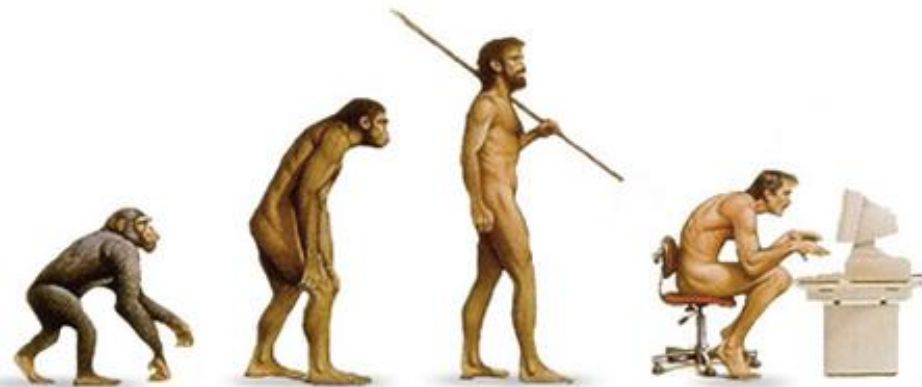
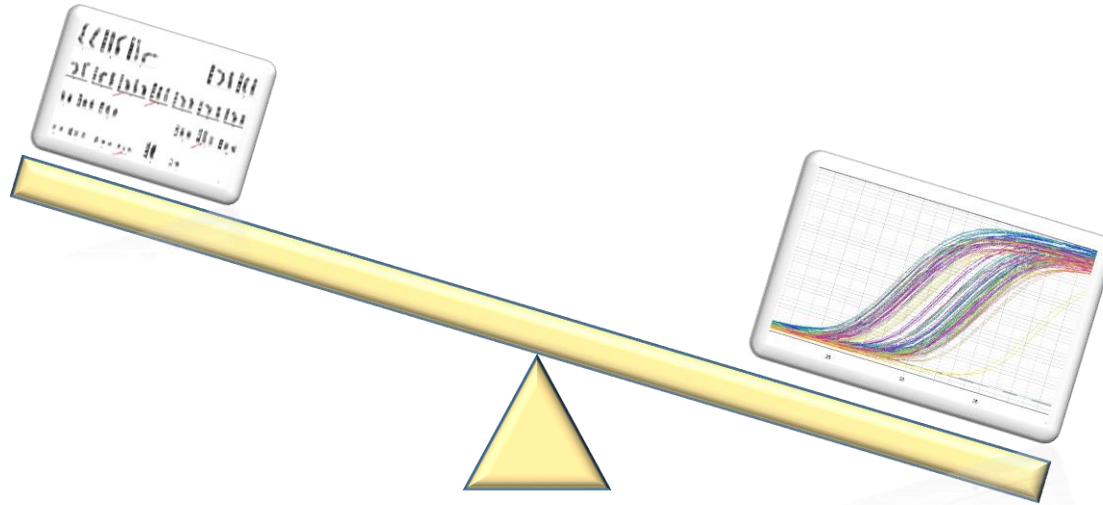
BCR-ABL1 RQ-PCR



A MOLEKULÁRIS MONITOROZÁS EVOLÚCIÓJA (2006-13)

CITOGENETIKA

BCR-ABL1 RQ-PCR



- RQ-PCR elterjedése/ 2G-TKI elsővonalbeli alkalmazása,
- Molekuláris válasz egyenrangú szerepe
- Válasz kategóriák BCR-ABL1^{IS} (IS: nemzetközi skála) alapján
- ~~Komplett~~-molekuláris válasz helyett MR4.0, MR4.5, MR5.0
- Mérföldkövek a másodvonalbeli terápia értékelésére

Review Article

European LeukemiaNet recommendations for the management of chronic myeloid leukemia: 2013

Michele Baccarani,¹ Michael W. Deininger,² Gianantonio Rosti,³ Andreas Hochhaus,⁴ Simona Soverini,³ Jane F. Apperley,⁵

CML: ÚJ ELNET AJÁNLÁS (2013)

Table 5. Definition of the response to TKIs (any TKI) as first-line treatment

	Optimal	Warning	Failure
Baseline	NA	High risk Or CCA/Ph+, major route	NA
3 mo	BCR-ABL1 \leq 10% and/or Ph+ \leq 35%	BCR-ABL1 $>$ 10% and/or Ph+ 36-95%	Non-CHR and/or Ph+ $>$ 95%
6 mo	BCR-ABL1 $<$ 1% and/or Ph+ 0	BCR-ABL1 1-10% and/or Ph+ 1-35%	BCR-ABL1 $>$ 10% and/or Ph+ $>$ 35%
12 mo	BCR-ABL1 \leq 0.1%	BCR-ABL1 $>$ 0.1-1%	BCR-ABL1 $>$ 1% and/or Ph+ $>$ 0
Then, and at any time	BCR-ABL1 \leq 0.1%	CCA/Ph- (-7, or 7q-)	Loss of CHR Loss of CCyR Confirmed loss of MMR* Mutations CCA/Ph+

MOLEKULÁRIS MONITOROZÁS - Mit, mikor?

- RQ-PCR: 3 havonta
- MMR után 6 havonta

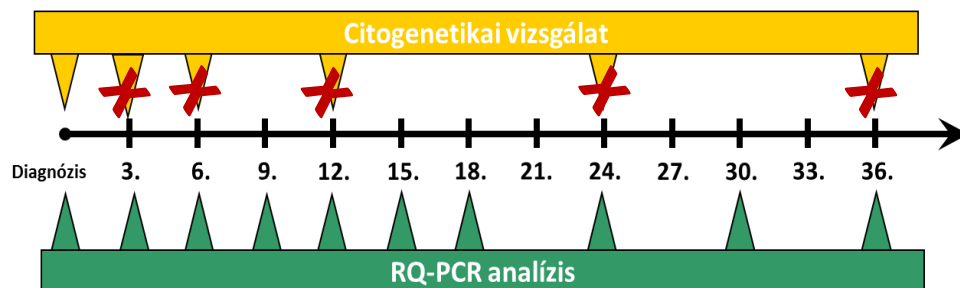


Table 9. Recommendations for cytogenetic and molecular monitoring

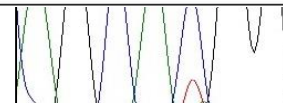
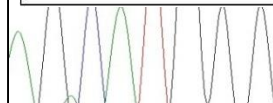
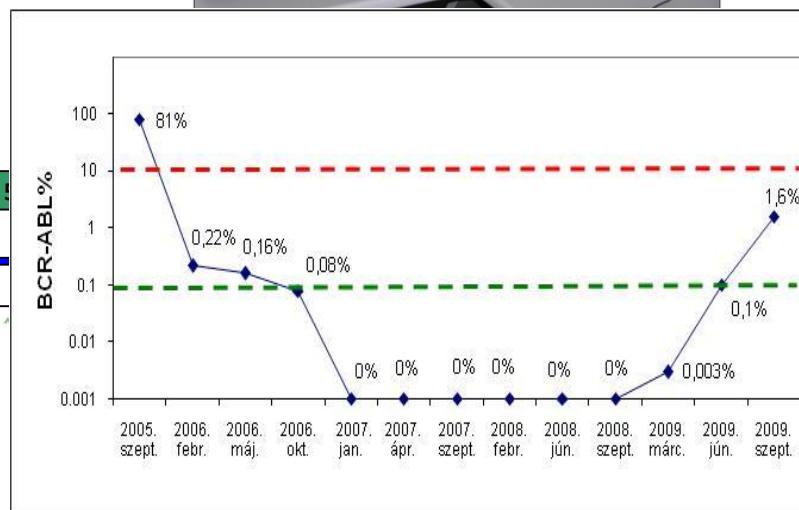
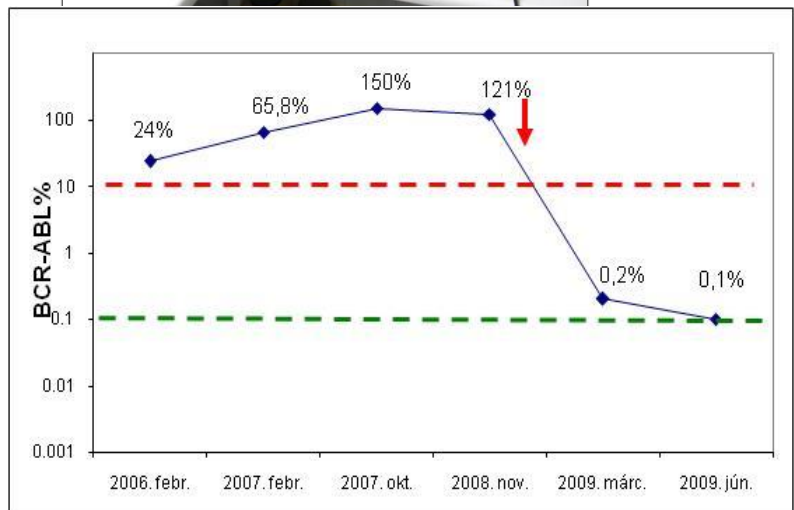
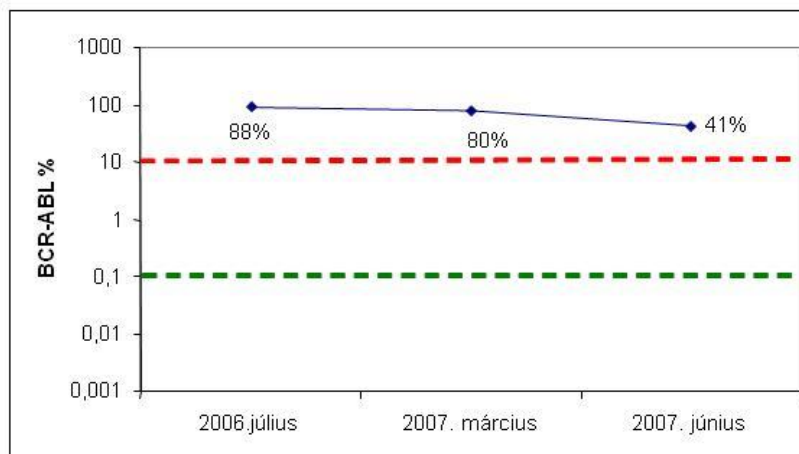
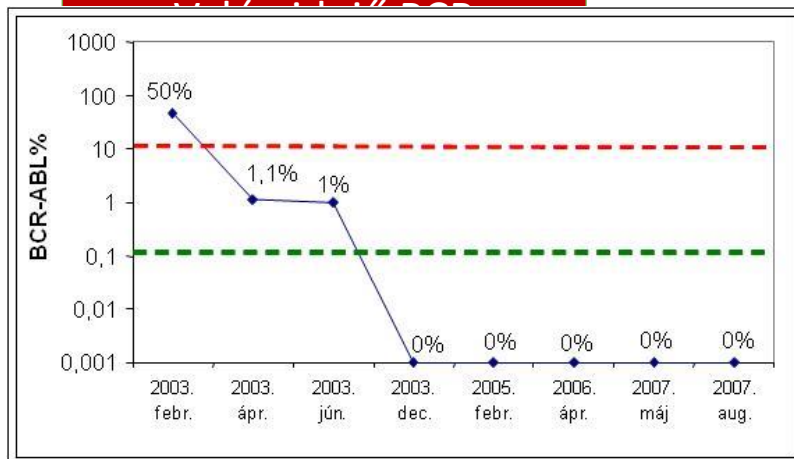
At diagnosis	Chromosome banding analysis (CBA) of marrow cell metaphases FISH in case of Ph negativity to identify variant, cryptic translocations Qualitative PCR (identification of transcript type)
During treatment	Quantitative real-time PCR (RQ-PCR) for the determination of <i>BCR-ABL1</i> transcripts level on the international scale, to be performed every 3 months until an MMR ($BCR-ABL \leq 0.1\%$, or $MR^{3.0}$) has been achieved, then every 3 to 6 months and/or CBA of marrow cell metaphases (at least 20 banded metaphases), to be performed at 3, 6, and 12 months until a CCyR has been achieved, then every 12 months. Once a CCyR is achieved, FISH on blood cells can be done. <u>If adequate molecular monitoring can be ensured, cytogenetics can be spared.</u>
Failure, progression	RQ-PCR, mutational analysis, and CBA of marrow cell metaphases. Immunophenotyping in BP.
Warning	Molecular and cytogenetic tests to be performed more frequently. CBA of marrow cell metaphases recommended in case of myelodysplasia or CCA/Ph- with chromosome 7 involvement.

Terápiás hatás felmérése

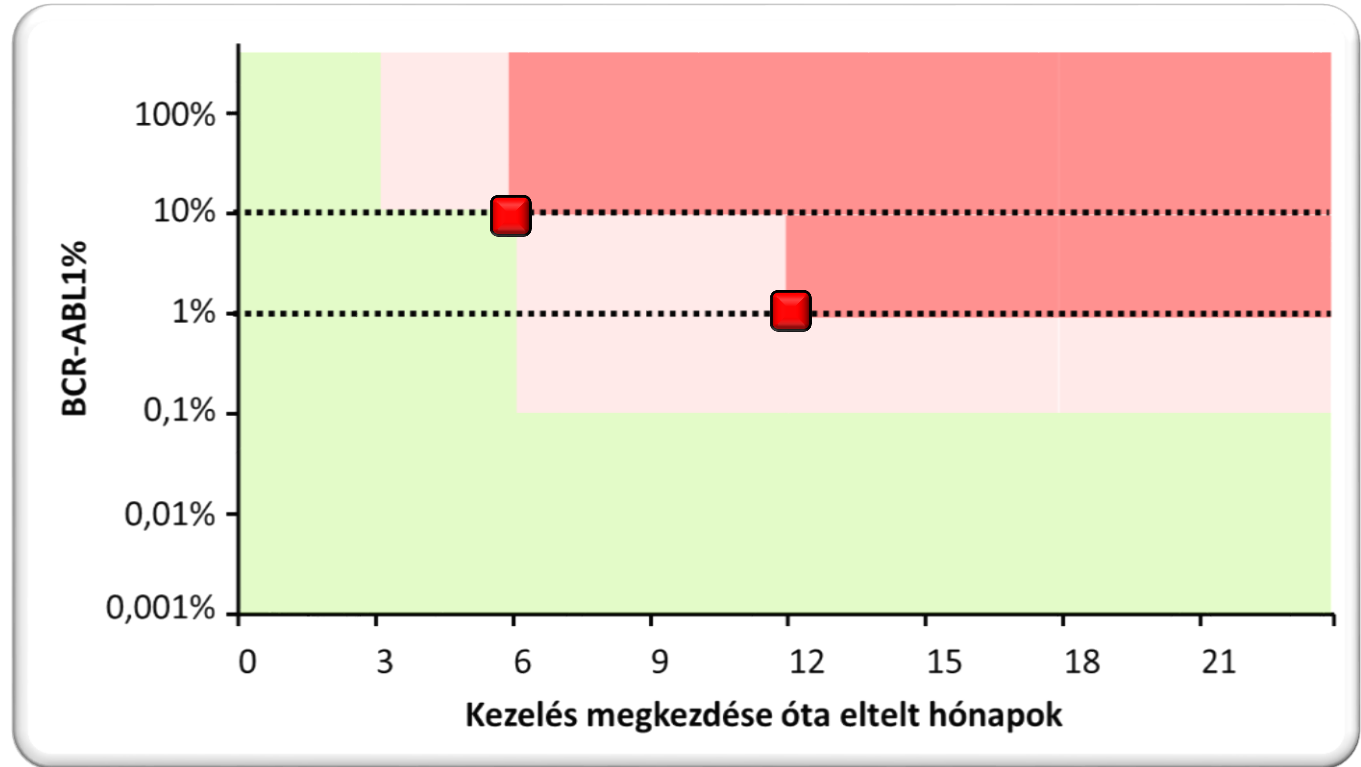
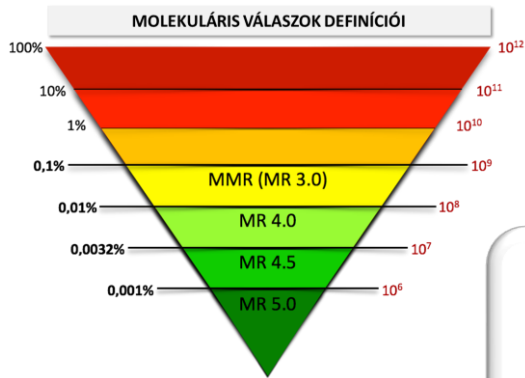
■ BCR-ABL fúziós transzkriptum

és

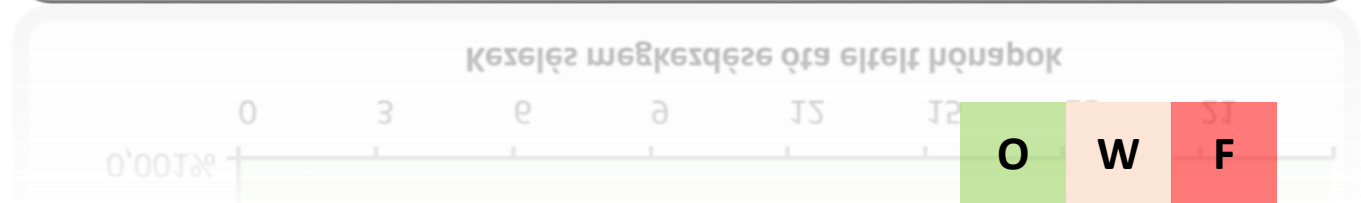
ABL kináz domén mutációk



Optimális Molekuláris Válasz



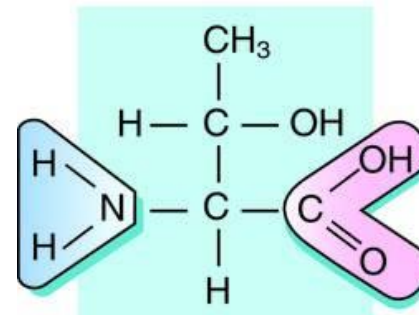
- RQ-PCR: 3 havonta
- MMR után 6 havonta



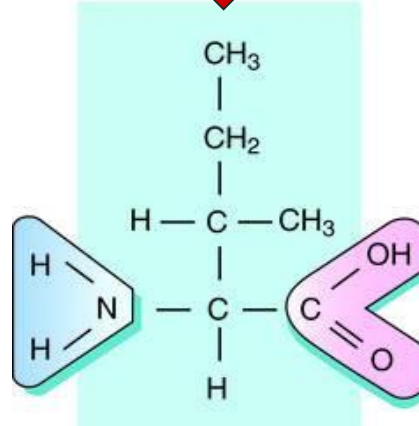
Rezisztencia mutációk az ABL kináz doménben

- „Gatekeeper” (Kapuőrző) mutáció : T 315 I (315 Thr > Ile)

Aminosav	3 betűs kód	1 betűs kód
Alanine	Ala	A
Arginine	Arg	R
Asparagine	Asn	N
Aspartic acid	Asp	D
Cysteine	Cys	C
Glutamic Acid	Glu	E
Glutamine	Gln	Q
Glycine	Gly	G
Histidine	His	H
Isoleucine	Ile	I
Leucine	Leu	L
Lysine	Lys	K
Methionine	Met	M
Phenylalanine	Phe	F
Proline	Pro	P
Serine	Ser	S
Threonine	Thr	T
Tryptophan	Trp	W
Tyrosine	Tyr	Y
Valine	Val	V

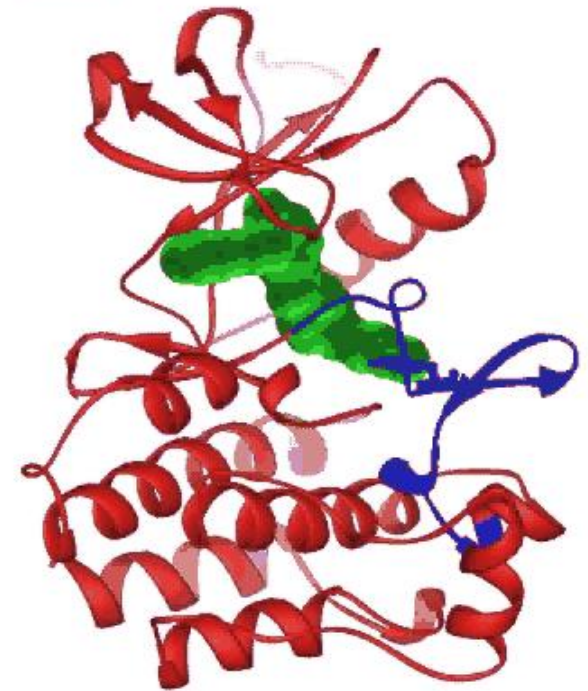


Threonin (T)

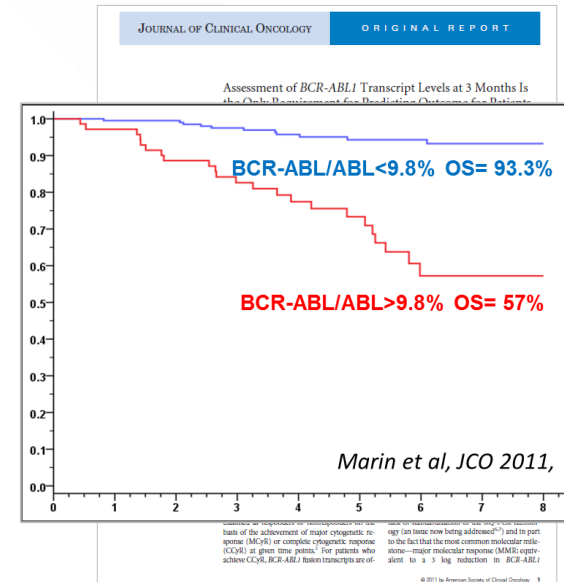
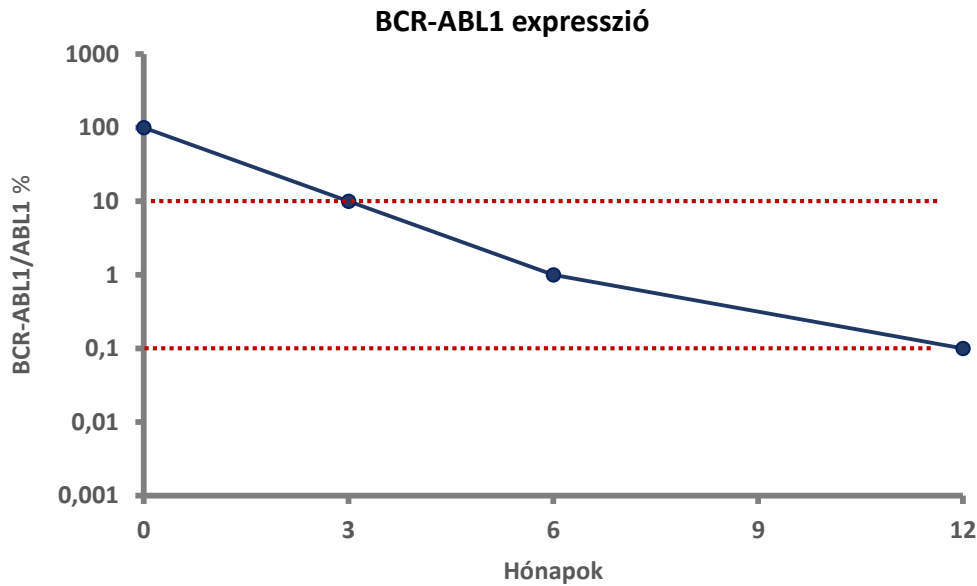
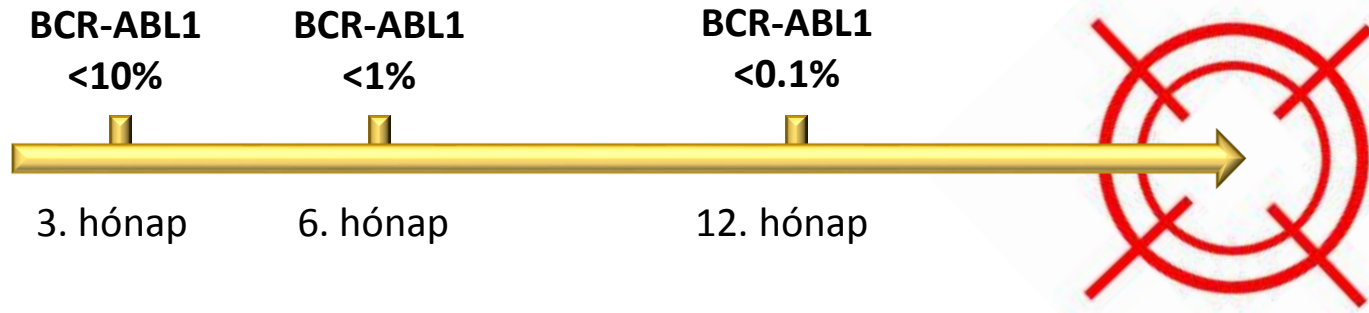


Izoleucin (I)

Abl

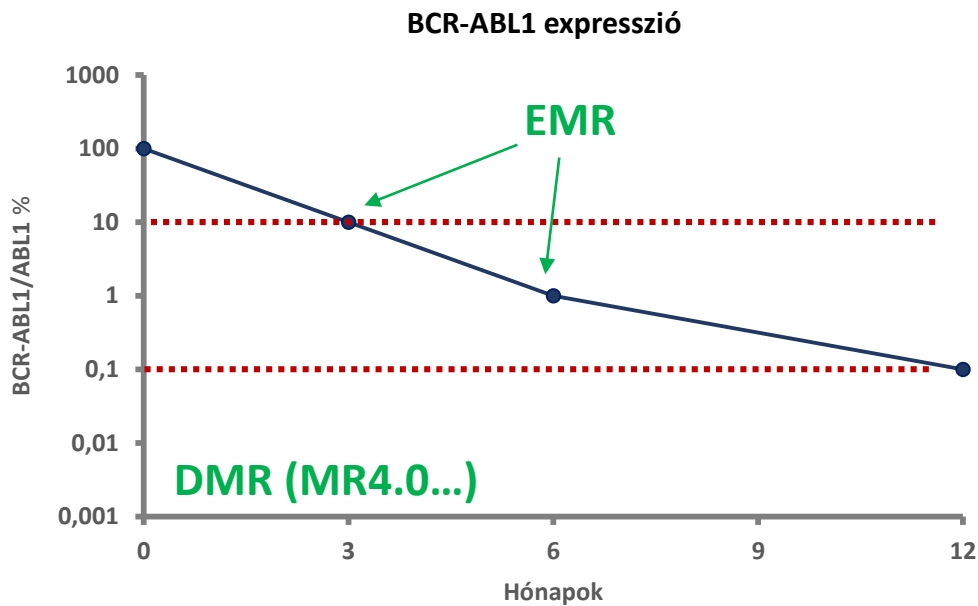
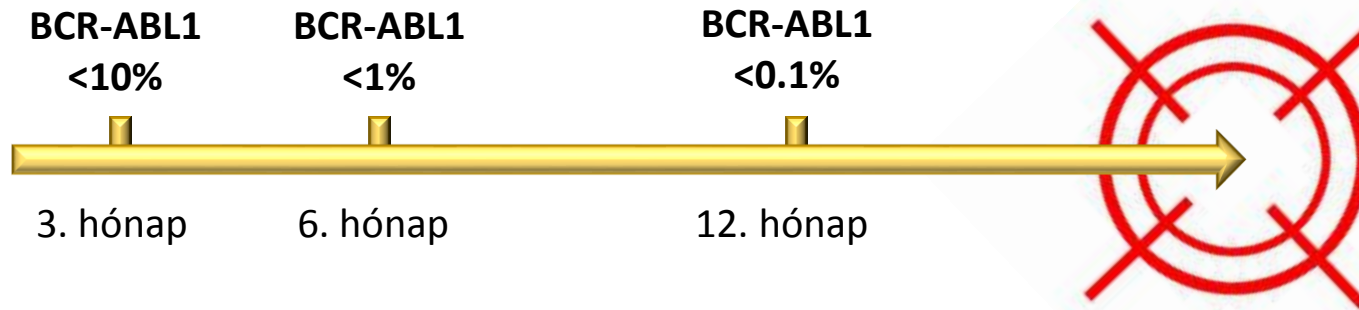


MOLEKULÁRIS MONITOROZÁS: ELNET 2013



Marin et al, JCO 2011

MOLEKULÁRIS MONITOROZÁS: ELNET 2013



„better outcome,
deeper response”

Hanfstein, Leukemia 2012;
Marin, Blood 2012;
Jain, Blood 2013;
Hughes, Blood 2014;
Jabbour, Blood 2014

EMR : early molecular response
DMR : deep molecular response

Blood Forum

The price of drugs for chronic myeloid leukemia (CML) is a reflection of the unsustainable prices of cancer drugs: from the perspective of a large group of CML experts

Experts in Chronic Myeloid Leukemia

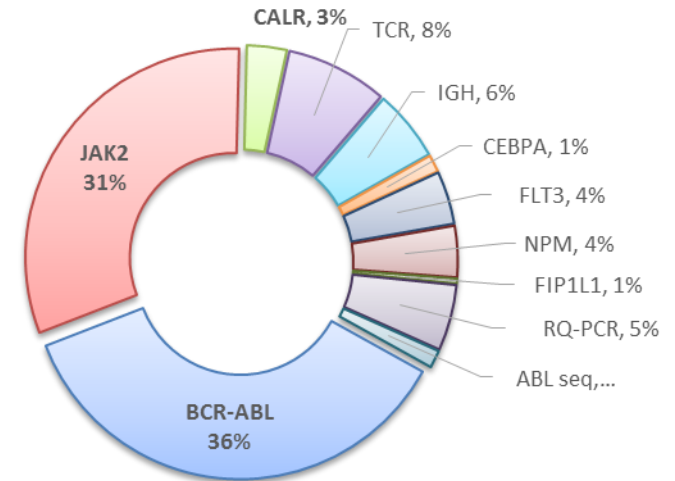
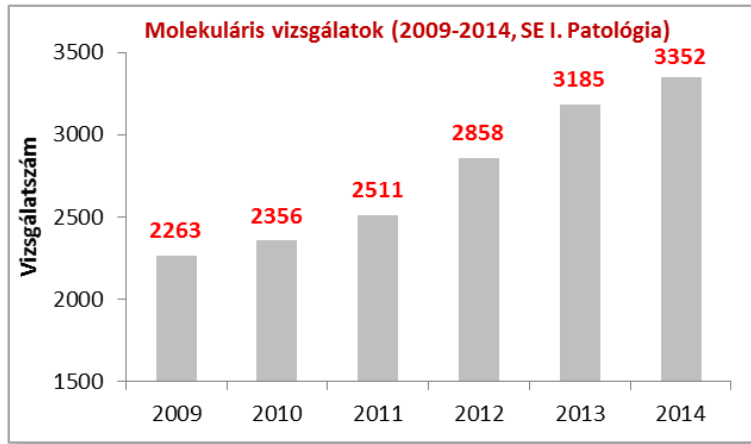
As a group of more than 100 experts in chronic myeloid leukemia (CML), we draw attention to the high prices of cancer drugs, with the particular focus on the prices of approved tyrosine kinase in-

hibitors for the treatment of CML. This editorial addresses the multiple factors involved in cancer drug pricing and their impact on individual patients and health care policies, and argues for the need to

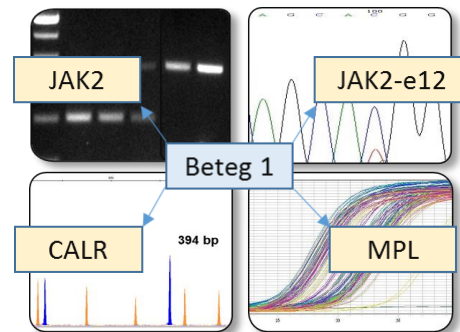
(1) lower the prices of cancer drugs to allow more patients to afford them and (2) maintain sound long-term health care policies. (*Blood*. 2013;121(22):4439-4442)



Molekuláris Diagnosztika – Onkohematológia

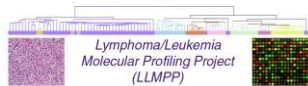


■ Jelenlegi stratégia



KÖSZÖNETNYILVÁNÍTÁS

- Király Péter
- Marosvári Dóra
- Bárányné Pallag Adrienne
- Gulácsi Edina
- Nagy Veronika
- Gángó Ambrus
- Kiss Richárd
- Boha Zsófi
- Dénes Kitti
- Fesüs Viki
- Rupnik Zsuzsi
- Rác Réka
- **Matolcsy András**
- **Csomor Judit**
- **Csernus Balázs**
- **Mózes Réka**
- **Rajnai Hajnalka**
- **Szepesi Ágota**
- **Timár Botond**



Bristol-Myers Squibb

