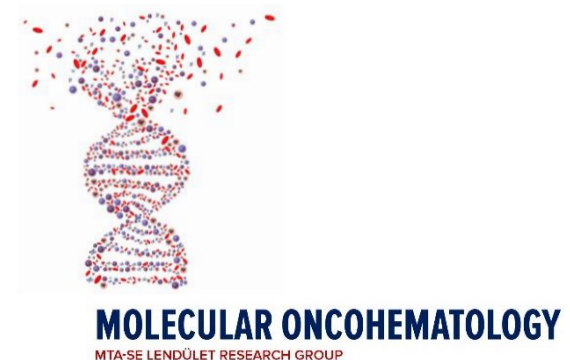


A myeloproliferatív betegségek molekuláris diagnosztikája az új vizsgálati módszerek és WHO felosztás tükrében

Bödör Csaba

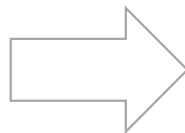


Semmelweis Egyetem
Patológiai és Kísérleti Rákkutató Intézet,
Molekuláris Diagnosztika Részleg
HCEMM-SE Molekuláris Onkohematológia Kutatócsoport

Genetikai/Diagnosztikai vizsgálómódszerek evolúciója



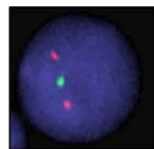
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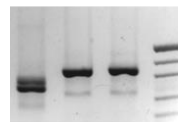
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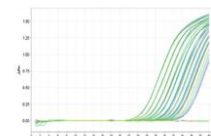
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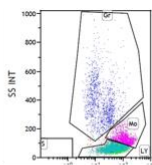
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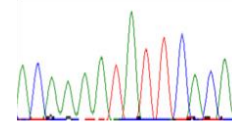
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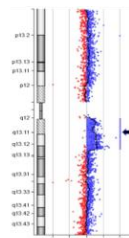
Q-PCR



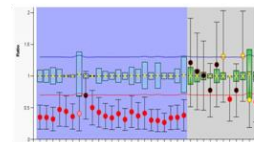
flow



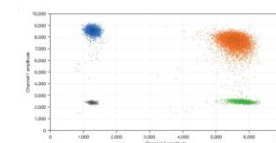
Sanger



array-CGH



MLPA, dMLPA



ddPCR

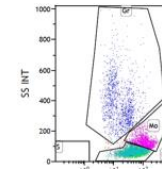


NGS

Molekuláris vizsgálatok szerepe az onkohematológiában

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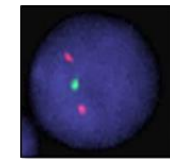
- Klonalitás kimutatás
- Diagnózis megerősítés
- Genetikai szubklasszifikáció
- Prognózis becslés
- Kezelés megválasztás
- MRD monitorozás
- Rezisztencia előrejelzés
- Mintaküldés, érzékenység fontossága
- „TAT” ?



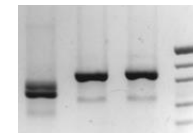
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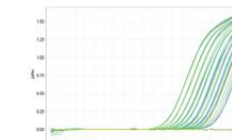
kariotipizálás



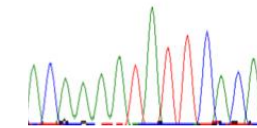
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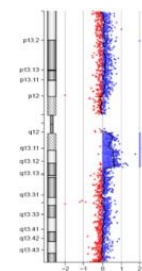
PCR



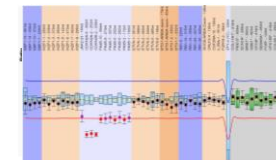
Q-PCR



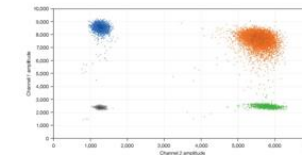
Sanger



array-CGH



MLPA, dMLPA



ddPCR

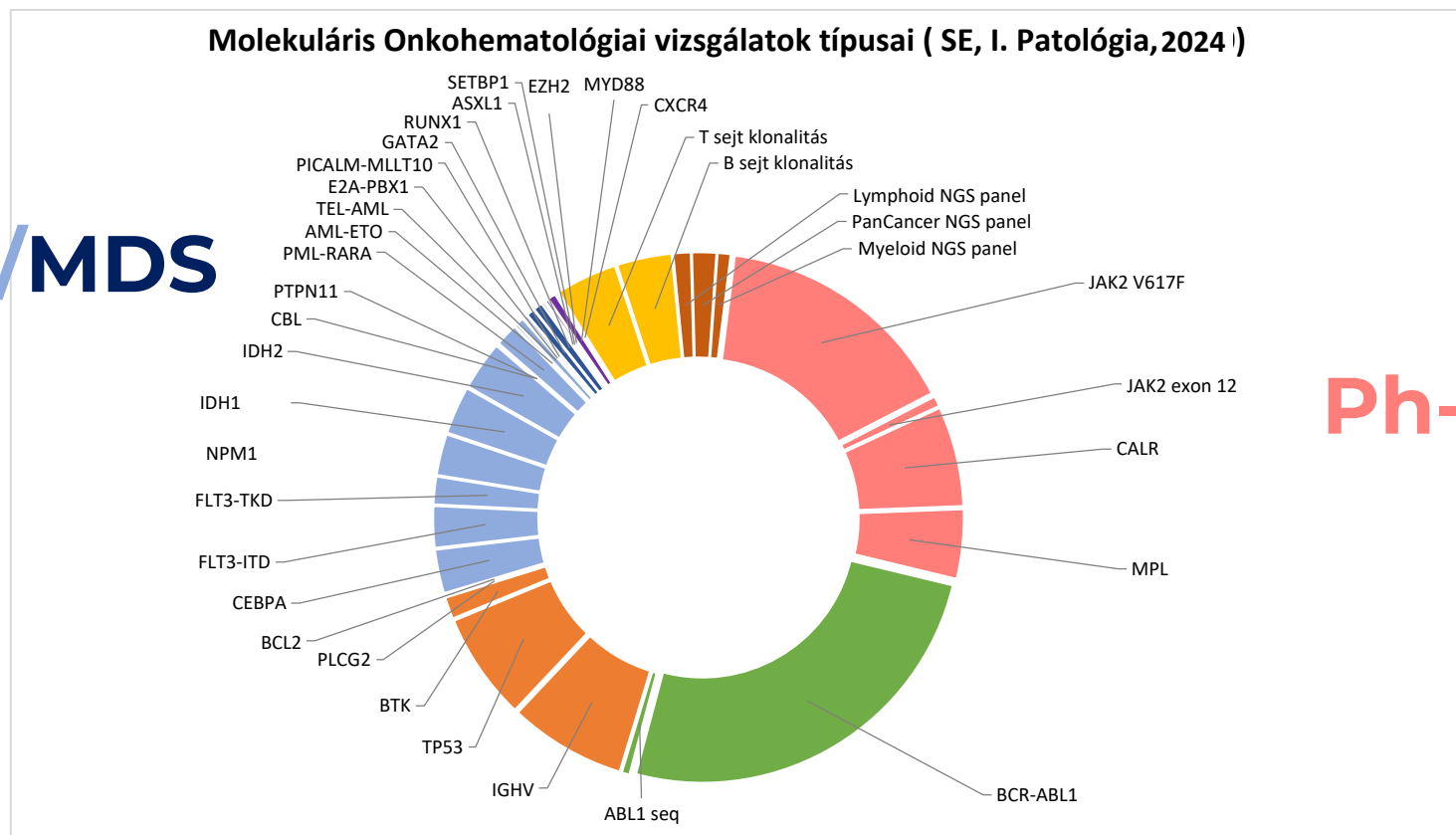


NGS

Molekuláris vizsgálatok Myeloproliferatív kórképekben

AML/MDS

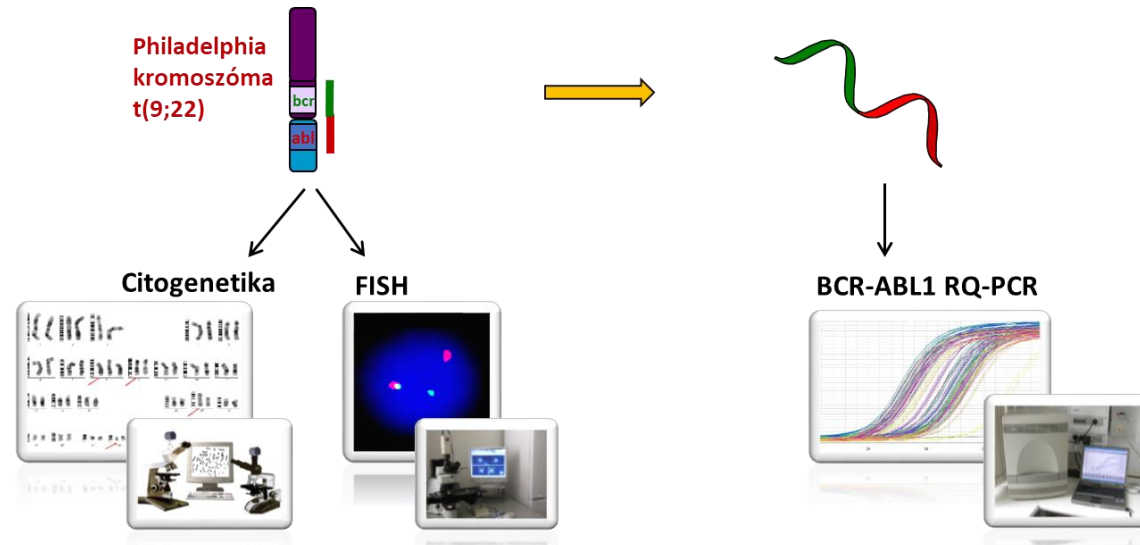
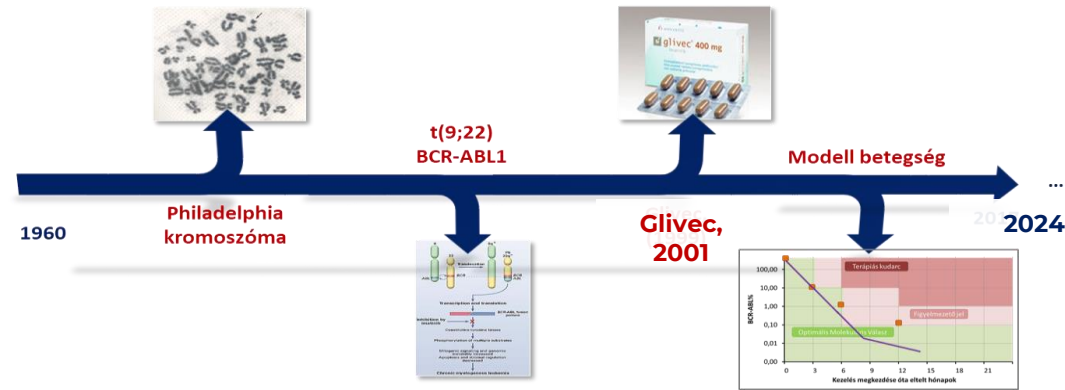
Molekuláris Onkohematológiai vizsgálatok típusai (SE, I. Patológia, 2024)



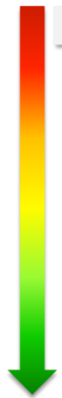
Ph-MPN

CML

Molekuláris vizsgálatok jelentősége CML-ben



Molekuláris vizsgálatok jelentősége CML-ben

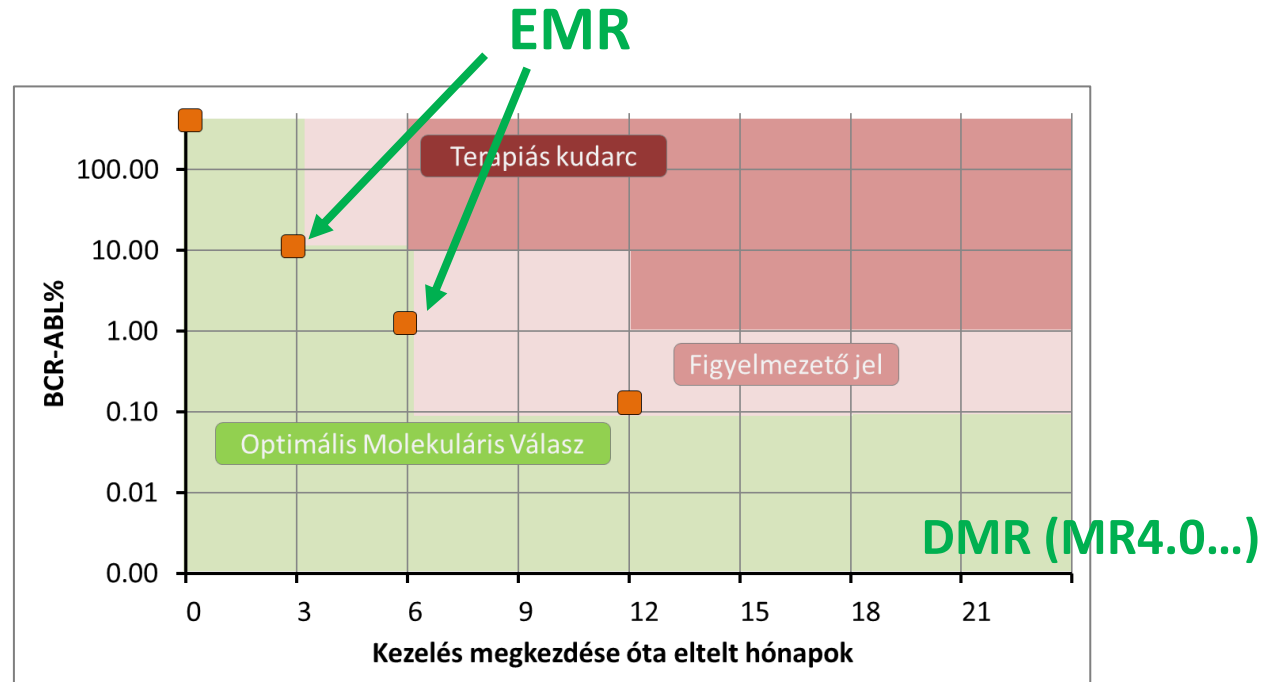


OPTIMÁLIS VÁLASZ

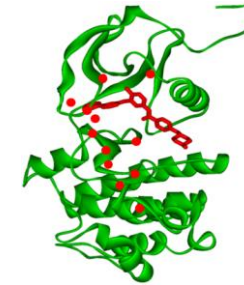
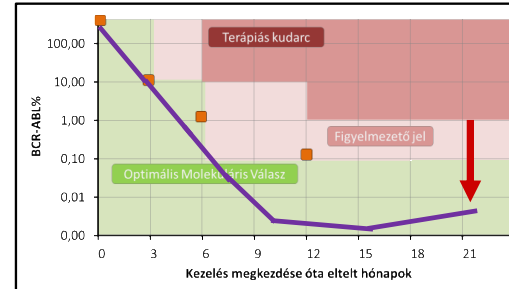
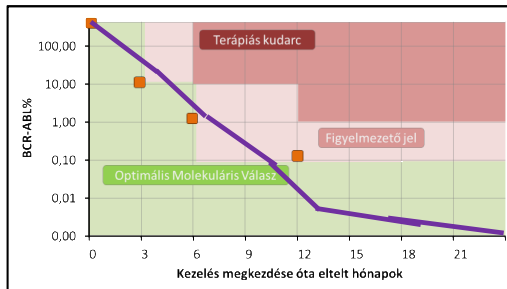
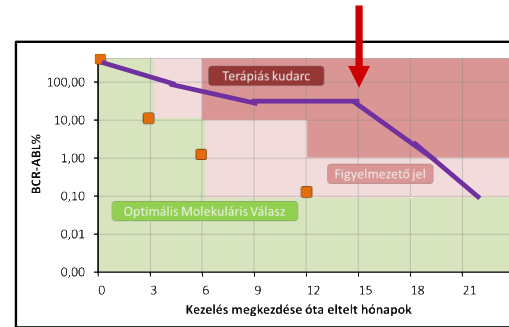
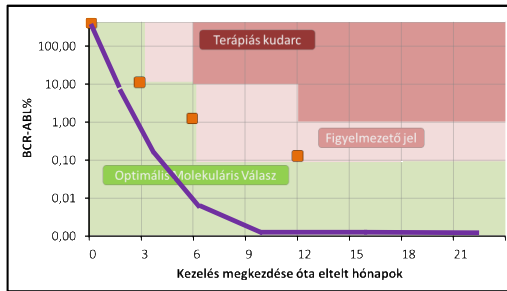
3. hónap	BCR-ABL1 <10%
6. hónap	BCR-ABL1 <1%
12. hónap	BCR-ABL1 <0,1%

Terápiás kudarc

6. hónap	BCR-ABL1 >10%
12. hónap	BCR-ABL1 >1%

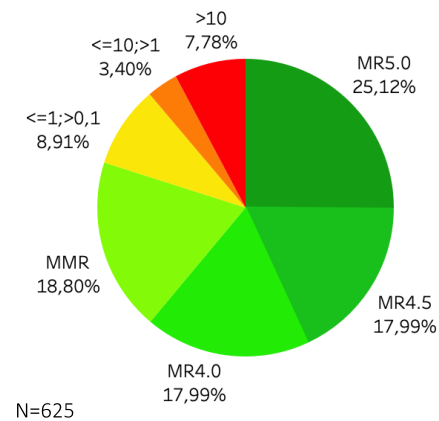


Molekuláris vizsgálatok jelentősége CML-ben

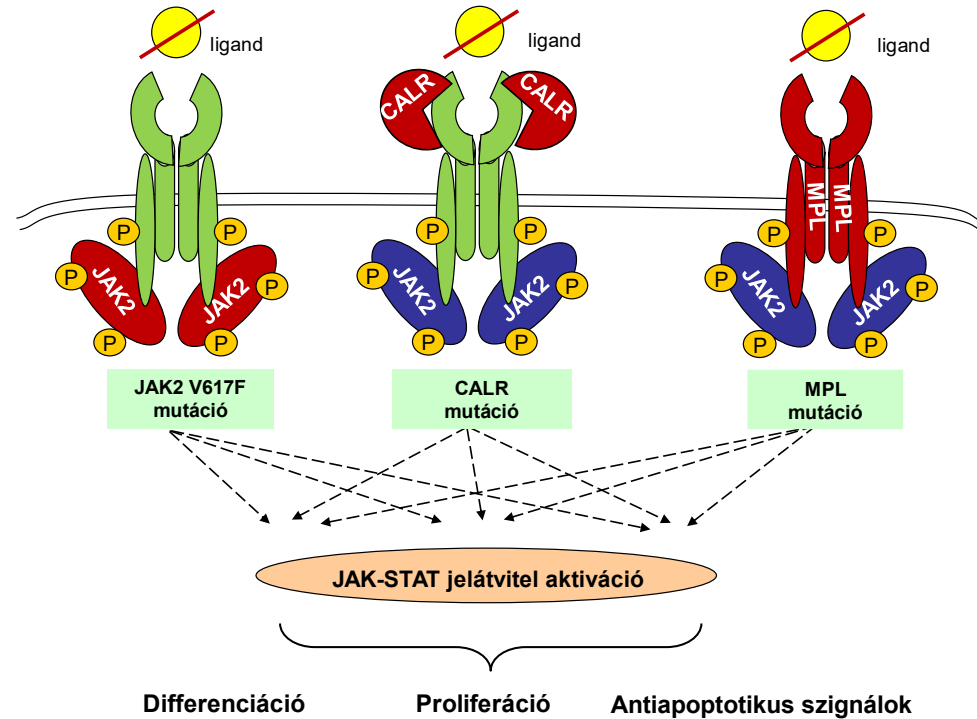
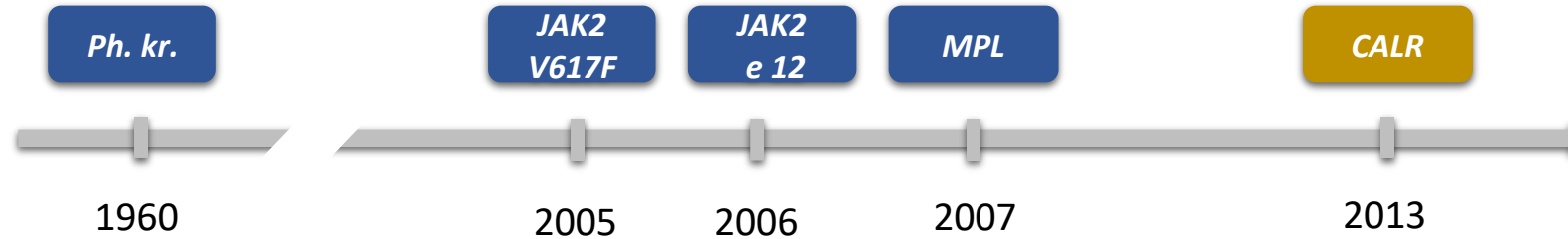


Mutáció	Dasatinib	Nilotinib
Q252H	Red	Green
Y253H	Green	Red
E255K/V	Green	Red
V299L	Red	Green
T315I	Red	Red
T315A	Red	Green
F317L/I	Red	Green
F359V/L	Green	Red

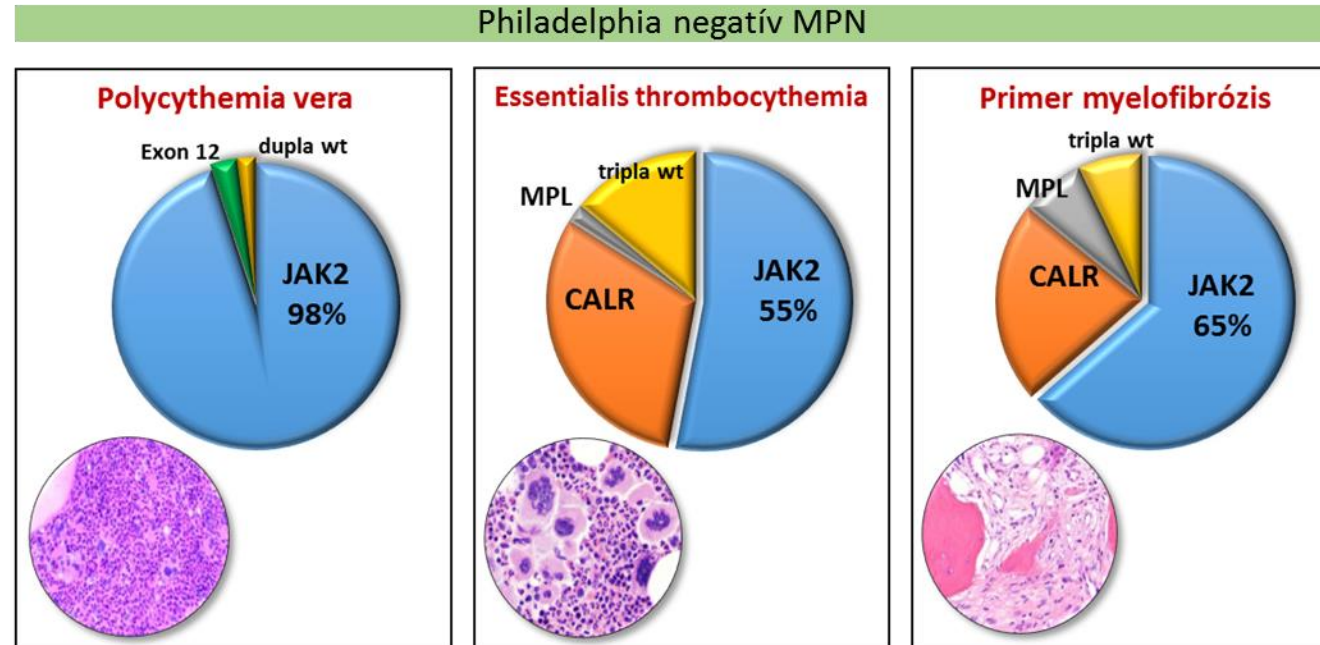
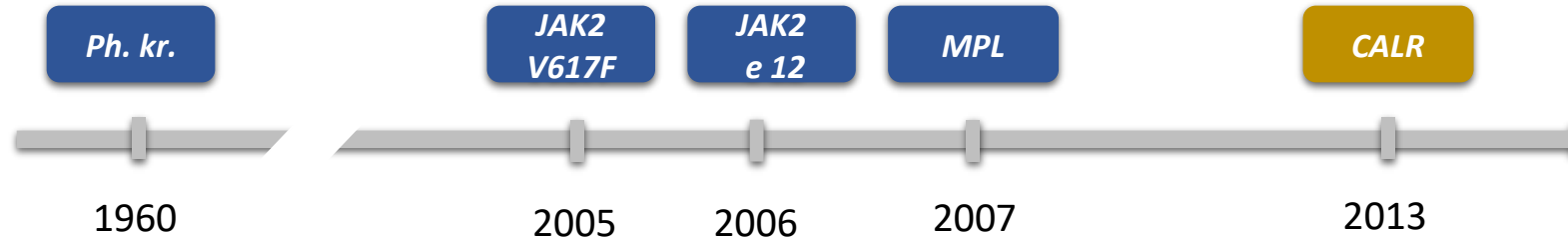
Molekuláris válaszok (n=625)



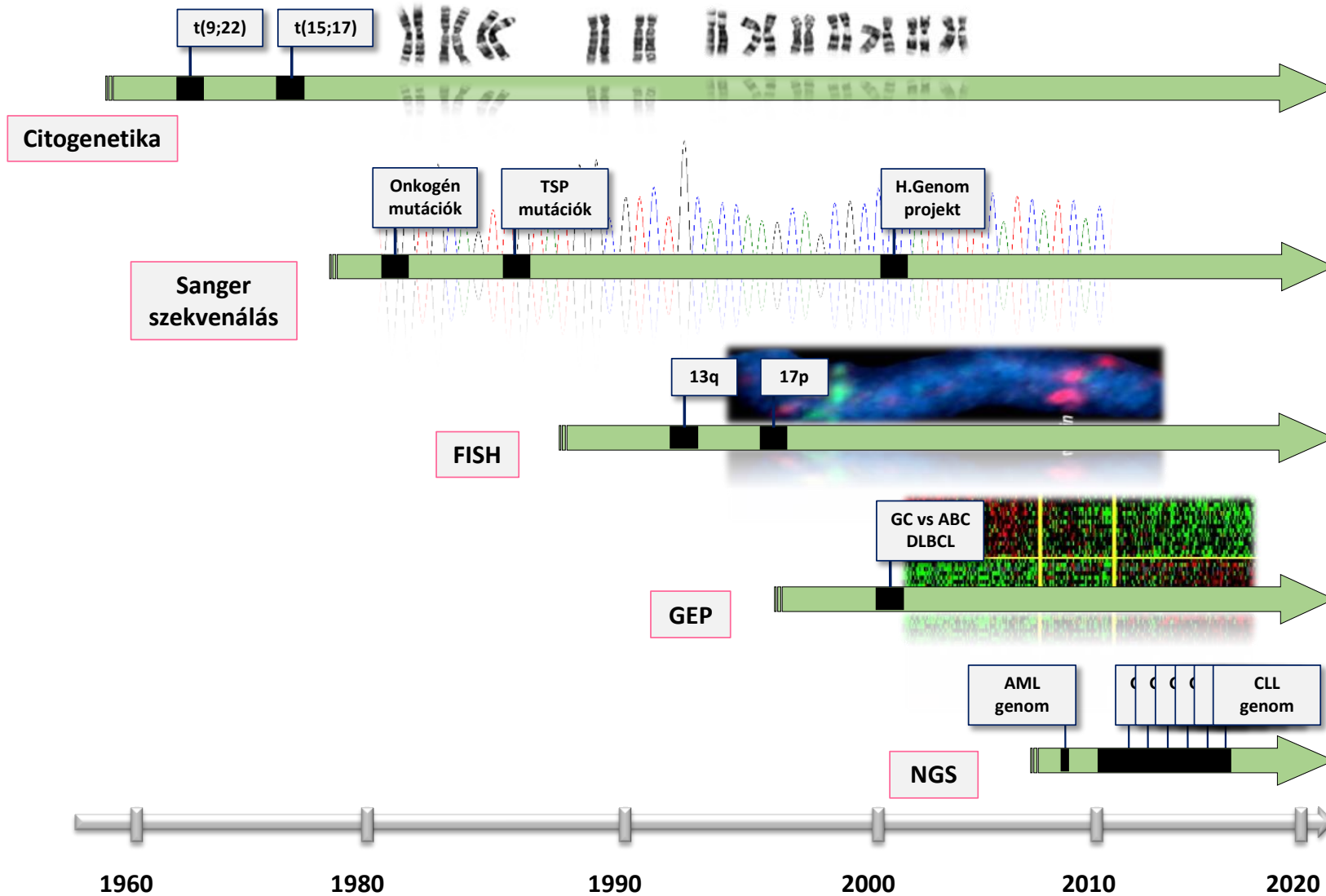
Molekuláris vizsgálatok jelentősége Ph- MPN-ben



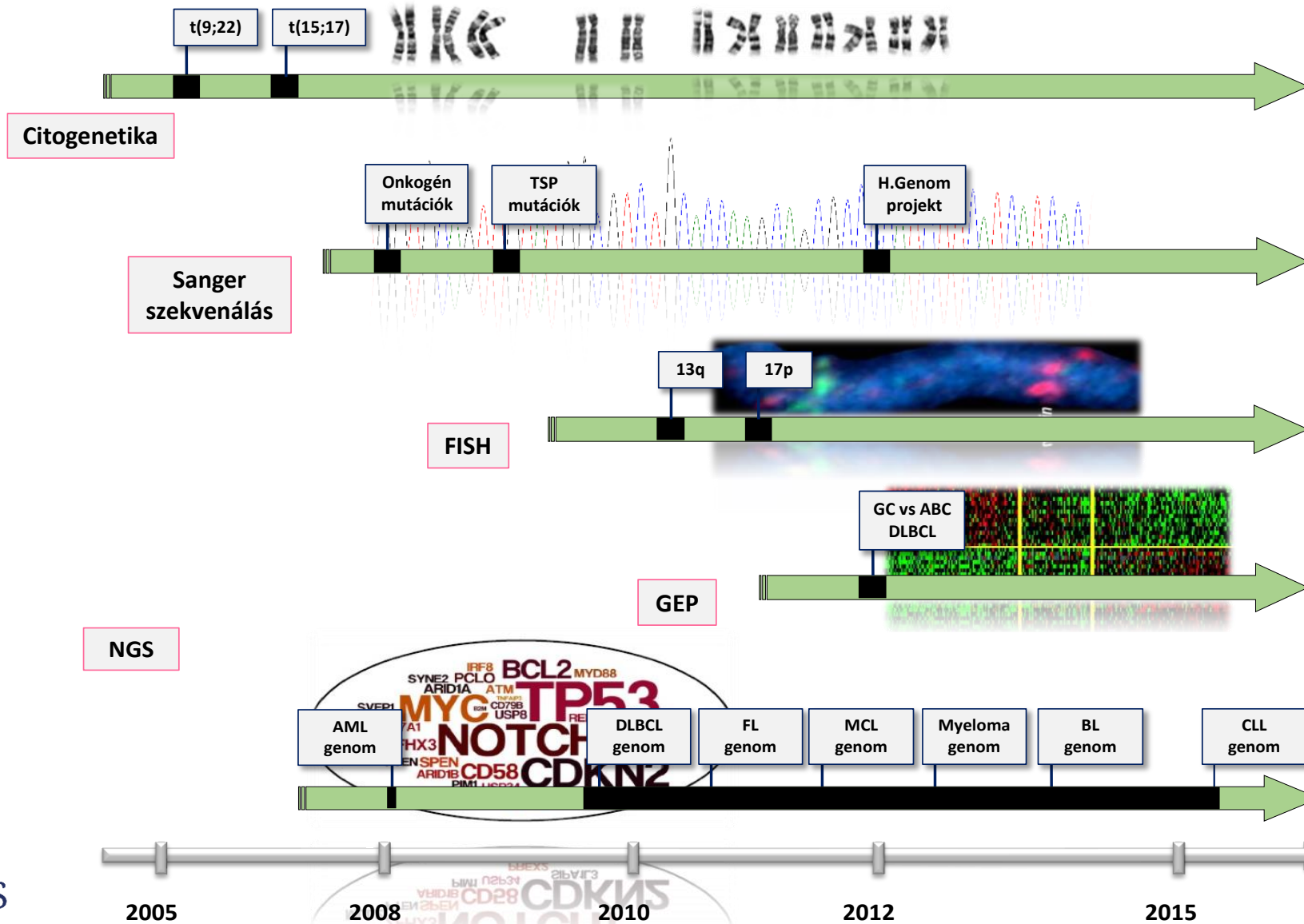
Molekuláris vizsgálatok jelentősége Ph- MPN-ben



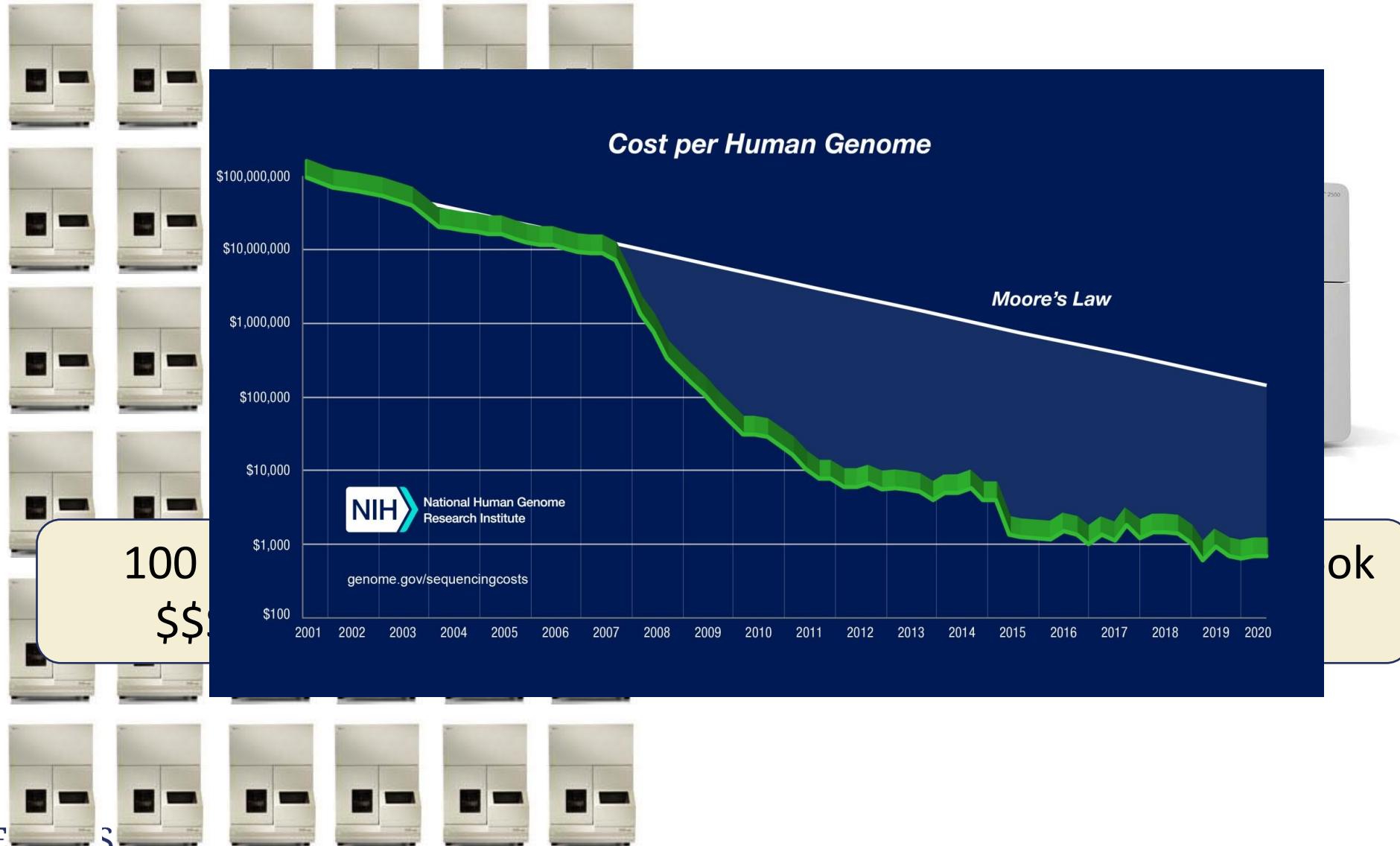
Genetikai vizsgálómódszerek evolúciója I.



Genetikai vizsgálómódszerek evolúciója II.

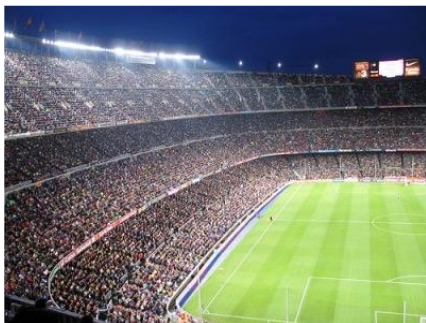


Az újgenerációs szekvenálás (NGS) forradalma



Az újgenerációs szekvenálás lehetőségei (NGS)

- Teljes genom szekvenálás (whole genome sequencing; **WGS**)
- Teljes exom szekvenálás (whole exome sequencing; **WES**)
- Célzott újraszekvenálás (targeted resequencing; **TS**) > ...”panel” ...

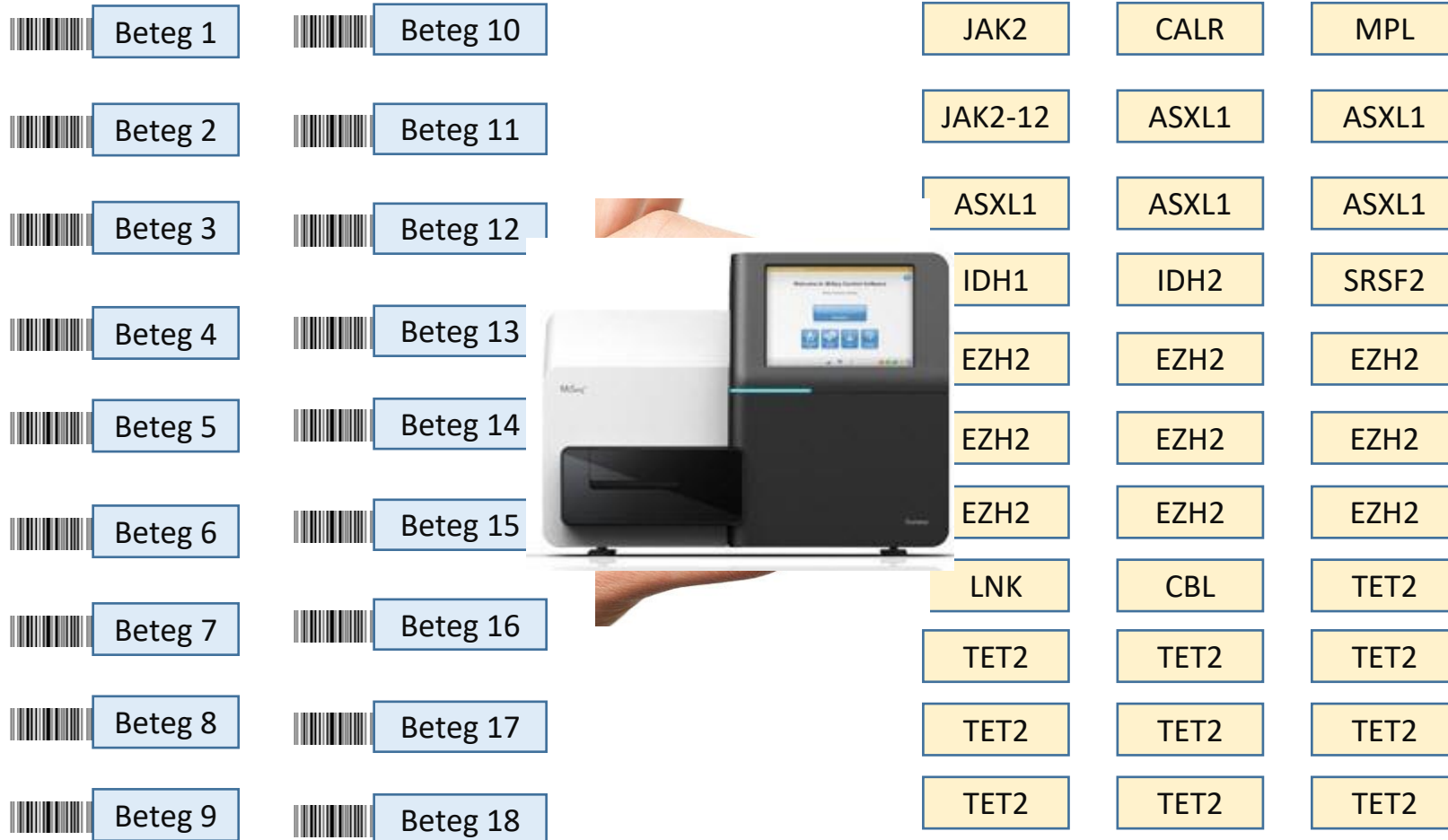


Teljes Genom WGS
3 milliárd nukleotid










```
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AAGCGGTTAAGTCAG  TACGAGTGGGCGT  TTGGCAGTGGGCGT  GG
  CGGTTAAGTCAGCTTTGGC  TGGGCGTGTGGCAGTGGGCGTAGG
AGCGGTTAAGTCAGCTTTGGCAGT  GCGTGTGGCA - - - GGCGTAG
  CGGTTAAGTCAGCTTTGGCAGTGGGC  TTGGCA - - - GGCGTAGG
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AAGCGGTTAAGTCAGCTTTGGCAGT  GCGTGTGGCA - - - GGCGTAGG
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  GCGGTTAA  CAGCTTTGGCAGTGGGCG  GTTGGCA - - - GGCGTAGG
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NGS stratégia



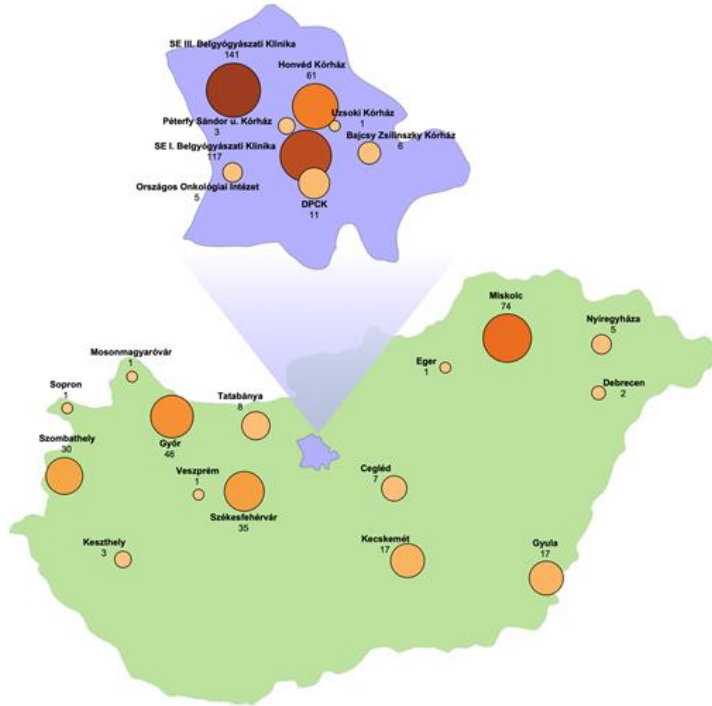
NGS stratégia

	Beteg 1	JAK2	CALR	MPL	JAK2-12	ASXL1	IDH1	IDH2	SRSF2	EZH2
	Beteg 2	JAK2	CALR	MPL	JAK2-12	ASXL1	IDH1	IDH2	SRSF2	EZH2
	Beteg 3	JAK2	CALR	MPL	JAK2-12	ASXL1	IDH1	IDH2	SRSF2	EZH2
	Beteg 4	JAK2	CALR	MPL	JAK2-12	ASXL1	IDH1	IDH2	SRSF2	EZH2
	Beteg 5	JAK2	CALR	MPL	JAK2-12	ASXL1	IDH1	IDH2	SRSF2	EZH2
	Beteg 6	JAK2	CALR	MPL	JAK2-12	ASXL1	IDH1	IDH2	SRSF2	EZH2
	Beteg 7	JAK2	CALR	MPL	JAK2-12	ASXL1	IDH1	IDH2	SRSF2	EZH2
	Beteg 8	JAK2	CALR	MPL	JAK2-12	ASXL1	IDH1	IDH2	SRSF2	EZH2
	Beteg 9	JAK2	CALR	MPL	JAK2-12	ASXL1	IDH1	IDH2	SRSF2	EZH2

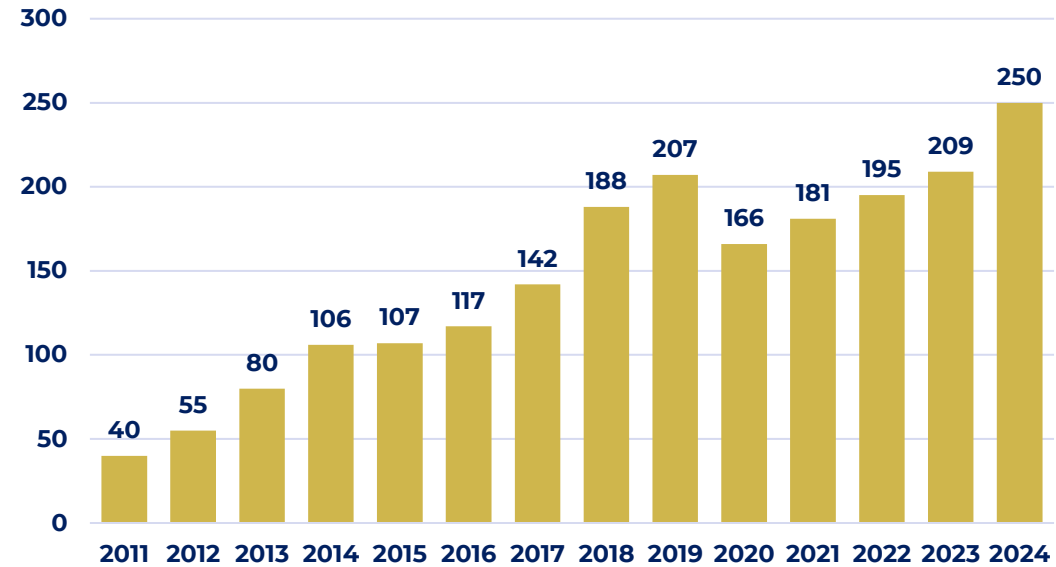
NGS stratégia

	Beteg 1	JAK2	CALR	MPL	JAK2-12	ASXL1	IDH1	IDH2	SRSF2	EZH2
	Beteg 2	JAK2	CALR	MPL	JAK2-12	ASXL1	IDH1	IDH2	SRSF2	EZH2
	Beteg 3	JAK2	CALR	MPL	JAK2-12	ASXL1	IDH1	IDH2	SRSF2	EZH2
	Beteg 4	JAK2	CALR	MPL	JAK2-12	ASXL1	IDH1	IDH2	SRSF2	EZH2
	Beteg 5	JAK2	CALR	MPL	JAK2-12	ASXL1	IDH1	IDH2	SRSF2	EZH2
	Beteg 6	JAK2	CALR	MPL	JAK2-12	ASXL1	IDH1	IDH2	SRSF2	EZH2
	Beteg 7	JAK2	CALR	MPL	JAK2-12	ASXL1	IDH1	IDH2	SRSF2	EZH2
	Beteg 8	JAK2	CALR	MPL	JAK2-12	ASXL1	IDH1	IDH2	SRSF2	EZH2
	Beteg 9	JAK2	CALR	MPL	JAK2-12	ASXL1	IDH1	IDH2	SRSF2	EZH2

Akut Myeloid Leukémia (AML)



AML diagnózisok 2011-2024 (SE, Patológiai Intézet)



Akut Myeloid Leukémia (AML)

EREDETI KÖZLEMÉNY

Visszatérő genetikai eltérések vizsgálata akut myeloid leukémiában az új célzott terápiák tükrében

Krizsán Szilvia¹, Dénes Zsófia¹, Gángó Ambrus¹, Gerecs Bence¹, Demeter Judit²,
Nagy Zsolt², Tárkányi Ilona², Masszi Tamás³, Farkas Péter³, Masszi András³,
Szombath Gergely³, Benedek Szabolcs³, Várkonyi Judit³, Horváth Laura³,
Nagy Zsolt⁴, Radványi Gáspár⁴, Takács István⁴, Hamed Aryan⁵, Lázár Zsolt⁵,
Süveges Erzsébet⁶, Kárpáti Ágnes⁶, Plander Márk⁷, Szendrei Tamás⁷, Pál Katalin⁸,
Gurzó Mihály⁹, Jakucs János¹⁰, Egyed Miklós¹¹, Bödör Csaba^{1,*}

¹Magyar Tudományos Akadémia, Semmelweis Egyetem Lendület Molekuláris Onkohematológia Kutatócsoport,
Semmelweis Egyetem, I. sz. Patológiai és Kísérleti Rákkutató Intézet, Budapest

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⁴Borsod-Abaúj-Zemplén Megyei Központi Kórház és Egyetemi Oktatókórház, Miskolc

⁵Petz Aladár Megyei Oktató Kórház, Győr

⁶Magyar Honvédség Egészségügyi Központ, Budapest

⁷Markusovszky Egyetemi Oktató Kórház, Szombathely

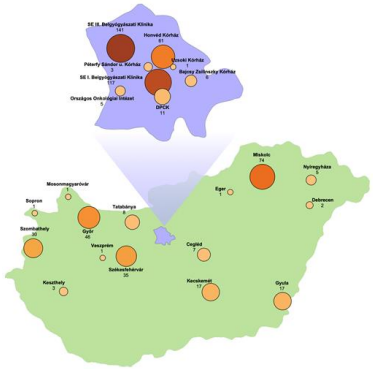
⁸Fejér Megyei Szent György Egyetemi Oktató Kórház, Székesfehérvár

⁹Bács-Kiskun Megyei Kórház, Kecskemét

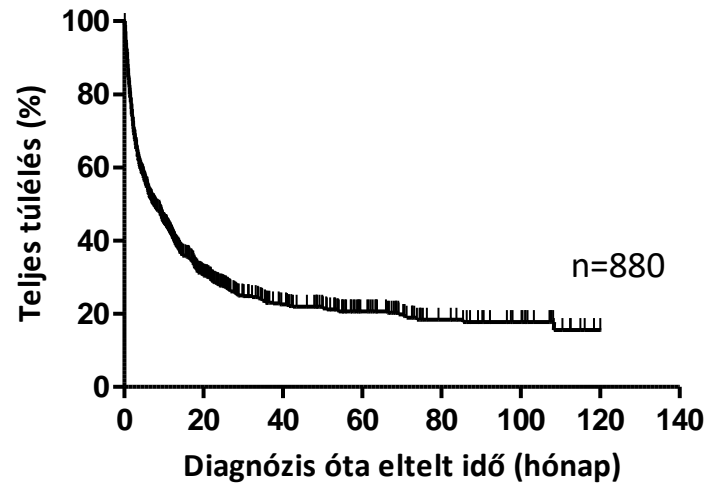
¹⁰Békés Megyei Pándy Kálmán Kórház, Gyula

¹¹Somogy Megyei Kaposi Mór Oktató Kórház, Kaposvár

Akut Myeloid Leukémia (AML)



I. sz. Patológiai és Kísérleti Rákkutató Intézetben
2008-2018 között diagnosztizált 880 AML-es
betegek teljes túlélése



5 éves teljes
túlélés

20,7%

Az AML molekuláris diagnosztikája

**WHO
osztályozás**

**ELN
rizikóstr.**

**Terápia-
tervezés**

Az AML molekuláris diagnosztikája

WHO
osztályozás

ELN
rizikóstr.

Terápia-
tervezés

Table 7. Acute myeloid leukaemia.

Acute myeloid leukaemia with defining genetic abnormalities
Acute promyelocytic leukaemia with <i>PML-RARA</i> fusion
Acute myeloid leukaemia with <i>RUNX1-RUNX1T1</i> fusion
Acute myeloid leukaemia with <i>CBFB-MYH11</i> fusion
Acute myeloid leukaemia with <i>DEK-NUP214</i> fusion
Acute myeloid leukaemia with <i>RBM15-MRTFA</i> fusion
Acute myeloid leukaemia with <i>BCR-ABL1</i> fusion
Acute myeloid leukaemia with <i>KMT2A</i> rearrangement
Acute myeloid leukaemia with <i>MECOM</i> rearrangement
Acute myeloid leukaemia with <i>NUP98</i> rearrangement
Acute myeloid leukaemia with <i>NPM1</i> mutation
Acute myeloid leukaemia with <i>CEBPA</i> 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

Table 8. Cytogenetic and molecular abnormalities defining acute myeloid leukaemia, myelodysplasia-related.

Defining cytogenetic abnormalities
Complex karyotype (≥ 3 abnormalities)
5q deletion or loss of 5q due to unbalanced translocation
Monosomy 7, 7q deletion, or loss of 7q due to unbalanced translocation
11q deletion
12p deletion or loss of 12p due to unbalanced translocation
Monosomy 13 or 13q deletion
17p deletion or loss of 17p due to unbalanced translocation
Isochromosome 17q
idic(X)(q13)
Defining somatic mutations
<i>ASXL1</i>
<i>BCOR</i>
<i>EZH2</i>
<i>SF3B1</i>
<i>SRSF2</i>
<i>STAG2</i>
<i>U2AF1</i>
<i>ZRSR2</i>

WHO klasszifikáció molekuláris alapokon

REVIEW ARTICLE OPEN

 Check for updates

The 5th edition of the World Health Organization Classification of Haematolymphoid Tumours: Myeloid and Histiocytic/Dendritic Neoplasms

Joseph D. Khoury ¹✉, Eric Solary ²✉, Oussama Ablal³, Yasmine Akkari ⁴, Rita Alaggio⁵, Jane F. Apperley ⁶, Rafael Bejar ⁷, Emilio Berti⁸, Lambert Busque ⁹, John K. C. Chan¹⁰, Weina Chen ¹¹, Xueyan Chen¹², Wee-Joo Chng¹³, John K. Choi ¹⁴, Isabel Colmenero ¹⁵, Sarah E. Coupland¹⁶, Nicholas C. P. Cross ¹⁷, Daphne De Jong¹⁸, M. Tarek Elghetany¹⁹, Emiko Takahashi ²⁰, Jean-Francois Emile ²¹, Judith Ferry²², Linda Fogelstrand²³, Michaela Fontenay²⁴, Ulrich Germing²⁵, Sumeet Gujral²⁶, Torsten Haferlach ²⁷, Claire Harrison²⁸, Jennelle C. Hodge²⁹, Shimin Hu ¹, Joop H. Jansen³⁰, Rashmi Kanagal-Shamanna ¹, Hagop M. Kantarjian ³¹, Christian P. Kratz ³², Xiao-Qiu Li³³, Megan S. Lim³⁴, Keith Loeb³⁵, Sanam Loghavi ¹, Andrea Marcogliese¹⁹, Soheil Meshinchi³⁶, Phillip Michaels³⁷, Kikkeri N. Naresh ³⁵, Yasodha Natkunam ³⁸, Reza Nejati³⁹, German Ott⁴⁰, Eric Padron ⁴¹, Keyur P. Patel¹, Nikhil Patkar ⁴², Jennifer Picarsic⁴³, Uwe Platzbecker ⁴⁴, Irene Roberts⁴⁵, Anna Schuh ⁴⁶, William Sewell⁴⁷, Reiner Siebert⁴⁸, Prashant Tembhare ⁴², Jeffrey Tyner ⁴⁹, Srdan Verstovsek ³¹, Wei Wang ¹, Brent Wood⁵⁰, Wenbin Xiao ⁵¹, Cecilia Yeung ³⁵ and Andreas Hochhaus ⁵²✉

WHO klasszifikáció molekuláris alapokon

Table 7. Acute myeloid leukaemia.

Acute myeloid leukaemia with defining genetic abnormalities
Acute promyelocytic leukaemia with <i>PML::RARA</i> fusion
Acute myeloid leukaemia with <i>R</i>
Acute myeloid leukaemia with <i>C</i>
Acute myeloid leukaemia with <i>D</i>
Acute myeloid leukaemia with <i>R</i>
Acute myeloid leukaemia with <i>B</i>
Acute myeloid leukaemia with <i>K</i>
Acute myeloid leukaemia with <i>M</i>
Acute myeloid leukaemia with <i>N</i>
Acute myeloid leukaemia with <i>N</i>
Acute myeloid leukaemia with <i>C</i>
Acute myeloid leukaemia, myelo
Acute myeloid leukaemia with o
Acute myeloid leukaemia, defined b
Acute myeloid leukaemia with <i>n</i>
Acute myeloid leukaemia without
Acute myeloid leukaemia with <i>n</i>
Acute basophilic leukaemia
Acute myelomonocytic leukaemia
Acute monocytic leukaemia
Acute erythroid leukaemia
Acute megakaryoblastic leukaemia

Table 8. Cytogenetic and molecular abnormalities defining acute myeloid leukaemia, myelodysplasia-related.

Defining cytogenetic abnormalities
location
balanced
inslocation
inslocation
<i>SRSF2</i>
<i>STAG2</i>
<i>U2AF1</i>
<i>ZRSR2</i>

Summary Box:

- AML is arranged into two families: AML with *defining genetic abnormalities* and AML *defined by differentiation*. AML, NOS is no longer applicable.
- Most AML with defining genetic abnormalities may be diagnosed with <20% blasts.
- AML-MR replaces the former term AML “with myelodysplasia-related changes”, and its diagnostic criteria are updated. AML transformation of MDS and MDS/MPN continues to be defined under AML-MR in view of the broader unifying biologic features.
- AML with rare fusions are incorporated as subtypes under AML with *other defined genetic alterations*.
- AML with somatic *RUNX1* mutation is not recognized as a distinct disease type due to lack of sufficient unifying characteristics.

WHO klasszifikáció molekuláris alapokon

Csíravonalbeli eltérések jelentősége

FAMILIÁRIS
AML/MDS

Mennyire ritka
a fehér holló?



Table 10. Subtypes of myeloid neoplasms associated with germline predisposition.

Myeloid neoplasms with germline predisposition without a pre-existing platelet disorder or organ dysfunction

- Germline *CEBPA* P/LP variant (CEBPA-associated familial AML)
- Germline *DDX41* P/LP variant^a
- Germline *TP53* P/LP variant^a (Li-Fraumeni syndrome)

Myeloid neoplasms with germline predisposition and pre-existing platelet disorder

- Germline *RUNX1* P/LP variant^a (familial platelet disorder with associated myeloid malignancy, FPD-MM)
- Germline *ANKRD26* P/LP variant^a (Thrombocytopenia 2)
- Germline *ETV6* P/LP variant^a (Thrombocytopenia 5)

Myeloid neoplasms with germline predisposition and potential organ dysfunction

- Germline *GATA2* P/LP variant (GATA2-deficiency)
- Bone marrow failure syndromes
 - Severe congenital neutropenia (SCN)
 - Shwachman-Diamond syndrome (SDS)
 - Fanconi anaemia (FA)
- Telomere biology disorders
- RASopathies (Neurofibromatosis type 1, CBL syndrome, Noonan syndrome or Noonan syndrome-like disorders^{a,b})
- Down syndrome^{a,b}
- Germline *SAMD9* P/LP variant (MIRAGE Syndrome)
- Germline *SAMD9L* P/LP variant (SAMD9L-related Ataxia Pancycytopenia Syndrome)^c
- Biallelic germline *BLM* P/LP variant (Bloom syndrome)

^aLymphoid neoplasms can also occur.

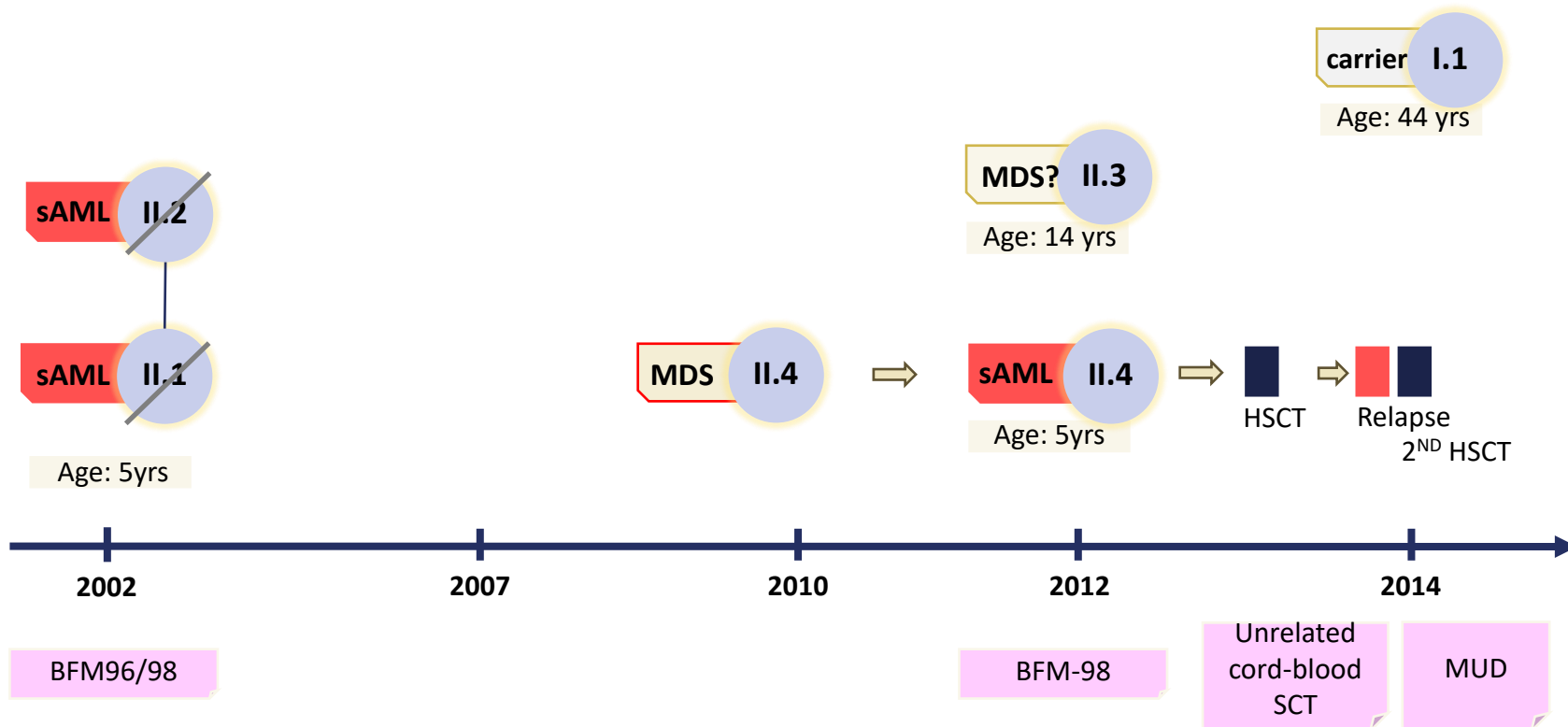
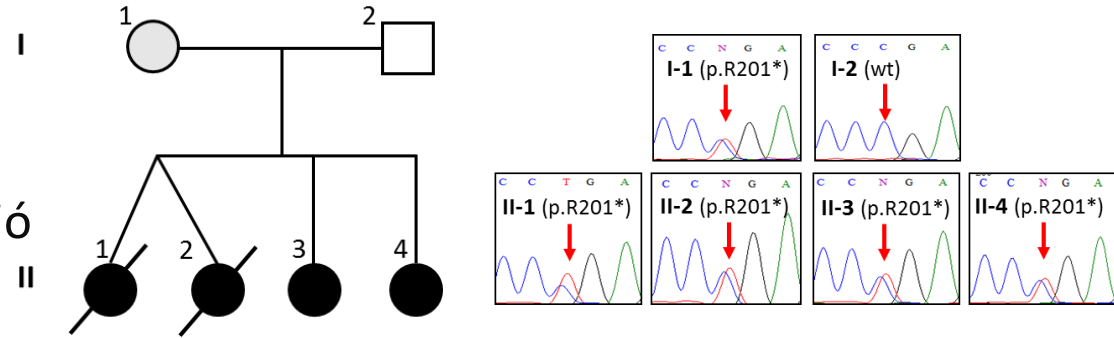
^bSee respective sections.

^cAtaxia is not always present.

P pathogenic, *LP* likely pathogenic.

Familiáris AML/MDS – FPD-AML *RUNX1* mutációval

- 4 érintett testvér
- 3 szekunder AML
- Germline ***RUNX1* p.R201*** mutáció



Az AML molekuláris diagnosztikája

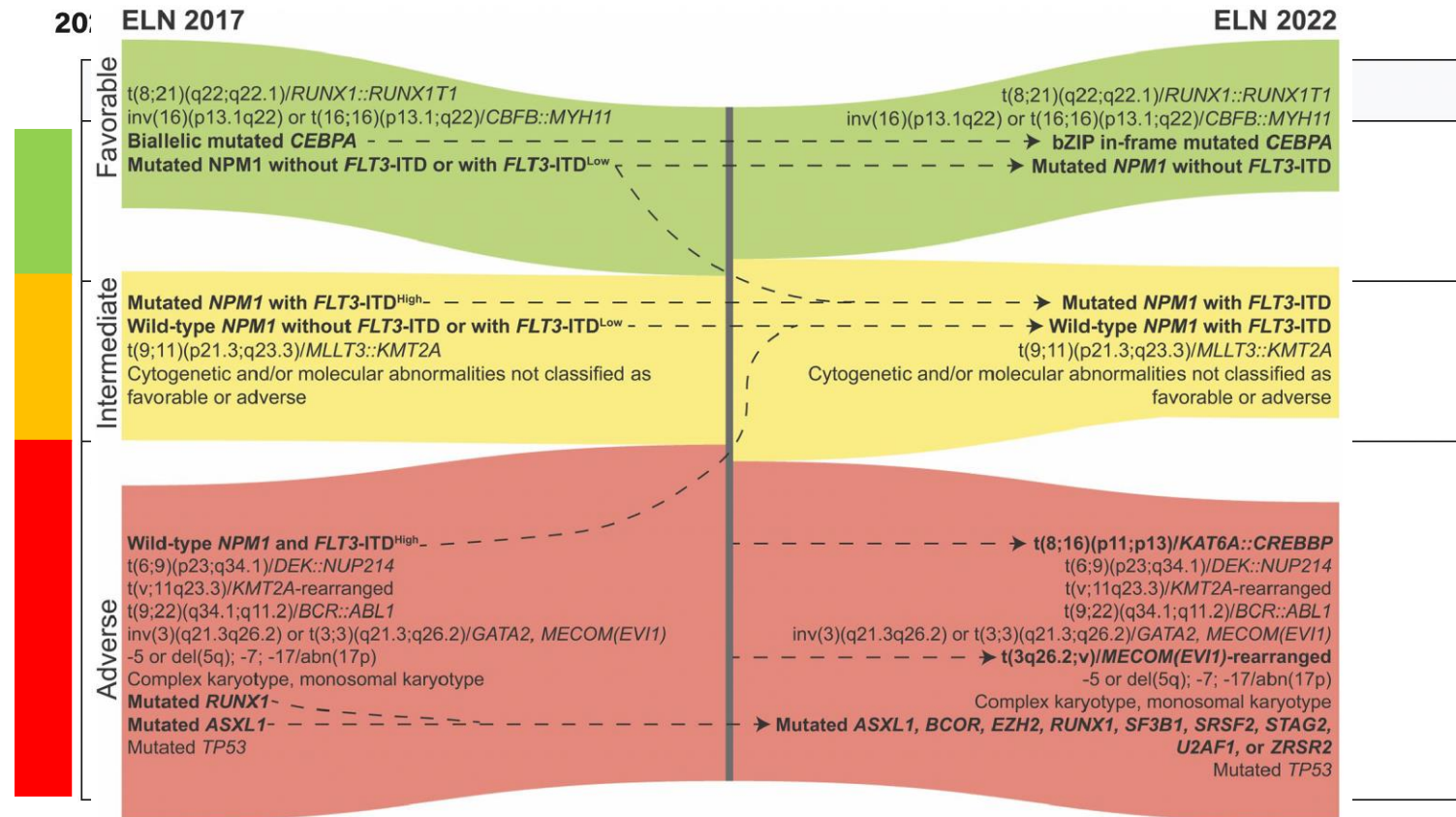


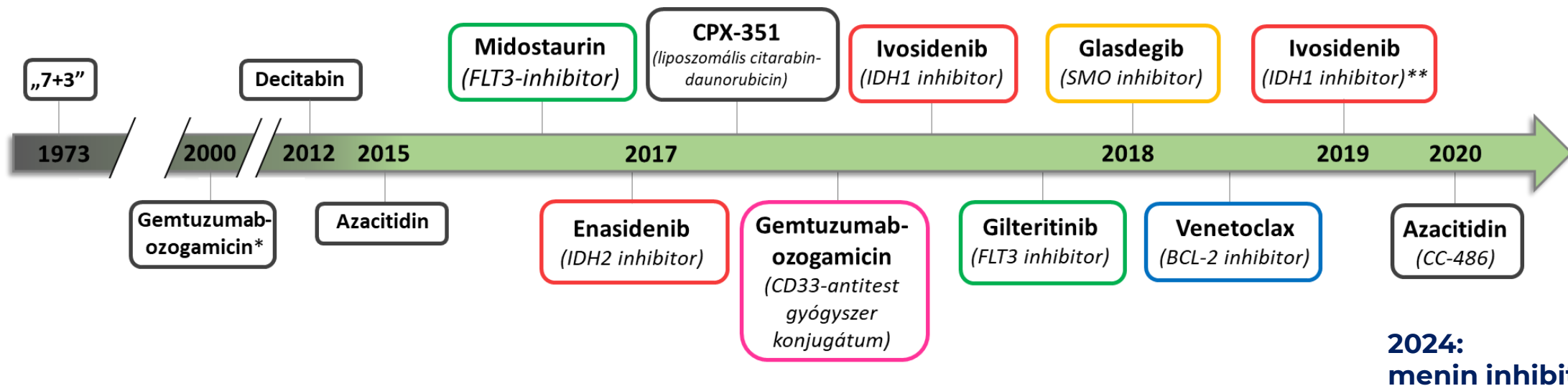
Fig. 3 Updates in ELN risk stratification of AML. A Sankey plot depicts changes in the 2017 [65] and 2022 [35] ELN risk stratification of AML.

Az AML molekuláris diagnosztikája

WHO
osztályozás

ELN
rizikóstr.

Terápia-
tervezés

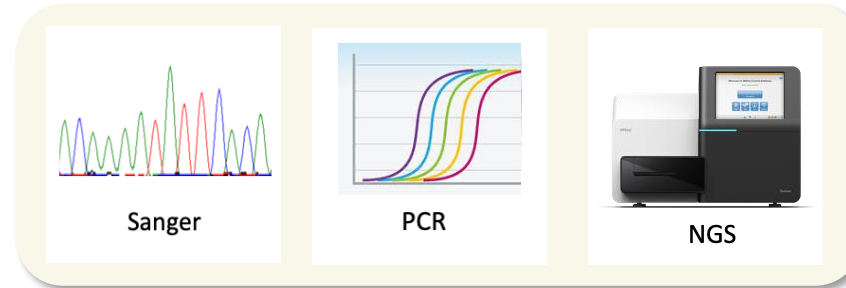


Az AML molekuláris diagnosztikája

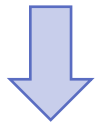
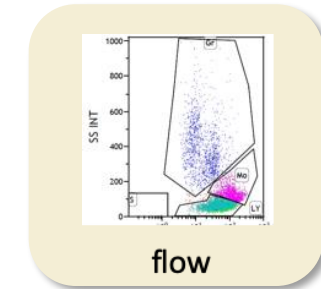
CITOGENETIKA



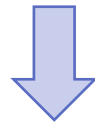
MOLEKULÁRIS VIZSGÁLATOK



ÁRAMLÁSI CITOMETRIA



DIAGNÓZIS



RIZIKÓBECSLÉS

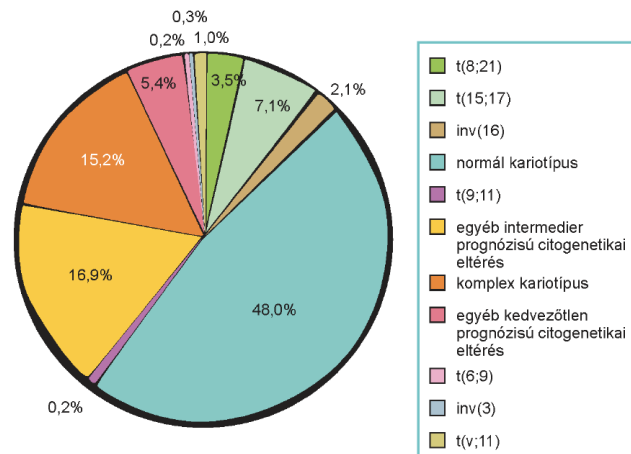
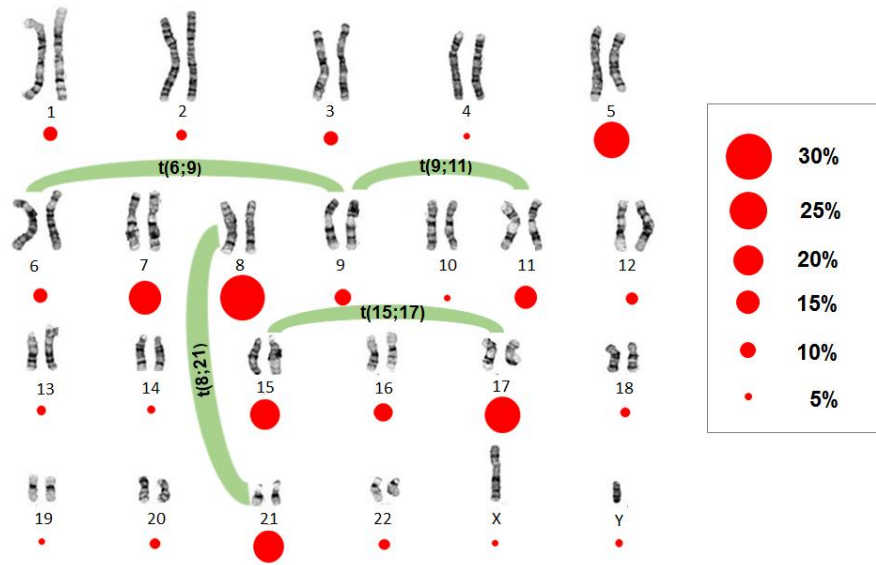


**TERÁPIA-
TERVEZÉS**



MONITOROZÁS

Citogenetikai eltérések AML-ben



Kariotípus	Betegszám (n=921)	%
t(8;21)	33	3,6%
inv(16)	19	2,1%
t(15;17)	65	7,1%
normál	444	48,2%
t(9;11)	5	0,5%
8+	77	8,4%
11+	19	2,1%
13+	8	0,9%
21+	18	2,0%
egyéb	116	12,6%
del(5q)/-5	127	13,8%
del(7q)/-7	47	5,1%
t(6;9)	2	0,2%
t(v;11q23.3)	6	0,7%
inv(3)	4	0,4%
t(9;22)	10	1,1%
-17	28	3,0%
komplex	142	15,4%

Az AML genetikai hátterének megismerése

DNA sequencing of a cytogenetically normal acute myeloid leukaemia genome

Timothy J. Ley^{1,2,3,4*}, Elaine R. Mardis^{2,3*}, Li Ding^{2,3}, Bob Fulton³, Michael D. McLellan³, Ken Chen³, David Dooling³, Brian H. Dunford-Shore³, Sean McGrath³, Matthew Hickenbotham³, Lisa Cook³, Rachel Abbott³, David E. Larson³, Dan C. Koboldt³, Craig Pohl³, Scott Smith³, Amy Hawkins³, Scott Abbott³, Devin Locke³, LaDeana W. Hillier^{3,8}, Tracie Miner³, Lucinda Fulton³, Vincent Magrini^{2,3}, Todd Wylie³, Jarret Glasscock³, Joshua Conyers³, Nathan Sander³, Xiaohi Shi³, John R. Osborne³, Patrick Minx³, David Gordon³, Asif Chinwalla³, Yu Zhao³, Rhonda E. Ries¹, Jacqueline E. Payton³, Peter Westervelt^{1,4}, Michael H. Tomasson^{1,4}, Mark Watson^{3,4,5}, Jack Baty⁶, Jennifer Ivanovich^{4,7}, Sharon Heath^{1,4}, William D. Shannon^{1,4}, Rakesh Nagarajan^{4,5}, Matthew J. Walter^{1,4}, Daniel C. Link^{1,4}, Timothy A. Graubert^{1,4}, John F. DiPersio^{1,4} & Richard K. Wilson^{2,3,4}

Ley et al, Nature 2008

- 1 beteg teljes genom szekvenálása
- 2+8 mutáció
- Az első humán „onkogenom”

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Genomic and Epigenomic Landscapes of Adult De Novo Acute Myeloid Leukemia

The Cancer Genome Atlas Research Network

TCGA, NEJM 2013

- 200 teljes genom/exom szekvenálása
- Legalább 1 mutáció szinte minden esetben
- 23 szignifikánsan mutált gén
- Nagymértékű heterogenitás, mintázatok

The NEW ENGLAND
JOURNAL of MEDICINE

ESTABLISHED IN 1812

JUNE 9, 2016

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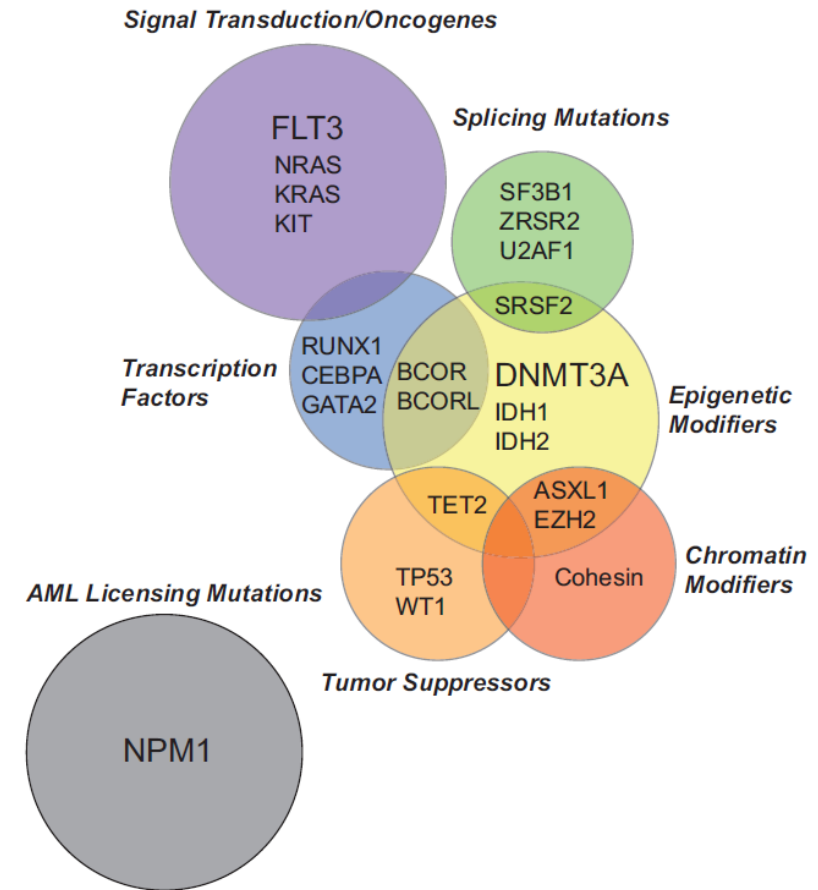
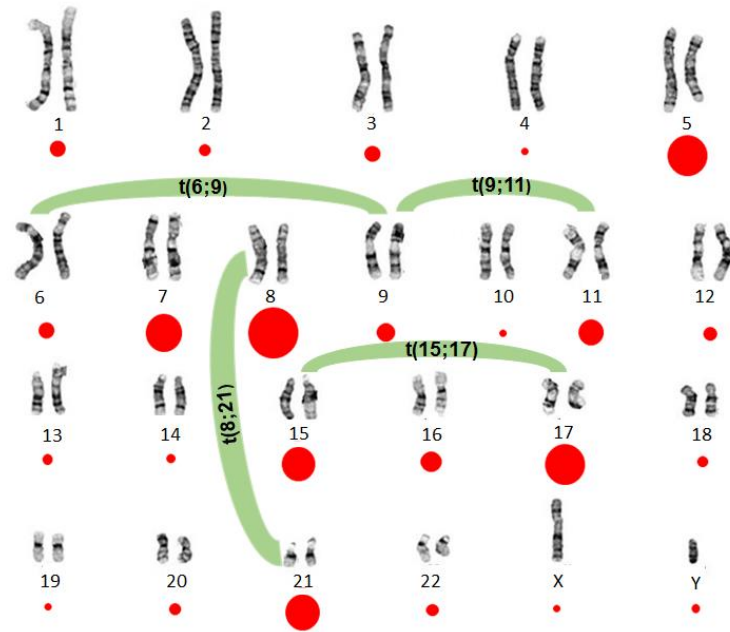
Genomic Classification and Prognosis in Acute Myeloid Leukemia

Elli Papaemmanuil, Ph.D., Moritz Gerstung, Ph.D., Lars Bullinger, M.D., Verena I. Gaidzik, M.D., Peter Paschke, M.D., Nicola D. Roberts, B.Sc., Nicola F. Potter, Ph.D., Michael Haughey, M.D., Felicitas Thiel, M.D.

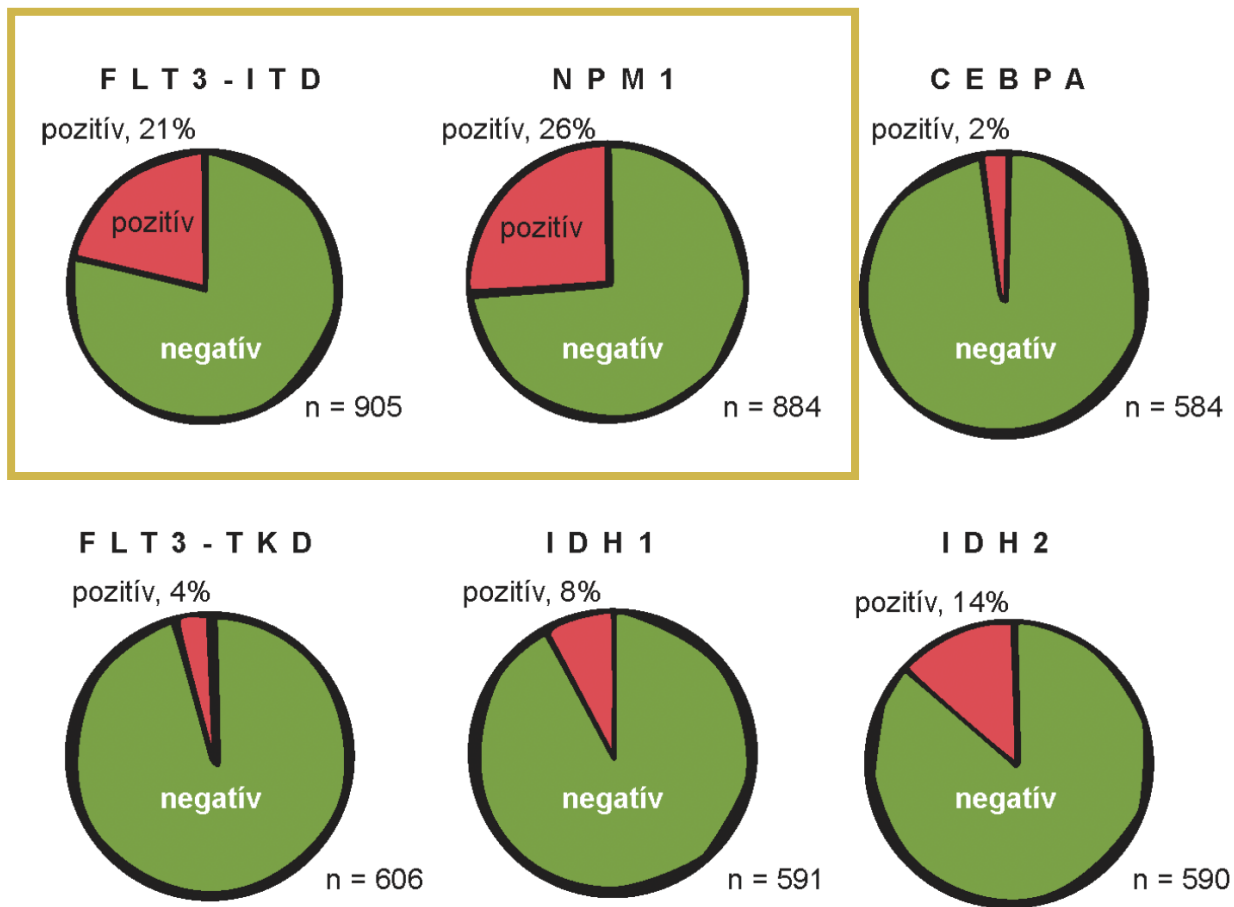
Papaemmanuil, NEJM 2016

- 1540 beteg, 111 gén vizsgálata
- 5234 mutáció 76 génben
- 2 vagy több mutáció a betegek 86%-ában
- Molekuláris altípusok, eltérő prognózissal

Az AML (cito)genetikai háttere



Szomatikus mutációk AML-ben (saját adatok)



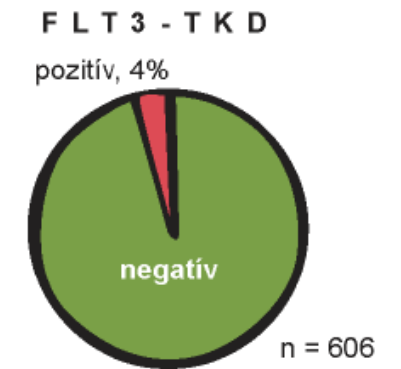
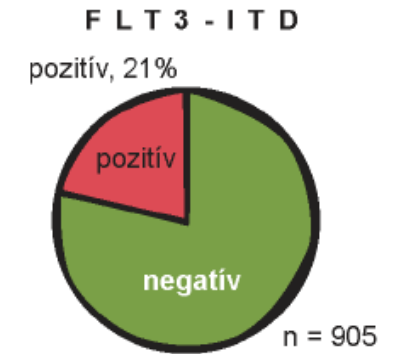
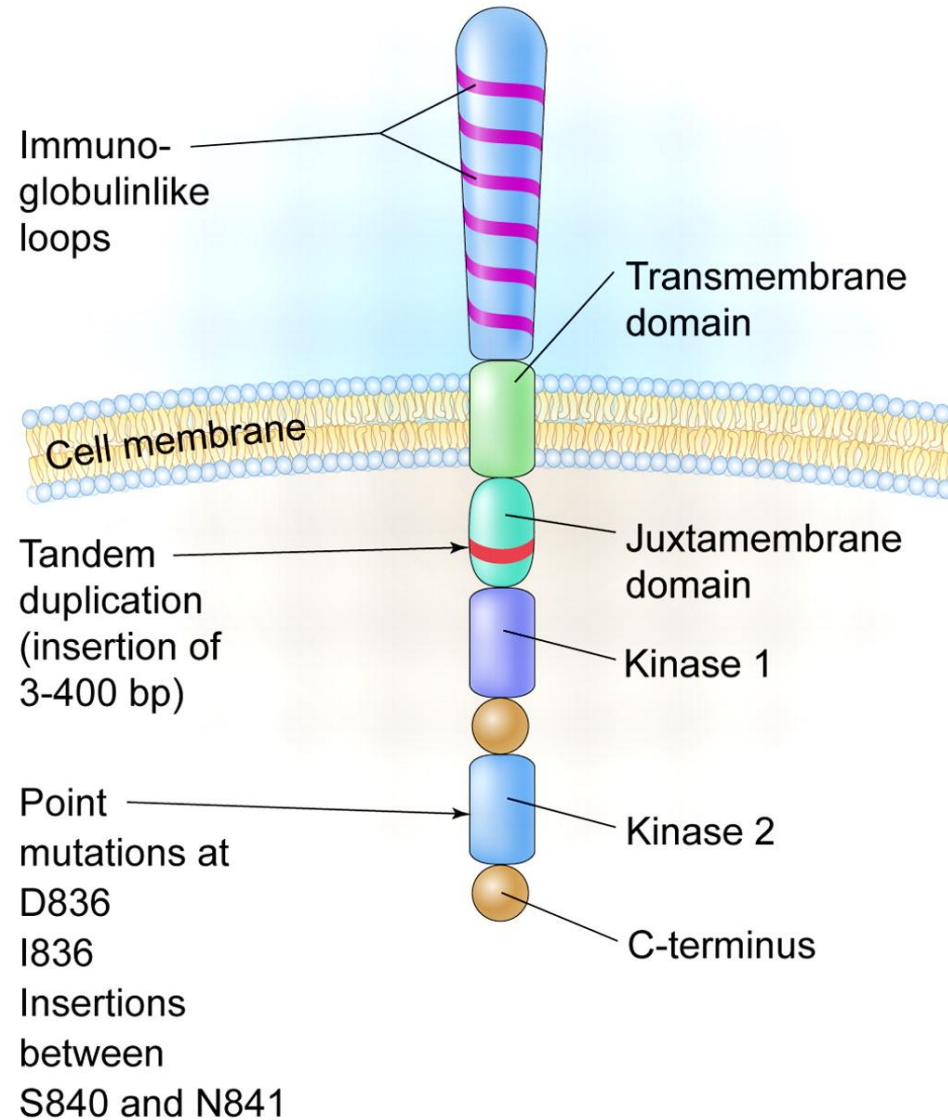
7. ábra: Intézetünkben rutinszerűen vizsgált génekben detektált mutációk gyakorisága. A betegekben leggyakrabban az NPM1 (26%), az FLT3-ITD (21%) és az IDH2 (14%) mutációját lehetett kimutatni. A CEBPA mutációk esetében csak a kedvező prognózist jelentő biallélikus mutációt tüntettük fel az ábrán.

FLT3 ITD és TKD mutációk

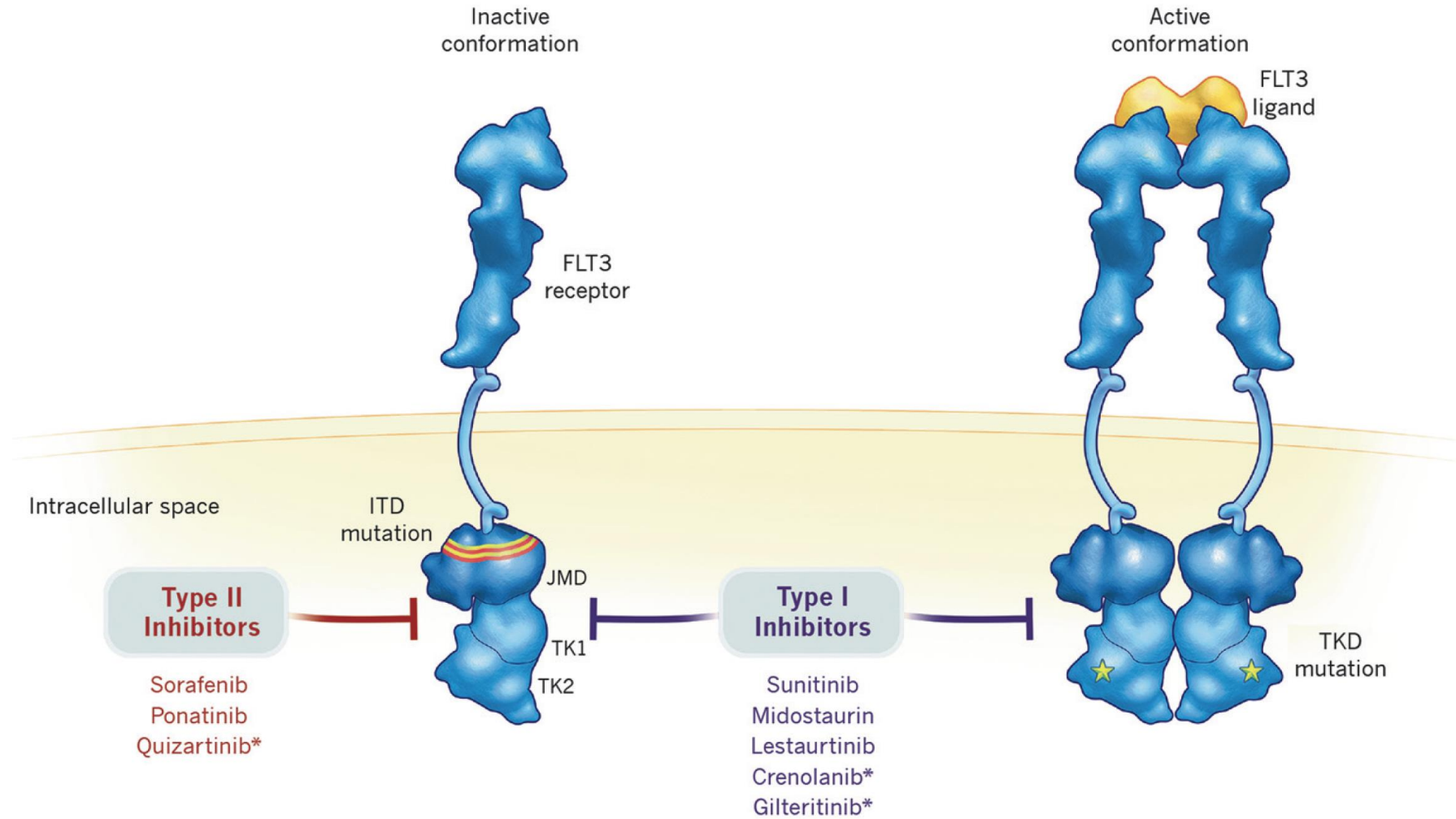
FLT3 ITD mutációk



FLT3 TKD mutációk



FLT3 gátlás



Az *IDH1* mutációk felfedezése

The NEW ENGLAND JOURNAL of MEDICINE

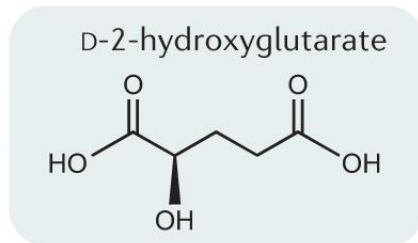
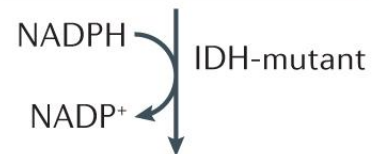
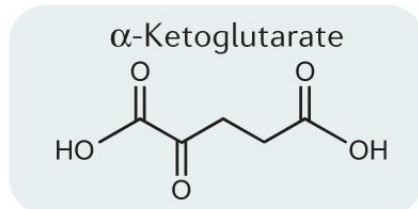
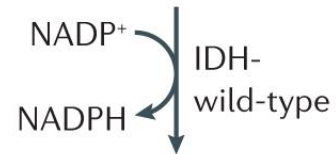
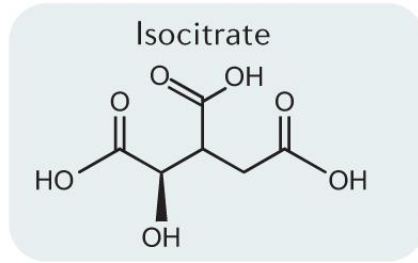
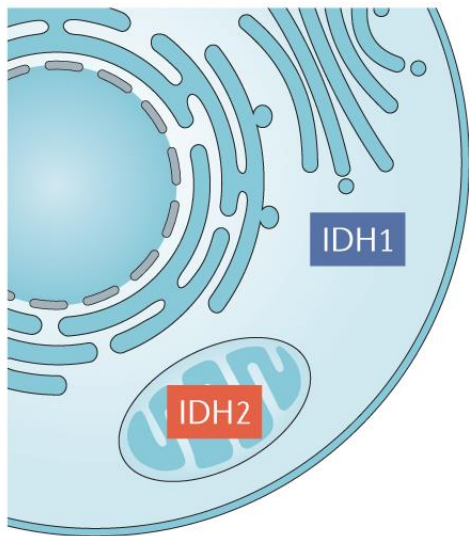
ORIGINAL ARTICLE

Recurring Mutations Found by Sequencing an Acute Myeloid Leukemia Genome

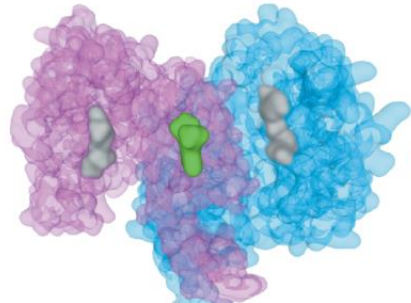
Elaine R. Mardis, Ph.D., Li Ding, Ph.D., David J. Dooling, Ph.D.,
David E. Larson, Ph.D., Michael D. McLellan, B.S., Ken Chen, Ph.D.,
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Lynn Carmichael, B.S., James M. Eldred, Christopher C. Harris, B.S.,
Jason Walker, B.A., B.S., Joshua B. Peck, M.B.A., Feiyu Du, M.S.,
Adam F. Dukes, B.A., Gabriel E. Sanderson, B.S., Anthony M. Brummett,
Eric Clark, Joshua F. McMichael, B.S., Rick J. Meyer, M.S.,
Jonathan K. Schindler, B.S., B.A., Craig S. Pohl, M.S., John W. Wallis, Ph.D.,
Xiaoqi Shi, M.S., Ling Lin, M.S., Heather Schmidt, B.S., Yuzhu Tang, M.D.,
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John F. DiPersio, M.D., Ph.D., Richard K. Wilson, Ph.D., and Timothy J. Ley, M.D.

- 1 beteg WGS analízise
- Majd 15/187= **8%**

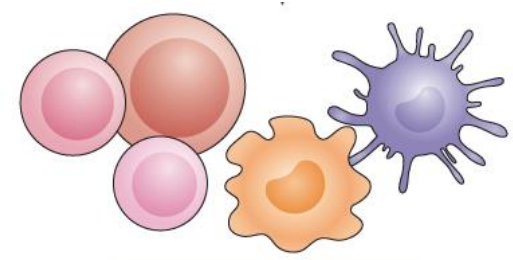
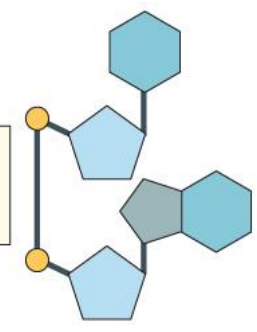
IDH1/2 mutációk az onkológiában



Allosteric mutant IDH inhibitor

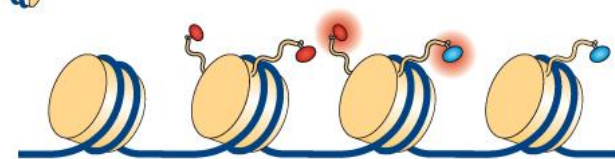
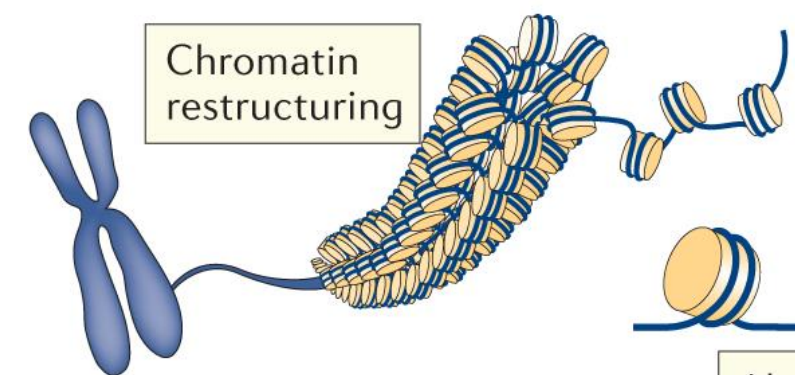


Altered metabolism



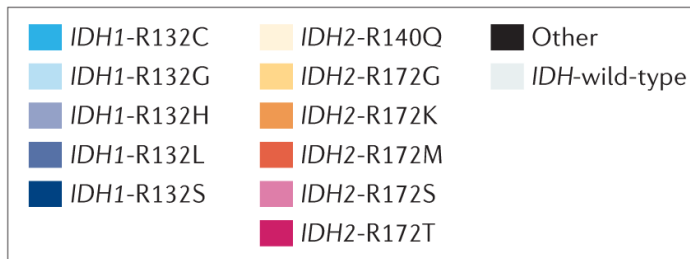
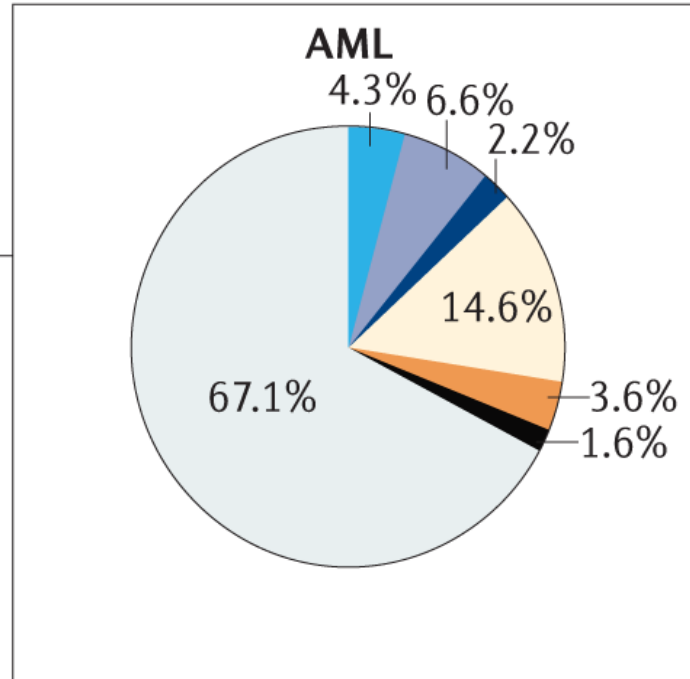
Differentiation blocks

Chromatin restructuring

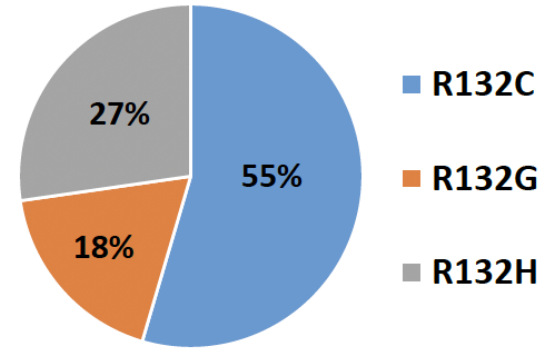


Aberrant methylation

IDH1/2 mutációk az AML-ben

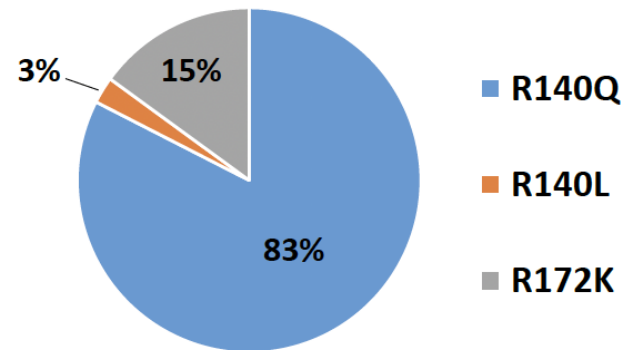


IDH1-mutációk megoszlása



Saját adatok

IDH2-mutációk megoszlása



Újgenerációs szekvenálás (NGS) AML-ben



MHTT konszenzus myeloid panel

Hotspots					Whole CDS		
<i>ABL1</i>	<i>ETNK1</i>	<i>KIT</i>	<i>PTPN11</i>	<i>TERT</i>	<i>ASXL1</i>	<i>IKZF1</i>	<i>ZRSR2</i>
<i>ACD</i>	<i>FLT3</i>	<i>KRAS</i>	<i>SAMD9</i>	<i>U2AF1</i>	<i>BCOR</i>	<i>PHF6</i>	<i>TERC</i>
<i>ANKRD26</i>	<i>GATA1</i>	<i>MPL</i>	<i>SAMD9L</i>	<i>WT1</i>	<i>CEBPA</i>	<i>RAD21</i>	
<i>BRAF</i>	<i>GNAS</i>	<i>MYC</i>	<i>SETBP1</i>		<i>DDX41</i>	<i>RUNX1</i>	
<i>CALR</i>	<i>GNB1</i>	<i>NF1</i>	<i>SF3B1</i>		<i>DNMT3A</i>	<i>SETD2</i>	
<i>CBL</i>	<i>IDH1</i>	<i>NPM1</i>	<i>SH2B3</i>		<i>ETV6</i>	<i>SRSF2</i>	
<i>CSF3R</i>	<i>IDH2</i>	<i>NRAS</i>	<i>SMC1A</i>		<i>EZH2</i>	<i>STAG2</i>	
<i>CUX1</i>	<i>JAK2</i>	<i>PDGFRA</i>	<i>SMC3</i>		<i>GATA1</i>	<i>TET2</i>	
<i>ERCC6L2</i>	<i>KDM6A</i>	<i>PRPF8</i>	<i>SRP72</i>		<i>GATA2</i>	<i>TP53</i>	

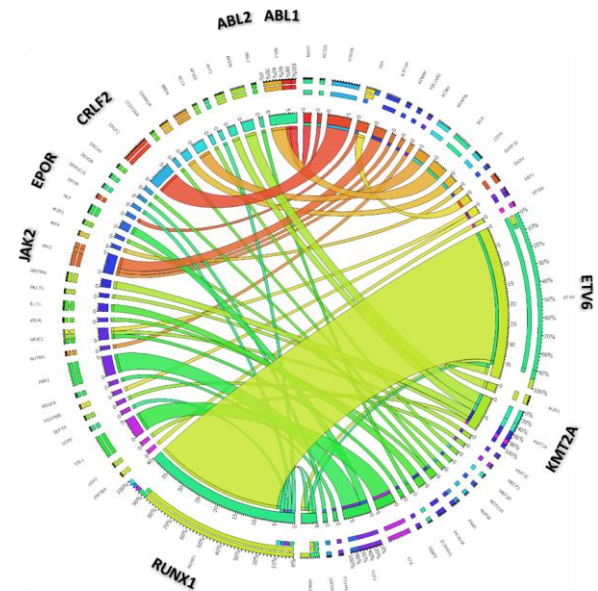
AML/MDS

Familiáris MDS/AML

JMML

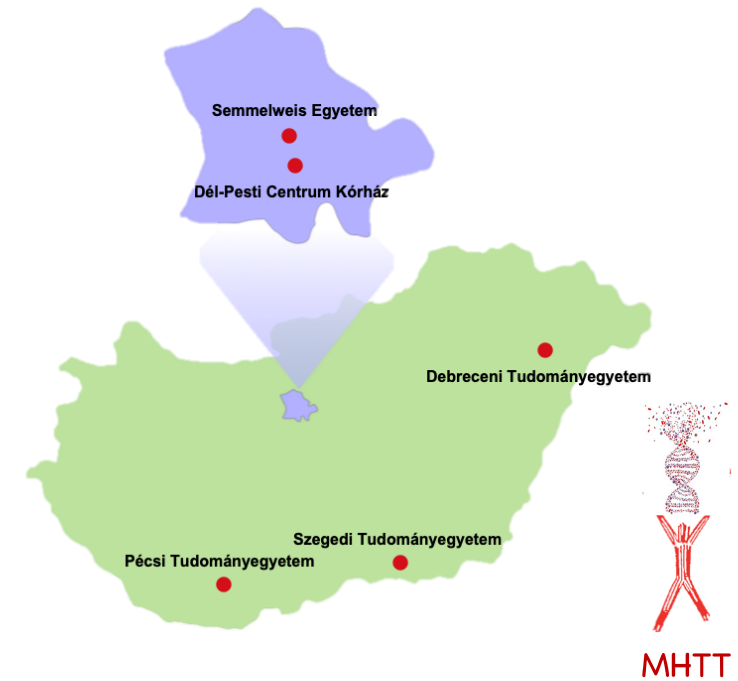
CML

Myeloid NGS panel (59 genes)

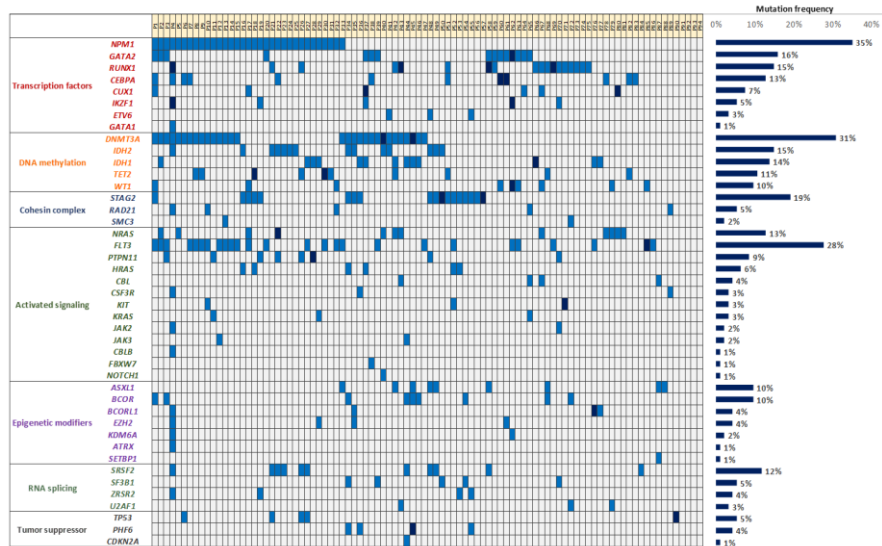


Illumina PanCancer Panel (1380 genes)

MHTT Molekuláris Munkacsoport (2013-2025)



Újgenerációs szekvenálás (NGS) AML-ben (saját adatok)



Esetszám	Myeloid NGS	Lymphoid NGS	Pan-Cancer	Összesen
2019.	40	0	63	103
2020.	48	64	77	189
2021.	40	88	98	226
2022.	120	104	105	329
2023.	118	90	77	285
2024.	143	99	70	312

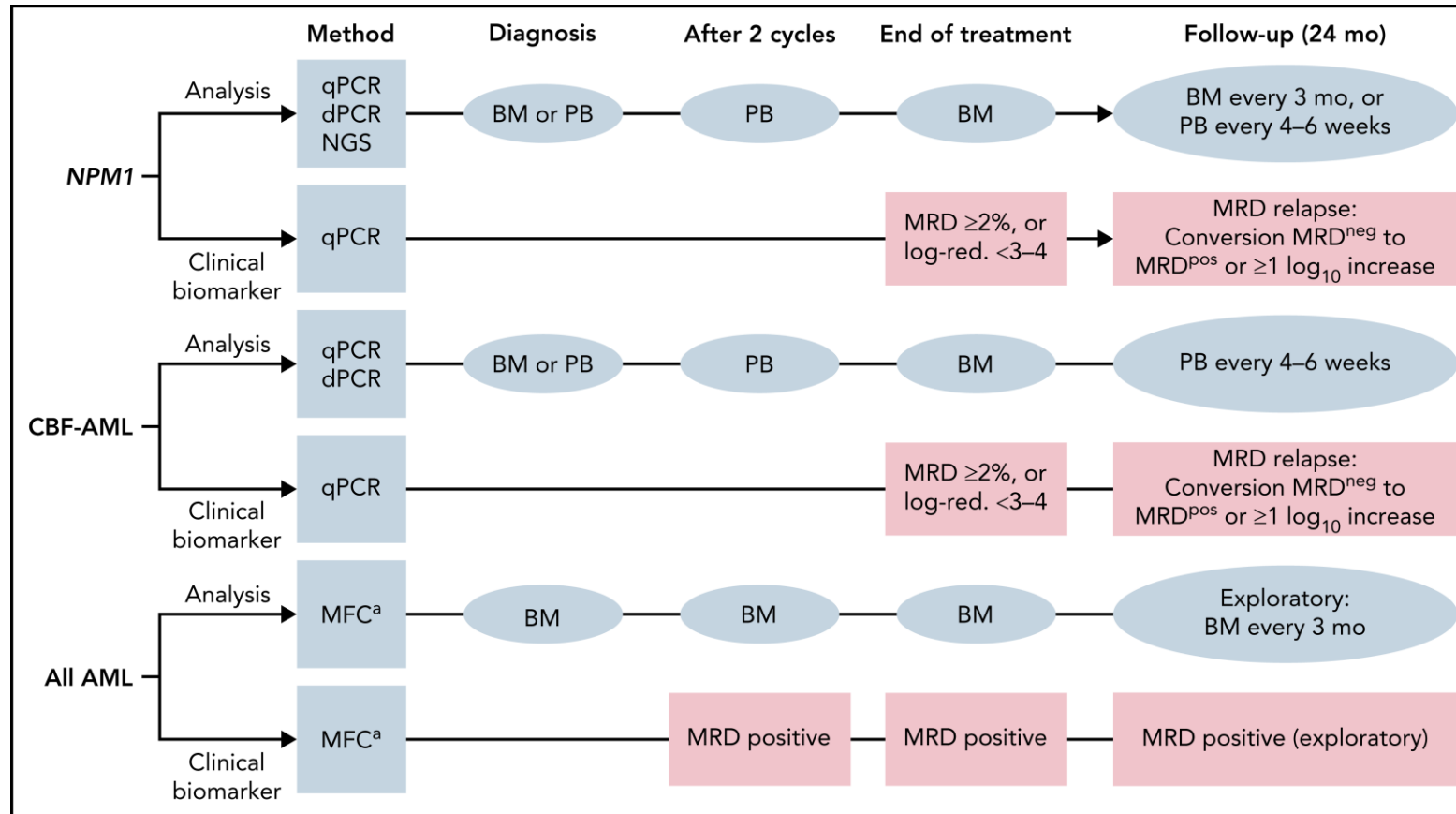
- **Betegek 37,8%-ában változott a rizikóbesorolás!**
- NPM1 (35%), DNMT3A (31%), FLT3 (28%), STAG2 (19%), GATA2 (16%), IDH2 (15%), RUNX1 (15%) and IDH1 (14%)
- Potenciálisan célozható eltérések (FLT3, IDH1, IDH2, NRAS) a betegek 53%-ában

Mérhető Reziduális Betegség (MRD) AML-ben

Methods for detection of MRD in AML

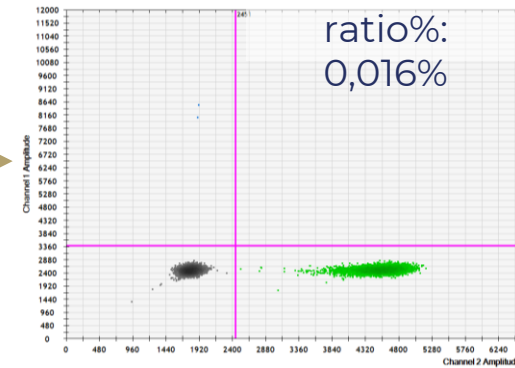
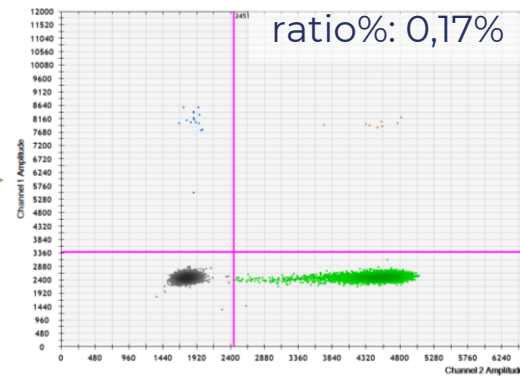
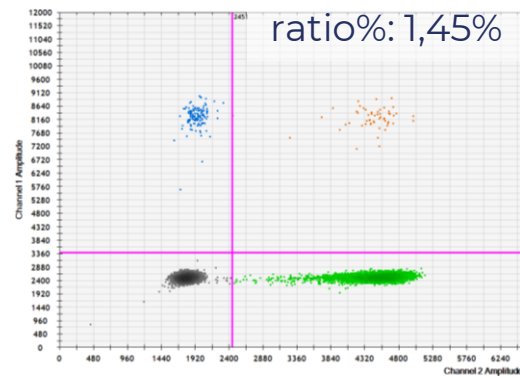
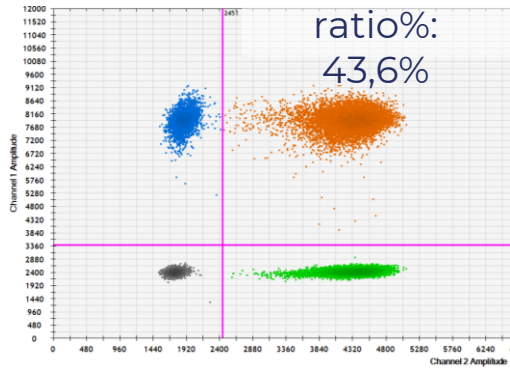
	Method	Target	Sensitivity	Applicable in % of AML	Turn-around time (days)	Limitations/problems
Established	Multi-parameter flow cytometry (MFC)	Leukemia-associated immunophenotype (LAIP) or different from normal (DfN)	10^{-3} to 10^{-4}	85-90	2	Less sensitive, more subjective analysis
Established	Real-time quantitative PCR (RT-qPCR)	Robust data: <i>NPM1</i> , <i>CBFB::MYH11</i> , <i>RUNX1::RUNX1T1</i> Less validated: <i>KMT2A::MLL3</i> , <i>DEK::NUP214</i> , <i>BCR::ABL1</i> , <i>WT1</i>	10^{-4} to 10^{-5}	40-50*	3-5	Limited applicability
Exploratory	Next-generation sequencing (NGS)†,‡	Potentially any somatic mutation†	10^{-2} to 10^{-4}	~100	5-10	Less sensitive, costly, technically challenging
Exploratory	Digital PCR (dPCR)	Specific targeted mutations	10^{-3} to 10^{-4}	~70	3-5	Specific assay necessary for every mutation, limited sensitivity

Mérhető Reziduális Betegség (MRD) AML-ben



Mérhető Reziduális Betegség (MRD) AML-ben

NPM1 mutációk nyomonkövetése ddPCR-rel ($10^{-4}/-5$)



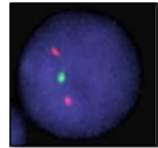
- Elérhető 2023 januártól (SE, DPCK)

Az AML molekuláris diagnosztikája

CITOGENETIKA

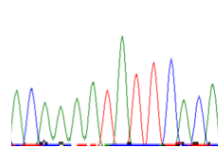


kariotipizálás

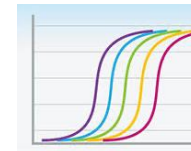


iFISH

MOLEKULÁRIS VIZSGÁLATOK



Sanger

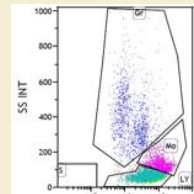


PCR



NGS

ÁRAMLÁSI CITOMETRIA



flow

DIAGNÓZIS

RIZIKÓBECSLÉS

TERÁPIATERVEZÉS

MONITOROZÁS

Új (valós) lehetőség: Digitális Citogenomika



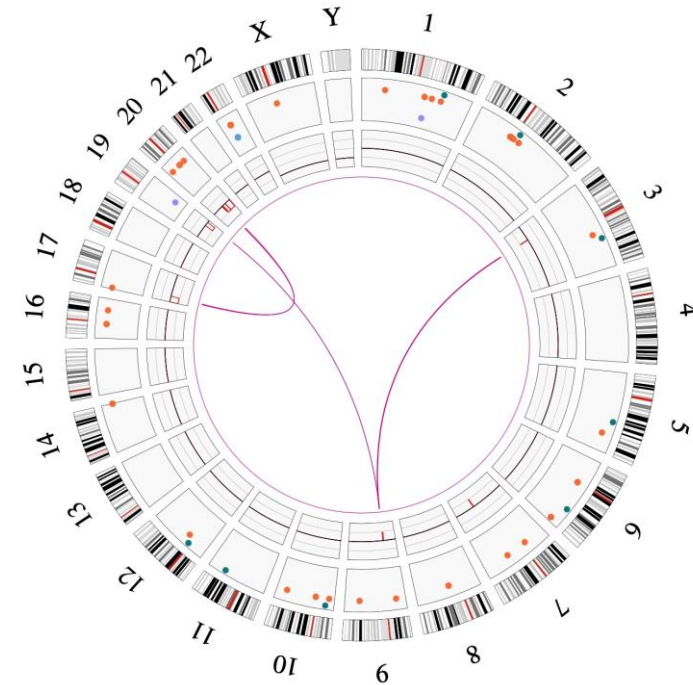
A citogenetika fejlődése



1956

Tijo és Levan

- 46 humán kromoszóma



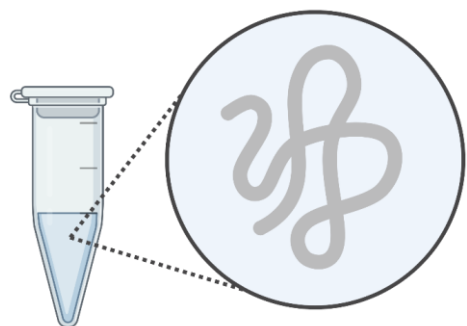
2017

Bionano Genomics

- Teljes genom térképezés
- Strukturális variánsok azonosítása (≥ 500 bp)
- Kópiaszámeltérések detektálása

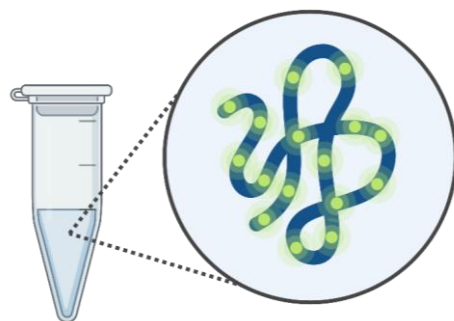
Optikai genom térképezés

1. nap



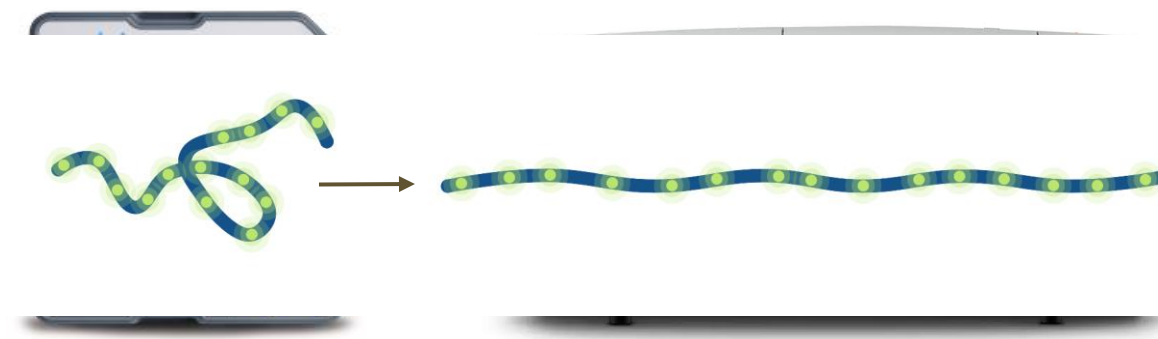
- Ultra nagy molekulású DNS izolálása
- Molekulahossz >150 kbp

2. nap

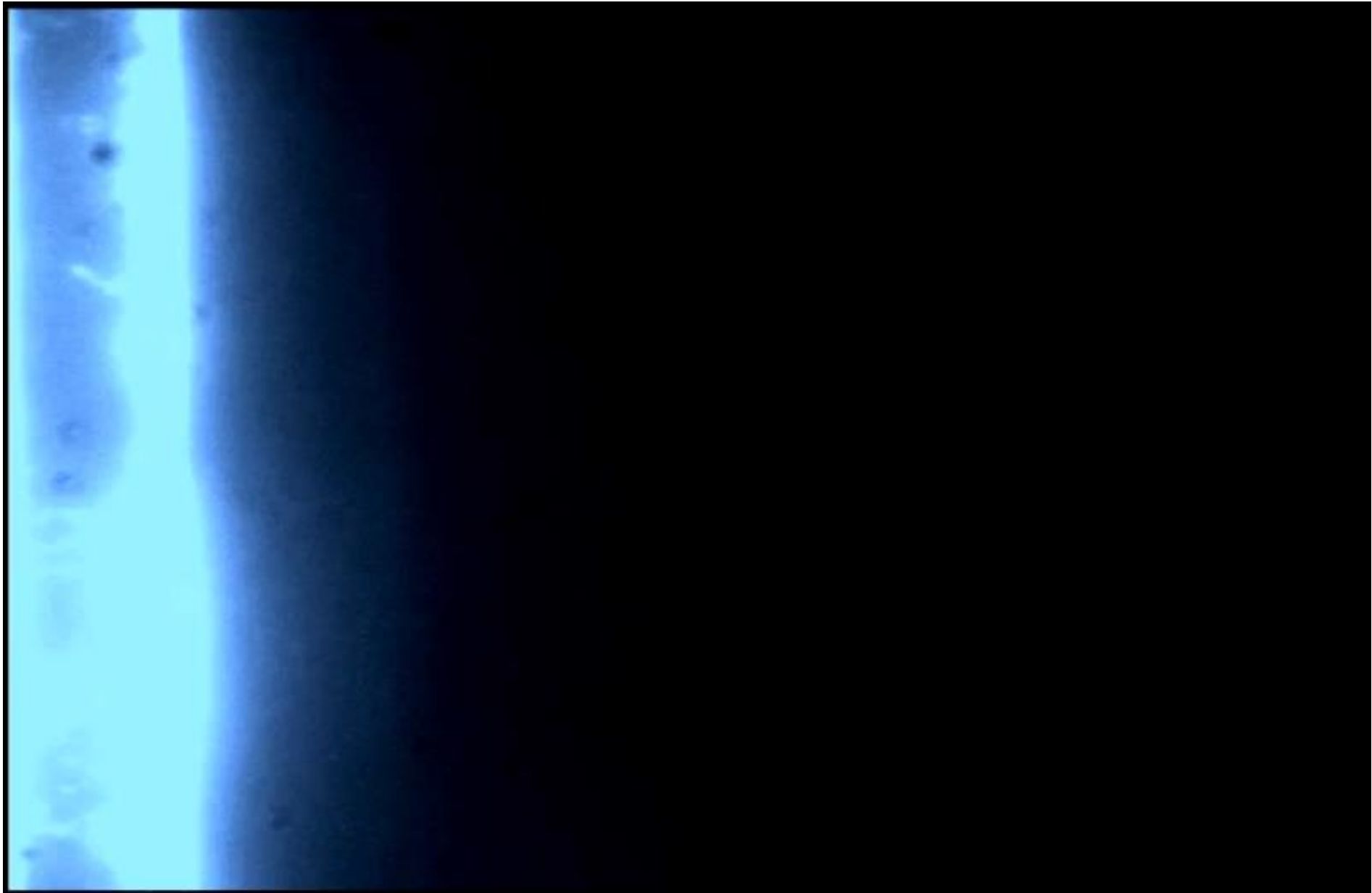


- Enzimatiszus jelölés
 - Specifikus nukleotid szekvencia (CTTAAG)
- DNS-váz festése

3. nap



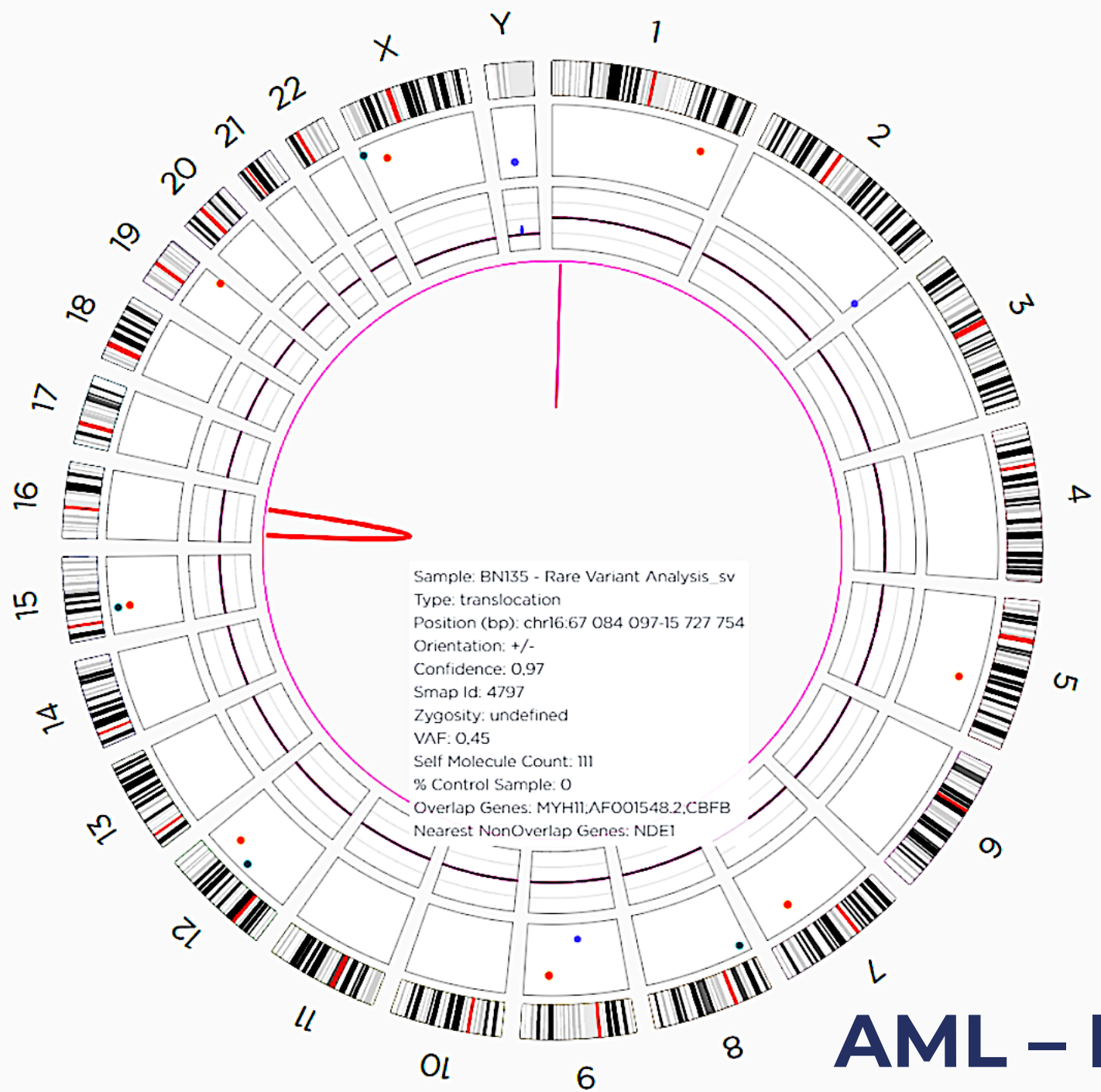
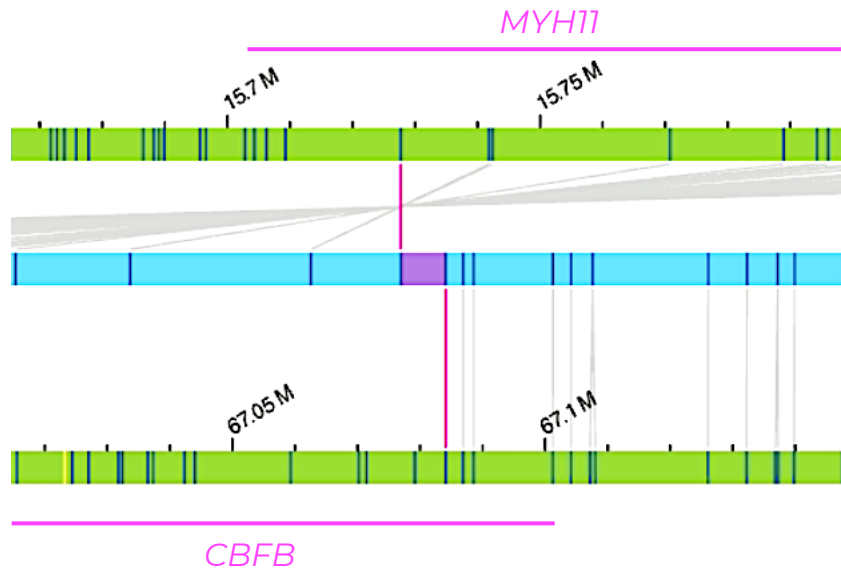
- Chip-re töltés
- DNS molekula linearizálódása
- DNS molekula nanocsatornába áramlása
- DNS molekulák fényképezése



Saját eredmények – I

2022-Pa10/60609

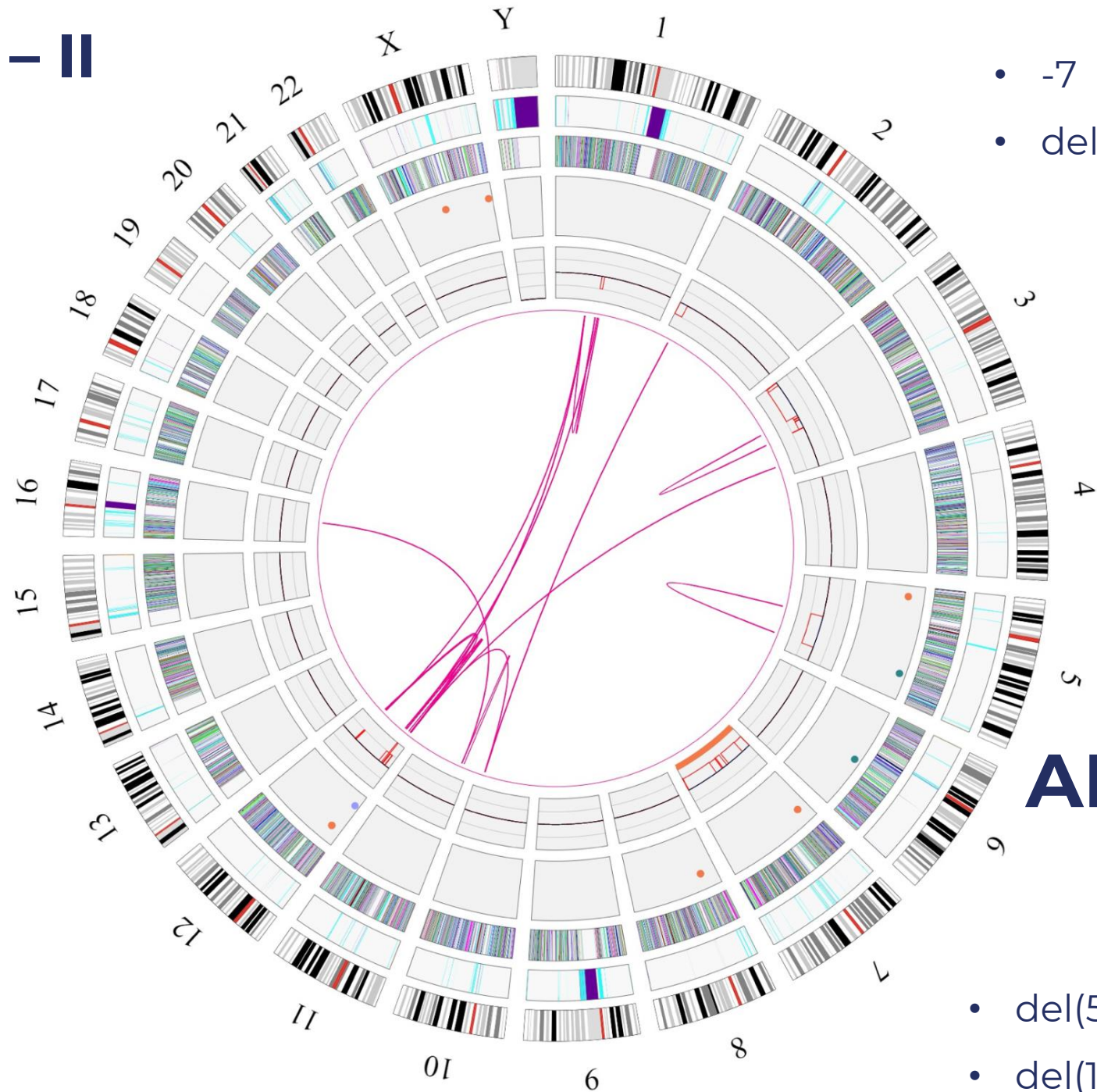
- *CBFB::MYH11* translocation



Saját eredmények – II

2022-Pa10/60251

- Komplex kariotípus
 - t(1;12)(p21.1;p12.1)
 - t(1;12)(p31.1;q22)
 - t(10;12)(q25.3;p13.2)
 - t(11;16)(p15.1;q12.1)
 - t(2;10)(p24.2;q25.3)
 - t(3;12)(q26.2;p13.2)
 - fus(1;1)(p31.1;p21.3)
 - fus(11;11)(p15.2;p15.1)
 - fus(12;12)(p13.2;p12.1)
 - fus(3;3)(p12.2;q13.13)
 - fus(5;5)(q14.3;q33.1)

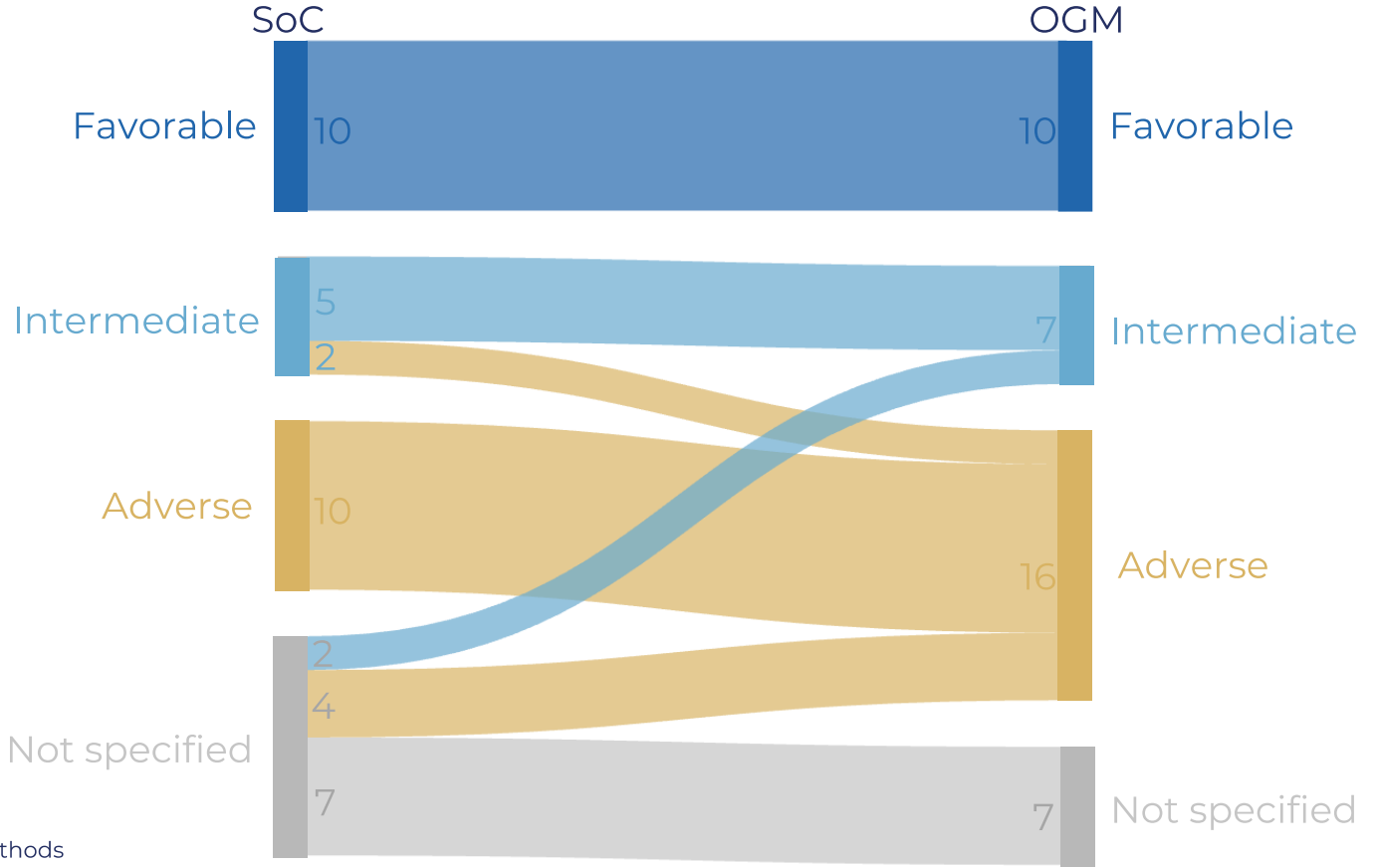
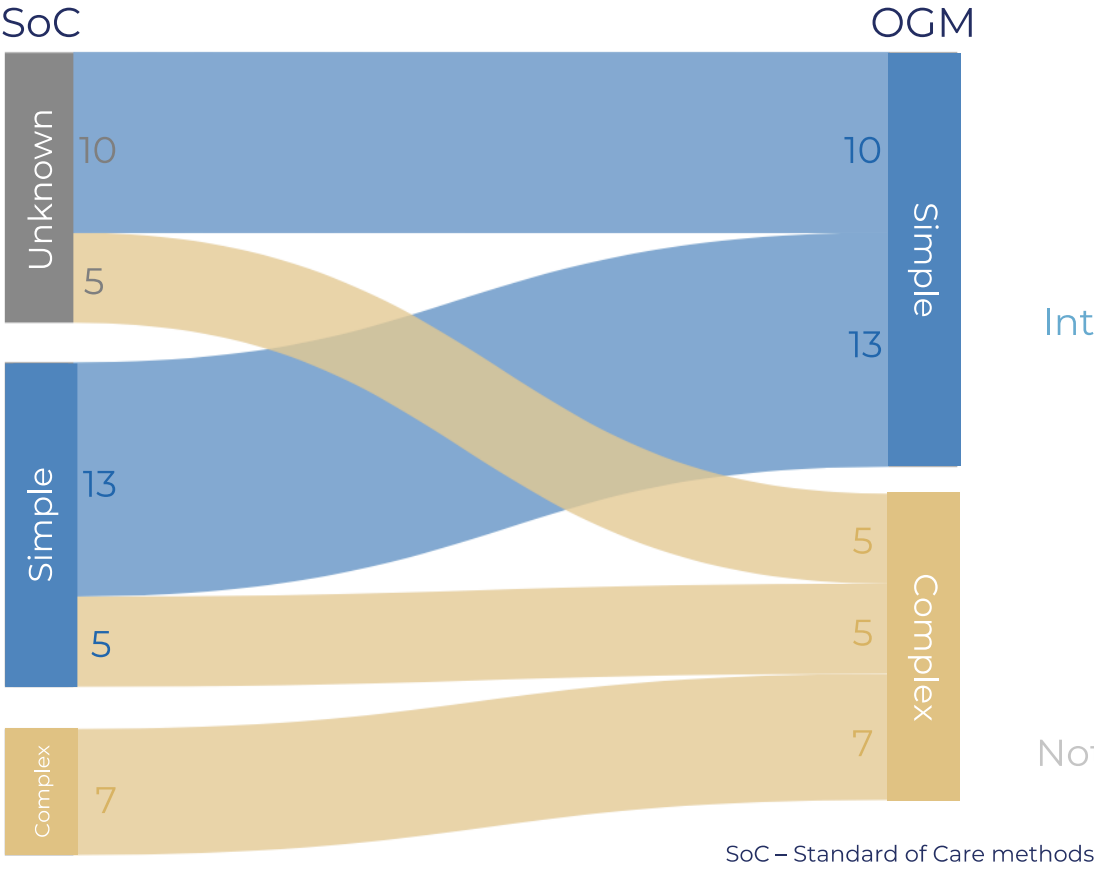


- -7
- del(3)(p26.3p11.1)

AML – II

- del(5)(q14.3q33.1)
- del(12)(p13.2p11.23)v

Saját OGM adatok AML-ben



Myelodysplasias szindrómák (MDS)

MDS klasszifikáció

Myelodysplastic syndromes (MDS)
MDS with single lineage dysplasia
MDS with ring sideroblasts (MDS-RS)
MDS-RS and single lineage dysplasia
MDS-RS and multilineage dysplasia
MDS with multilineage dysplasia
MDS with excess blasts
MDS with isolated del(5q)
MDS, unclassifiable
Provisional entity: Refractory cytopenia of childhood
Myeloid neoplasms with germ line predisposition

MDS diagnosztikai kritériumok

MDS/MPN diagnostic criteria

- Anemia associated with erythroid lineage dysplasia with or without multilineage dysplasia, $\geq 15\%$ ring sideroblasts,* $< 1\%$ blasts in PB and $< 5\%$ blasts in the BM
- Persistent thrombocytosis with platelet count $\geq 450 \times 10^9/L$
- Presence of a *SF3B1* mutation or, in the absence of *SF3B1* mutation, no history of recent cytotoxic or growth factor therapy that could explain the myelodysplastic/myeloproliferative features†
- No *BCR-ABL1* fusion gene, no rearrangement of *PDGFRA*, *PDGFRB*, or *FGFR1*; or *PCM1-JAK2*; no (3;3)(q21;q26), inv(3)(q21q26) or del(5q)‡
- No preceding history of MPN, MDS (except MDS-RS), or other type of MDS/MPN

*At least 15% ring sideroblasts required even if *SF3B1* mutation is detected.

†A diagnosis of MDS/MPN-RS-T is strongly supported by the presence of *SF3B1* mutation together with a mutation in *JAK2* V617F, *CALR*, or *MPL* genes.

‡In a case which otherwise fulfills the diagnostic criteria for MDS with isolated del(5q)-no or minimal absolute basophilia; basophils usually $< 2\%$ of leukocytes.

- Morfológia/klinikum központi szerepe
- del(5q) alcsoport definiáló (cito)genetikai eltérés
- Szomatikus mutációk limitált szerepe
- *SF3B1* mutációk jelentősége
- Csírvonalbeli mutációk jelentősége

Myelodysplasias neoplázia (MDS) -2022

REVIEW ARTICLE OPEN

Check for updates

The 5th edition of the World Health Organization Classification of Haematolymphoid Tumours: Myeloid and Histiocytic/Dendritic Neoplasms

Table 3. Classification and defining features of myelodysplastic neoplasms (MDS).

	Blasts	Cytogenetics	Mutations
MDS with defining genetic abnormalities			
MDS with low blasts and isolated 5q deletion (MDS-5q)	<5% BM and <2% PB	5q deletion alone, or with 1 other abnormality other than monosomy 7 or 7q deletion	
MDS with low blasts and <i>SF3B1</i> mutation ^a (MDS- <i>SF3B1</i>)		Absence of 5q deletion, monosomy 7, or complex karyotype	<i>SF3B1</i>
MDS with biallelic <i>TP53</i> inactivation (MDS-bi <i>TP53</i>)	<20% BM and PB	Usually complex	Two or more <i>TP53</i> mutations, or 1 mutation with evidence of <i>TP53</i> copy number loss or cnLOH
MDS, morphologically defined			
MDS with low blasts (MDS-LB)	<5% BM and <2% PB		
MDS, hypoplastic ^b (MDS-h)			
MDS with increased blasts (MDS-IB)			
MDS-IB1	5–9% BM or 2–4% PB		
MDS-IB2	10–19% BM or 5–19% PB or Auer rods		
MDS with fibrosis (MDS-f)	5–19% BM; 2–19% PB		

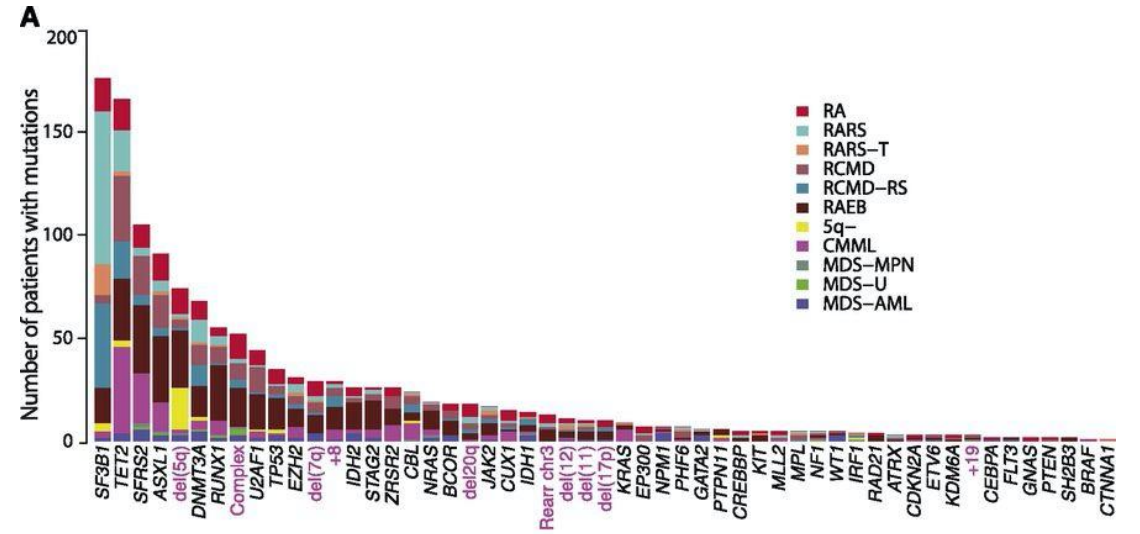
^aDetection of ≥15% ring sideroblasts may substitute for *SF3B1* mutation. Acceptable related terminology: MDS with low blasts and ring sideroblasts.

^bBy definition, ≤25% bone marrow cellularity, age adjusted.

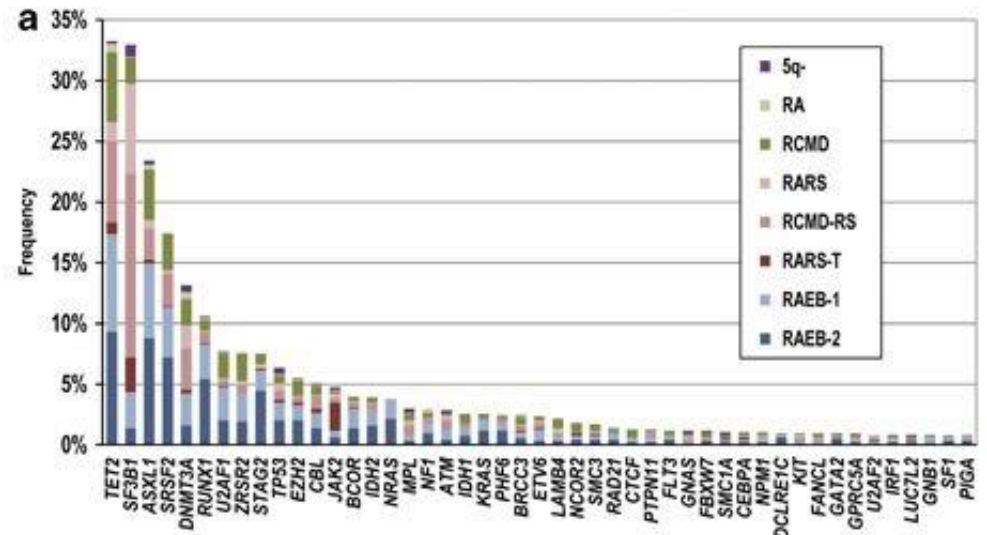
BM bone marrow, PB peripheral blood, cnLOH copy neutral loss of heterozygosity.

Az MDS genetikai háttérének feltérképezése

- Az esetek 80-85%-a legalább egy onkogén mutációt hordoz
- A mutációk leggyakrabban a DNS metilációért és az RNS splicingért felelős géneket érintik
- Egyes mutációk prognosztikus jelentőséggel is bírnak
- Átfedés az AML-ben ismert mutációkkal

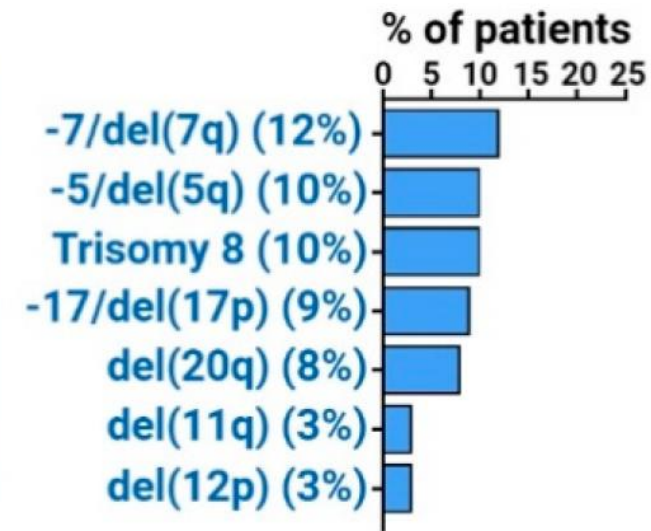
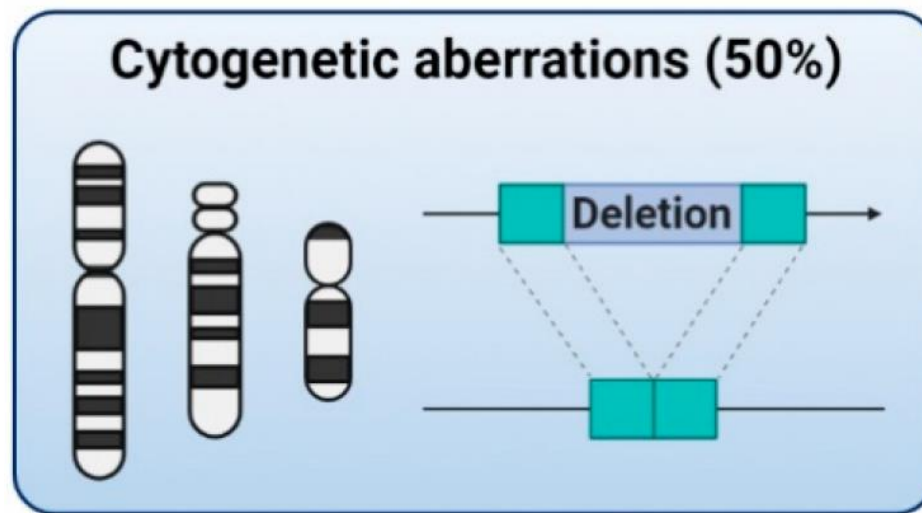


Pappaemmauil et al, NEJM, 2013

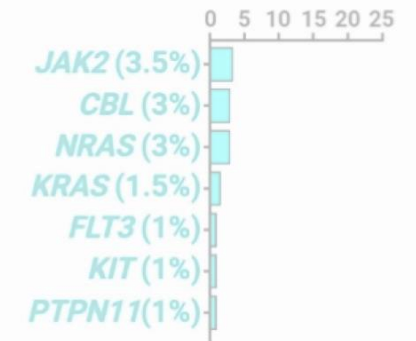
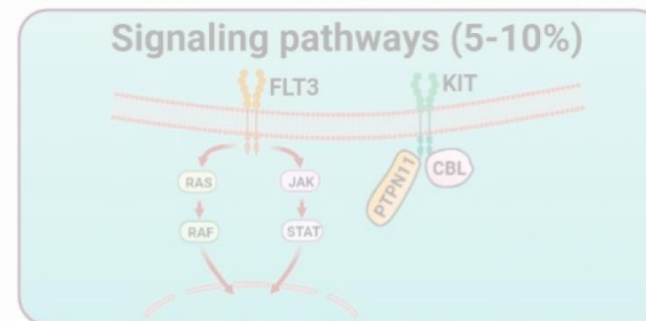
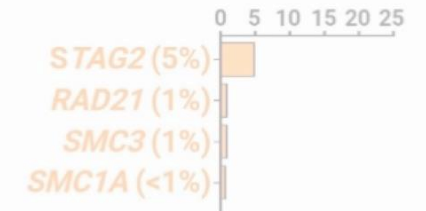
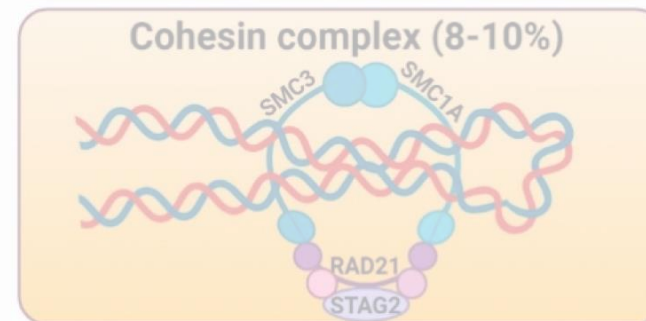
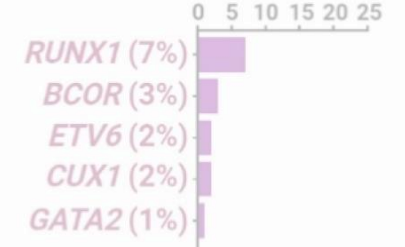
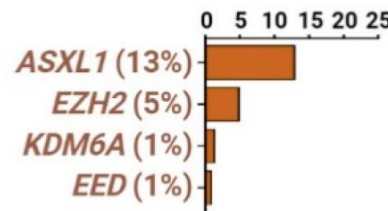
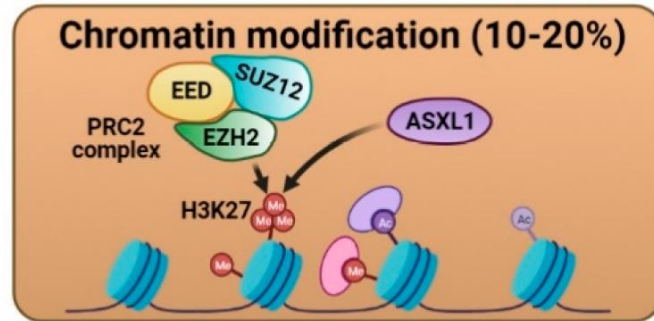
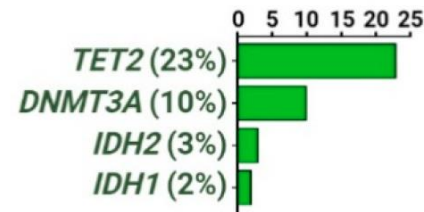
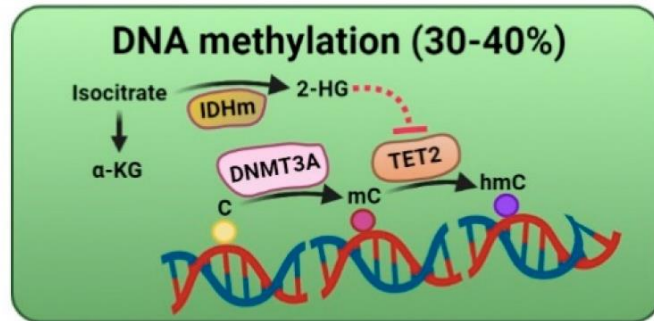
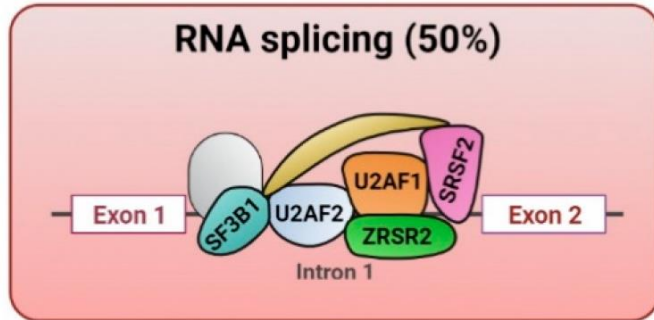


Bejar et al, NEJM, 2011

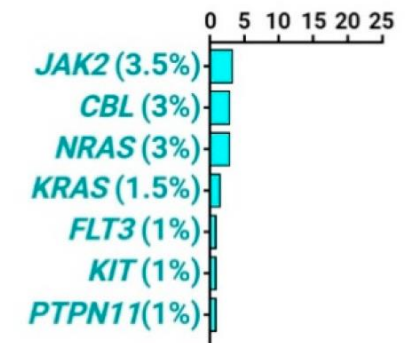
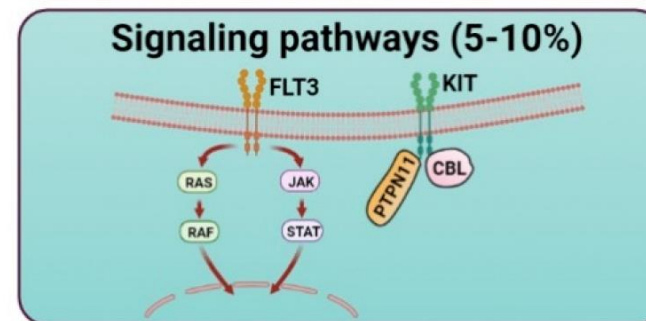
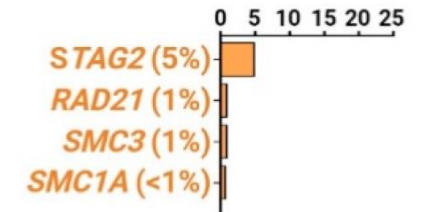
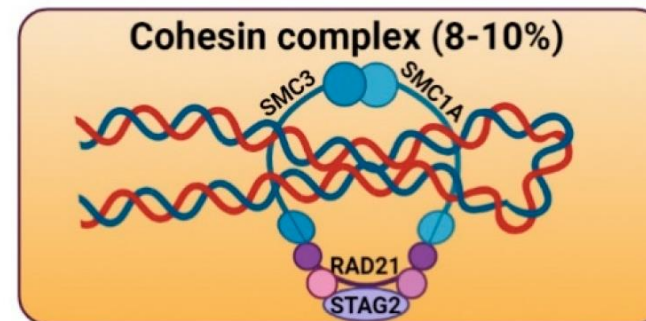
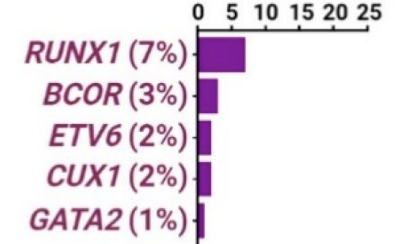
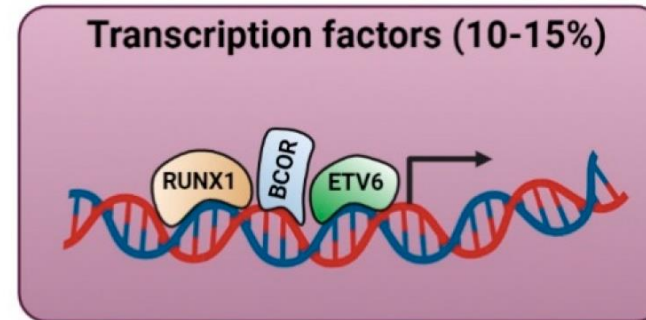
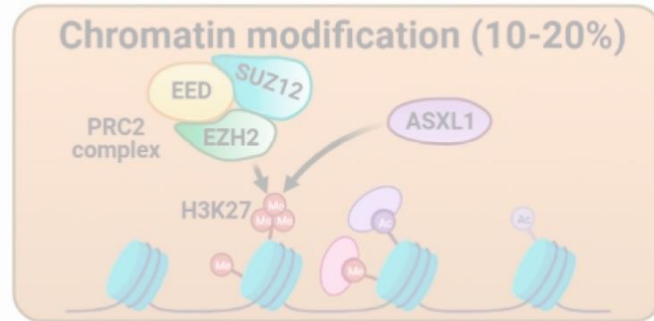
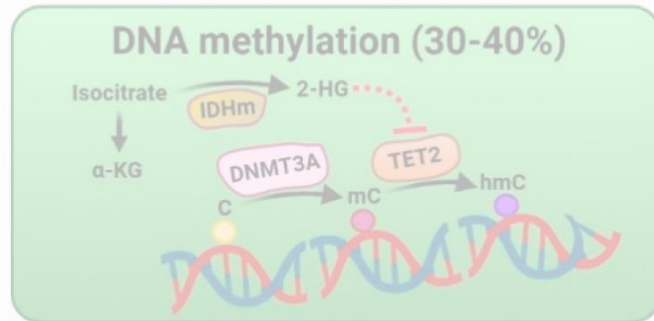
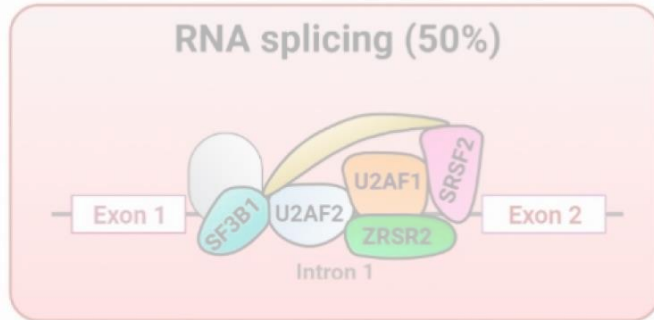
Az MDS genetikai hátterének feltérképezése



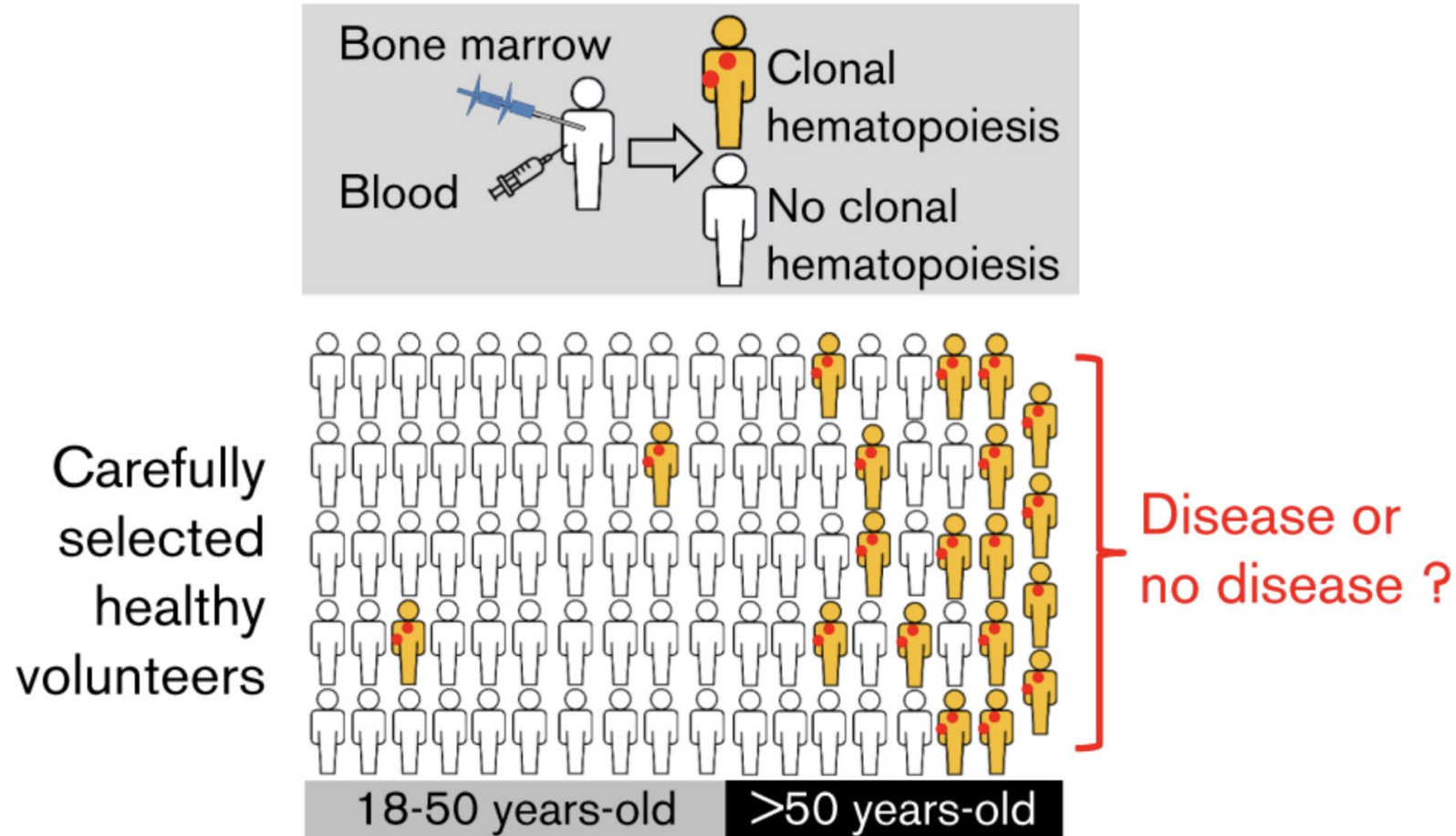
Az MDS genetikai háttérének feltérképezése



Az MDS genetikai hátterének feltérképezése

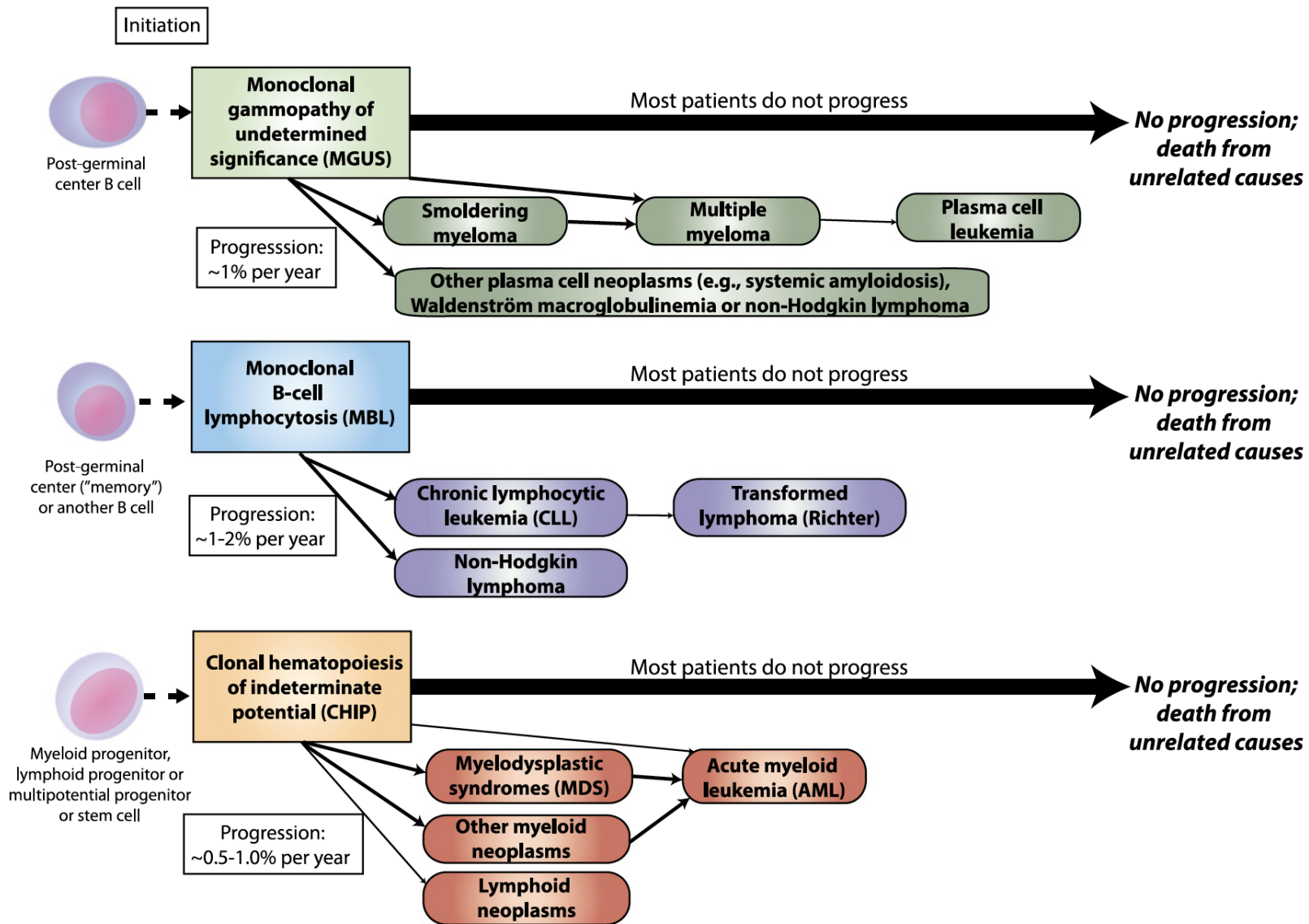


Klonális Hemopoezis („Chip mutciók)

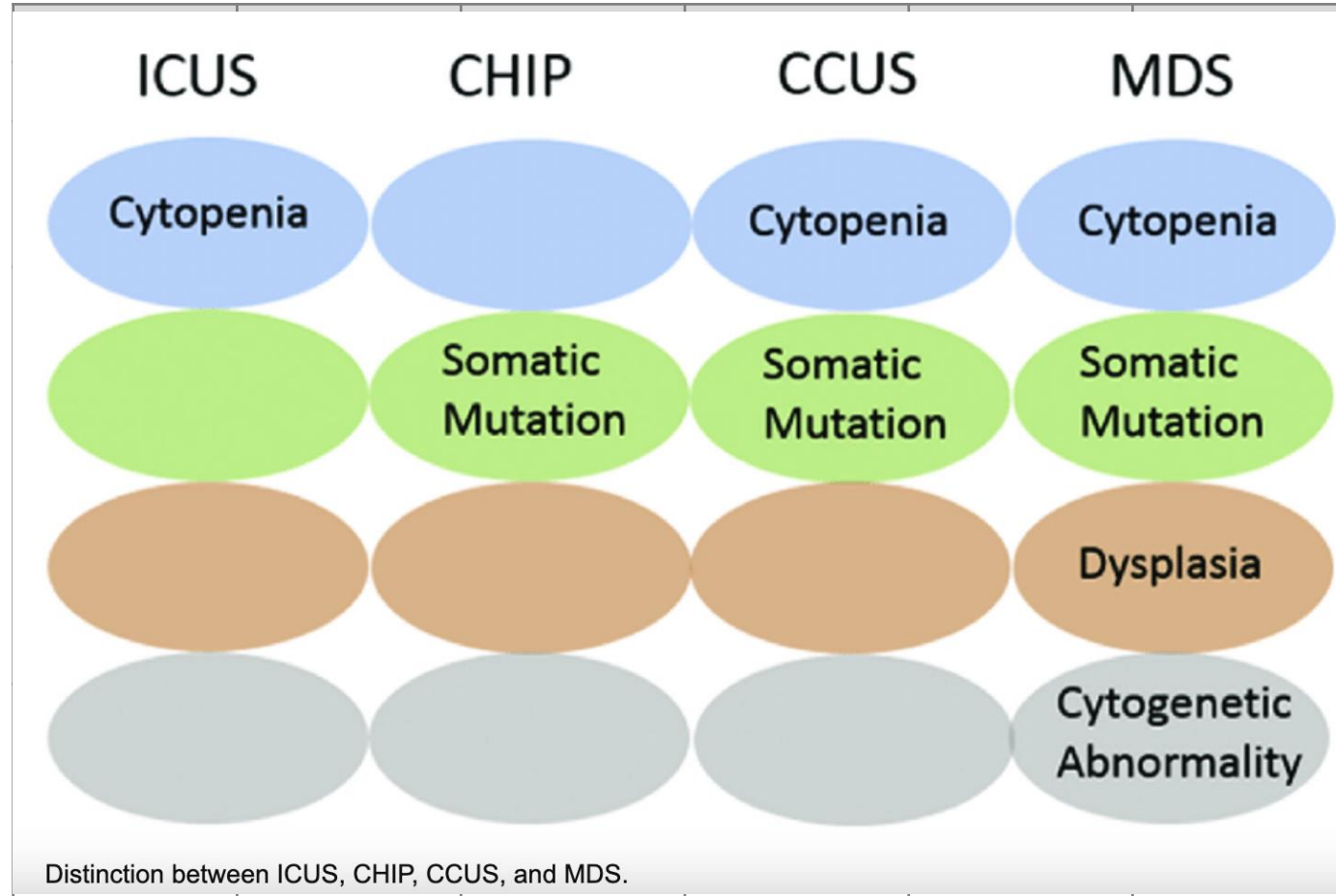


CHIP: clonal haematopoiesis of indeterminate potential

Premalignus léziók - Progresszió

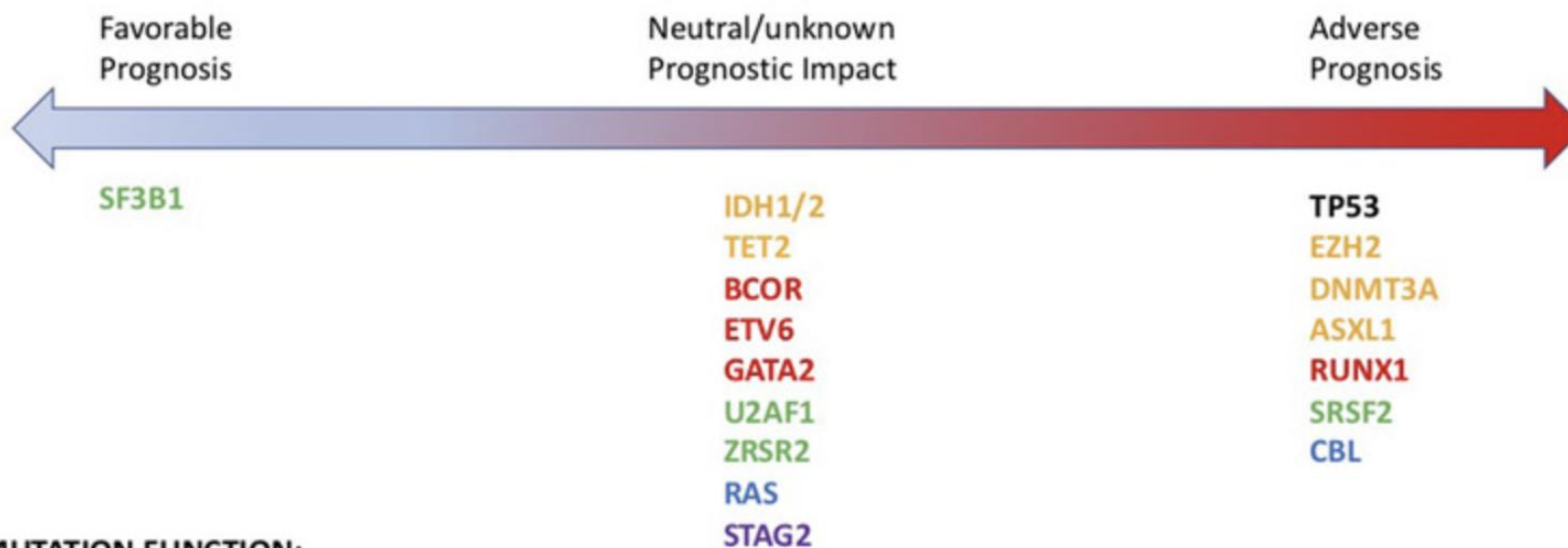


CHIP, CCUS, ICUS ...és a többiek



ICUS, idiopathic cytopenia of undetermined significance;
CCUS, clonal cytopenia of undetermined significance;
IDUS, idiopathic dysplasia of undetermined significance;
CHIP, clonal hematopoiesis of indeterminate potential.

Mutációk Prognosztikus Szerepe



MUTATION FUNCTION:

DNA Damage Response

Epigenetic/Chromatin Modifiers

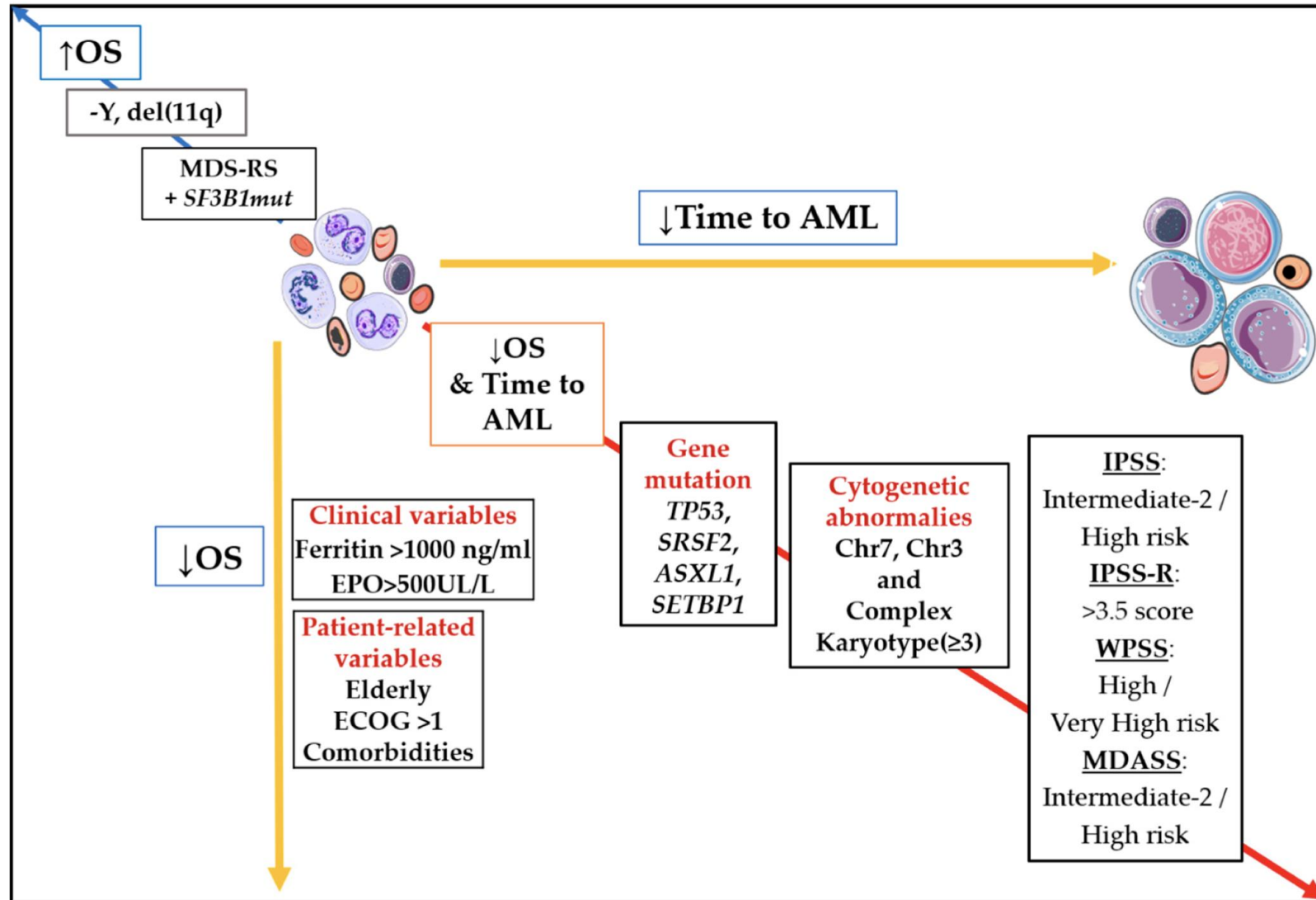
Transcription Factors

RNA Splicing

Signal Transduction

Cohesin Complex

Prognózt meghatározó tényezők



IPSS-M Risk Calculator for Myelodysplastic Syndromes (MDS)

MDS

NEJM
Evidence

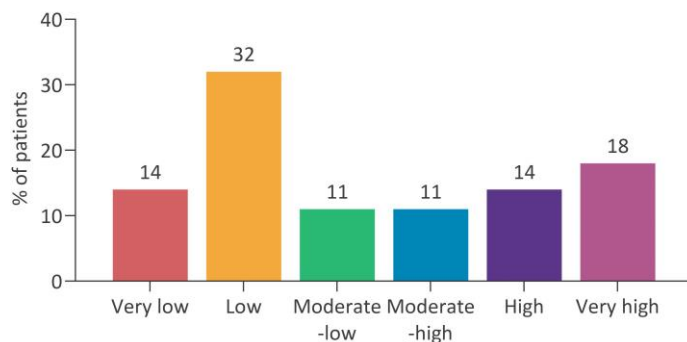
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ORIGINAL ARTICLE | FREE PREVIEW

Molecular International Prognostic Scoring System for Myelodysplastic Syndromes

Elsa Bernard, Ph.D.¹, Heinz Tüchler, Peter L. Greenberg, M.D.², Robert P. Hasserjian, M.D.³, Juan E. Arango Ossa, M.S.¹, Yasuhito Nannya, M.D., Ph.D.^{4,5}, Sean M. Devlin, Ph.D.¹, Maria Creignou, M.D.⁶, Philippe Pinel, M.S.¹, Lily Monnier, M.S.¹, ...
[Show More](#)



- 54% maradt az eredeti rizikócsoporthban
- 34% magasabb, 12% alacsonyabb csoportba került
- 7% legalább két kategóriát váltott

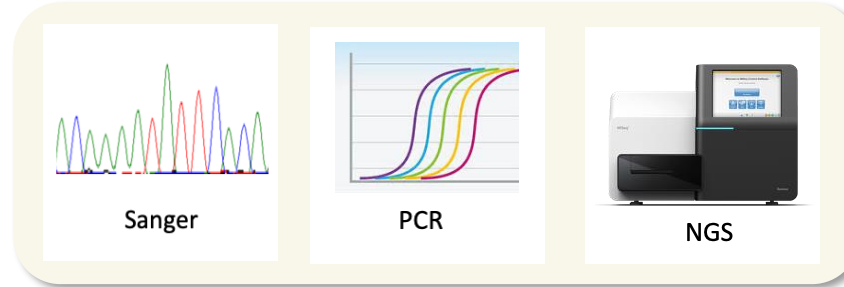
21 gén: TP53mutli, MLL^{PTD}, FLT3^{ITD/TKD}, SF3B1, NPM1, RUNX1, IDH2, NRAS, ETV6, EZH2, SETBP1, CBL, SRSF2, U2AF1, DNMT3A, ASXL1, KRAS, NF1, STAG2, BCOR, CEBPA

Összefoglalás

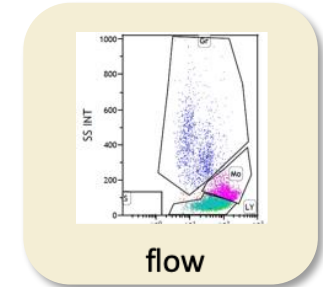
CITOGENETIKA



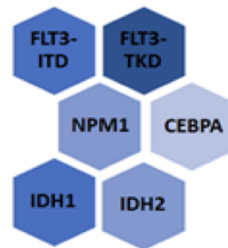
MOLEKULÁRIS VIZSGÁLATOK



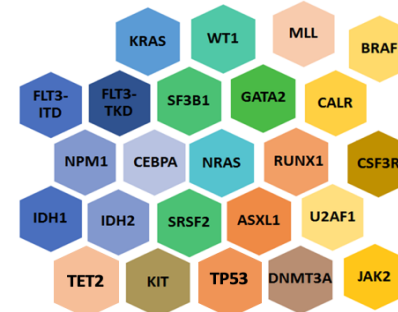
ÁRAMLÁSI CITOMETRIA



HAGYOMÁNYOS VIZSGÁLATOK KÖLTSÉGEI



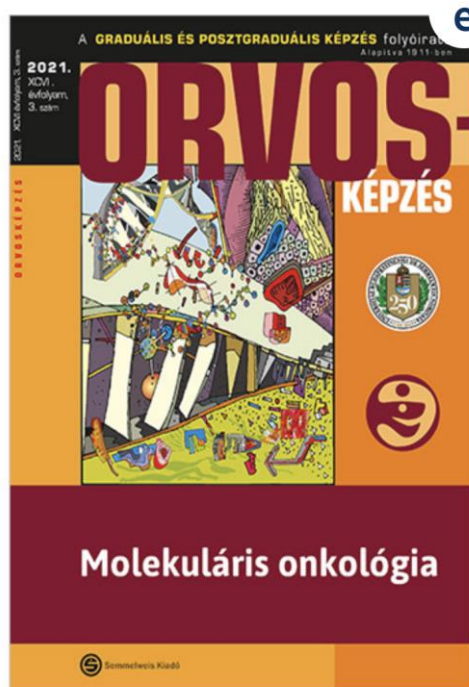
ÚJ-GENERÁCIÓS SZEKVENÁLÁS KÖLTSÉGEI



Összefoglalás

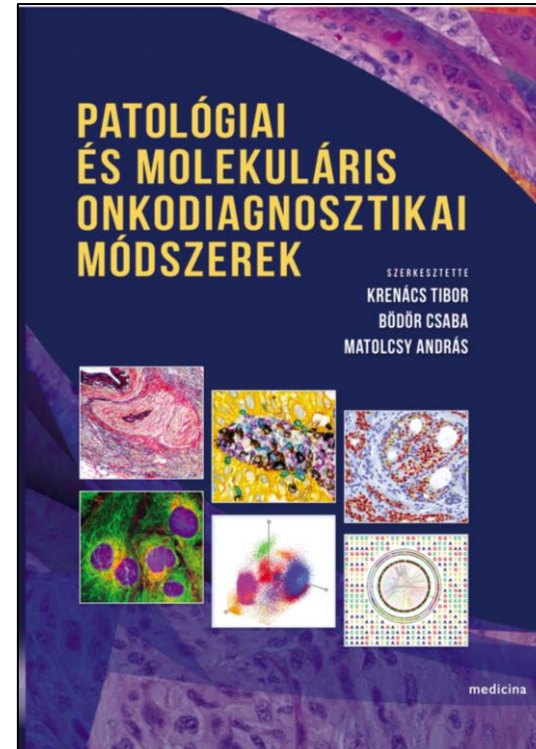
- Genetikai eltérések megjelenése a WHO klasszifikációban
- Genetikai eltérések szerepe a rizikóbecslésben (IPSS-M, ELNET)
- Biomarker vezérelt kezelési lehetőségek
- Csírvonalbeli mutációk jelentősége (*GATA2*, *DDX41* és társaik)
- NGS a mindennapi diagnosztikában Magyarországon
 - Mely betegcsoport számára?
 - Milyen kívánt leletátfordulási idővel
 - Megfelelő minta szükségessége

Ajánlott Irodalom



2021; XCVI. 3:301-620.

<https://www.semmelweiskiado.hu/t/ermekek/folyoiratok/orvoskepzes>



<https://www.medicina-kiado.hu/kiadvanyaink/szak-es-tankonyvek/patologia/patologiai-es-molekularis-onkodiagnostikai-modszerek/>

Molekuláris Onkohematológia csapat

