Molekularna dijagnostika akutnih leukemija

Margareta Radić Antolic
Klinički zavod za laboratorijsku dijagnostiku, KBC Zagreb

Kliničko pitanje?

Upućuje se na djelatnost 2550000 - Laboratorijska dijagnostika
(šifra i naziv djelatnosti)

u 006200621 - KLINIČKI BOLNIČKI CENTAR ZAGREB
(Šifra i naziv zdravstvene ustanove u koju se osiguranik upućuje, nije obavezan podatak)

Šifra dijagnoze prema MKB D 6 4 . 9 Hitno: NE Dijagnostički nalazi: NE U prilogu povijest bolesti: DA

Uputna dijagnoza Anemija, nespecificirana

Molim, traži se MOLEKULARNA IZ KOŠTANE SRŽI

u 006200621 - KLINIČKI BOLNIČKI CENTAR ZAGREB
(Šifra i naziv zdravstvene ustanove u koju se osiguranik upućuje, nije obavezan podatak)

Šifra dijagnoze prema MKB D 7 2 . 9 Hitno: NE Dijagnostički nalazi: NE U prilogu povijest bolesti: DA

Uputna dijagnoza Makrocitna anemija

Molim, traži se NGS

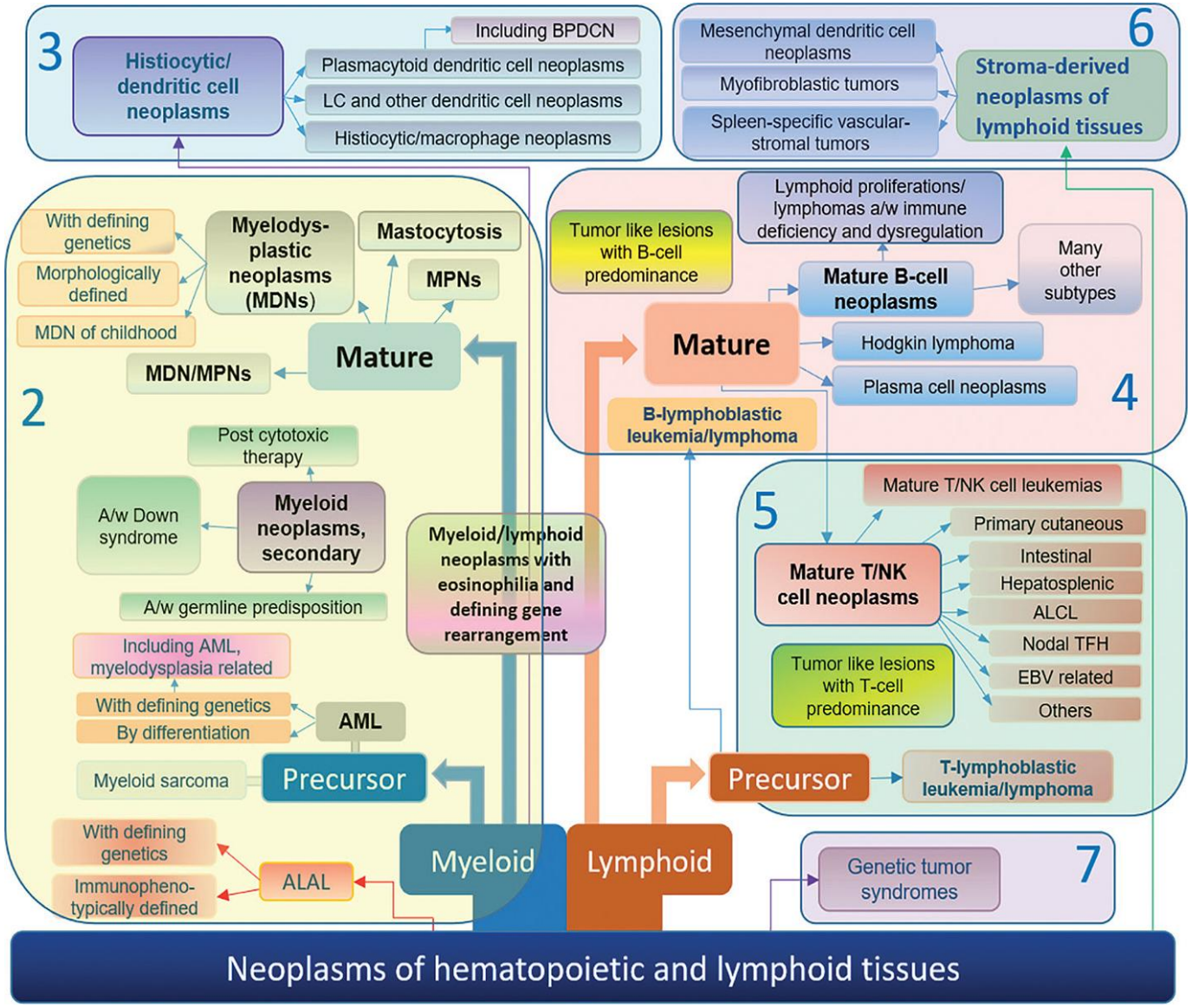
Dijagnostika hematoonkoloških bolesti

multidisciplinarni dijagnostički pristup

- citomorfologija/citokemija
- imunofenotipizacija
- citogenetika
- molekularna genetika/dijagnostika

Informacije iz anamneze i morfologije neophodne su za primarnu obradu, a pomažu u podjeli bolesnika prema:

- ***Dobi***
  - **Pedijatrijska ili odrasla populacija**
- ***Kliničkom stanju***
  - **Akutne ili kronične**
- ***Vrsti zahvaćene loze***
  - **Limfoproliferativne ili mijeloproliferativne**



Li W. The 5th Edition of the World Health Organization Classification of Hematolymphoid Tumors. In: Li W, editor. Leukemia [Internet]. Brisbane (AU): Exon Publications; 2022 Oct 16.

Akutne limfoblastične leukemije

WHO 2022

Precursor B-cell neoplasms
B-cell lymphoblastic leukaemias/lymphomas
B-lymphoblastic leukaemia/lymphoma, NOS
B-lymphoblastic leukaemia/lymphoma with high hyperdiploidy
B-lymphoblastic leukaemia/lymphoma with hypodiploidy
B-lymphoblastic leukaemia/lymphoma with iAMP21
B-lymphoblastic leukaemia/lymphoma with *BCR::ABL1* fusion
B-lymphoblastic leukaemia/lymphoma with *BCR::ABL1*-like features
B-lymphoblastic leukaemia/lymphoma with *KMT2A* rearrangement
B-lymphoblastic leukaemia/lymphoma with *ETV6::RUNX1* fusion
B-lymphoblastic leukaemia/lymphoma with *ETV6::RUNX1*-like features
B-lymphoblastic leukaemia/lymphoma with *TCF3::PBX1* fusion
B-lymphoblastic leukaemia/lymphoma with *IGH::IL3 fusion*
B-lymphoblastic leukaemia/lymphoma with *TCF3::HLF* fusion
B-lymphoblastic leukaemia/lymphoma with other defined genetic abnormalities

Precursor T-cell neoplasms
T-lymphoblastic leukaemia/lymphoma
T-lymphoblastic leukaemia / lymphoma, NOS
Early T-precursor lymphoblastic leukaemia / lymphoma

Khoury JD, et al. Leukemia. 2022;36(7): 1703-1719.

1. **Dijagnoza**
2. **Klasifikacija**
3. **Stratifikacija rizika**
4. **Terapija**
5. **Molekularni biljeg**
   - ✓ **Praćenje MRD**
   - ✓ **Rano predviđanje relapsa**
   - ✓ **Prevencija**

ICC 2022

B-ALL

- B-ALL with recurrent genetic abnormalities
- B-ALLwith t(9;22)(q34.1;q11.2)/*BCR::ABL1*
  - with lymphoid only involvement
  - with multilineage involvement
- B-ALLwith t(v;11q23.3)/*KMT2A* rearranged
- B-ALL with t(12;21)(p13.2;q22.1)/*ETV6::RUNX1*
- B-ALL, hyperdiploid
- B-ALL, low hypodiploid
- B-ALL, near haploid
- B-ALL with t(5;14)(q31.1;q32.3)/*IL3*::IGH
- B-ALL with t(1;19)(q23.3;p13.3)/*TCF3::PBX1*
- B-ALL, *BCR::ABL1*–like, ABL-1 class rearranged
- B-ALL, *BCR::ABL1*–like, JAK-STAT activated
- B-ALL, *BCR::ABL1*–like, NOS
- B-ALL with iAMP21
- B-ALL with *MYC* rearrangement
- B-ALL with *DUX4* rearrangement
- B-ALL with *MEF2D* rearrangement
- B-ALL with *ZNF384(362)* rearrangement
- B-ALL with *NUTM1* rearrangement
- B-ALL with *HLF* rearrangement
- B-ALL with *UBTF::ATXN7L3/PAN3,CDX2* ("CDX2/UBTF")
- B-ALL with mutated *IKZF1* N159Y
- B-ALLwith mutated *PAX5* P80R

- Provisional entity: B-ALL, *ETV6::RUNX1*-like
- Provisional entity: B-ALL, with *PAX5* alteration
- Provisional entity: B-ALL, with mutated *ZEB2* (p.H1038R)/ IGH::*CEBPE*
- Provisional entity: B-ALL, *ZNF384* rearranged-like
- Provisional entity: B-ALL, *KMT2A* rearranged-like

B-ALL, NOS

T-ALL

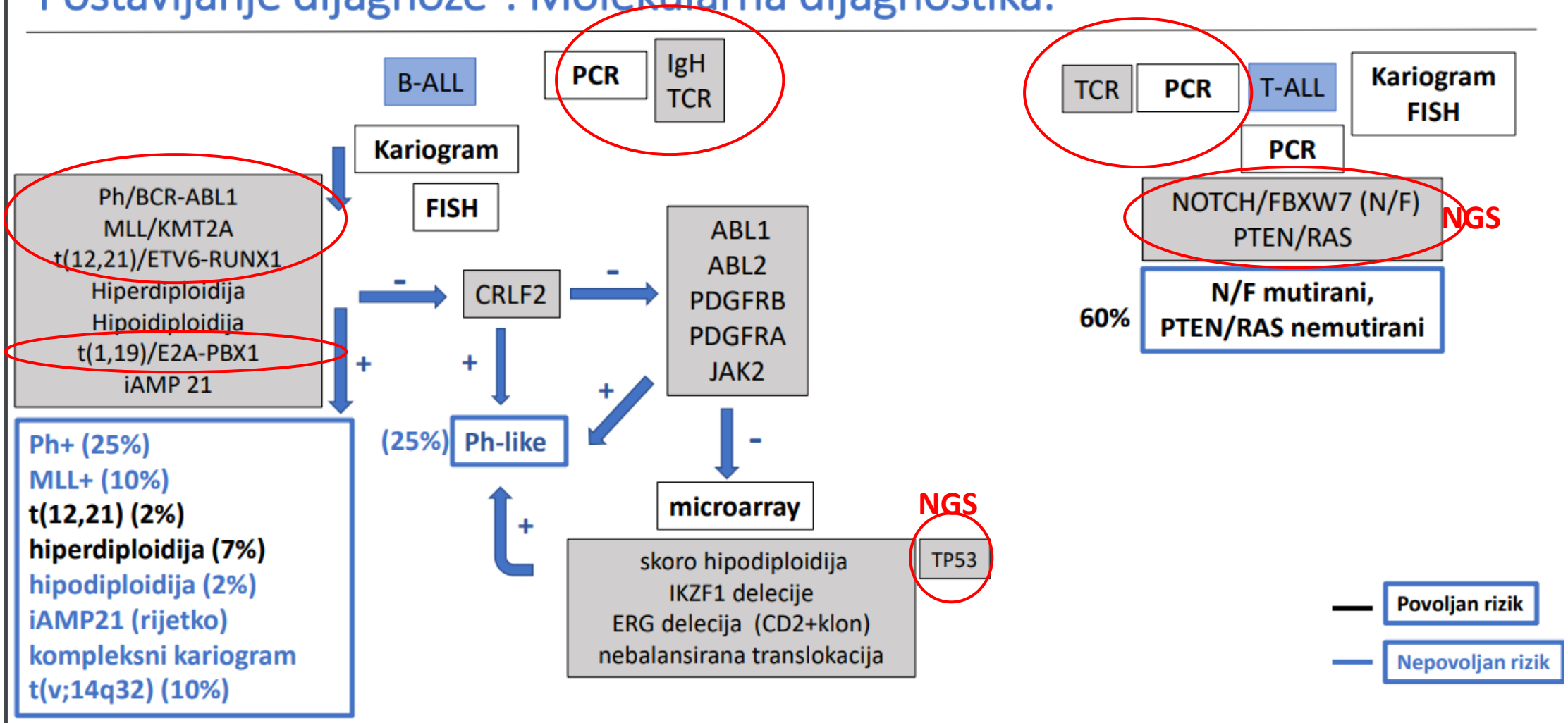
- Early T-cell precursor ALLwith *BCL11B* rearrangement
- Early T-cell precursor ALL, NOS

T-ALL, NOS

Arber, Daniel A et al. *Blood* vol. 140,11 (2022): 1200-1228.

KroHem

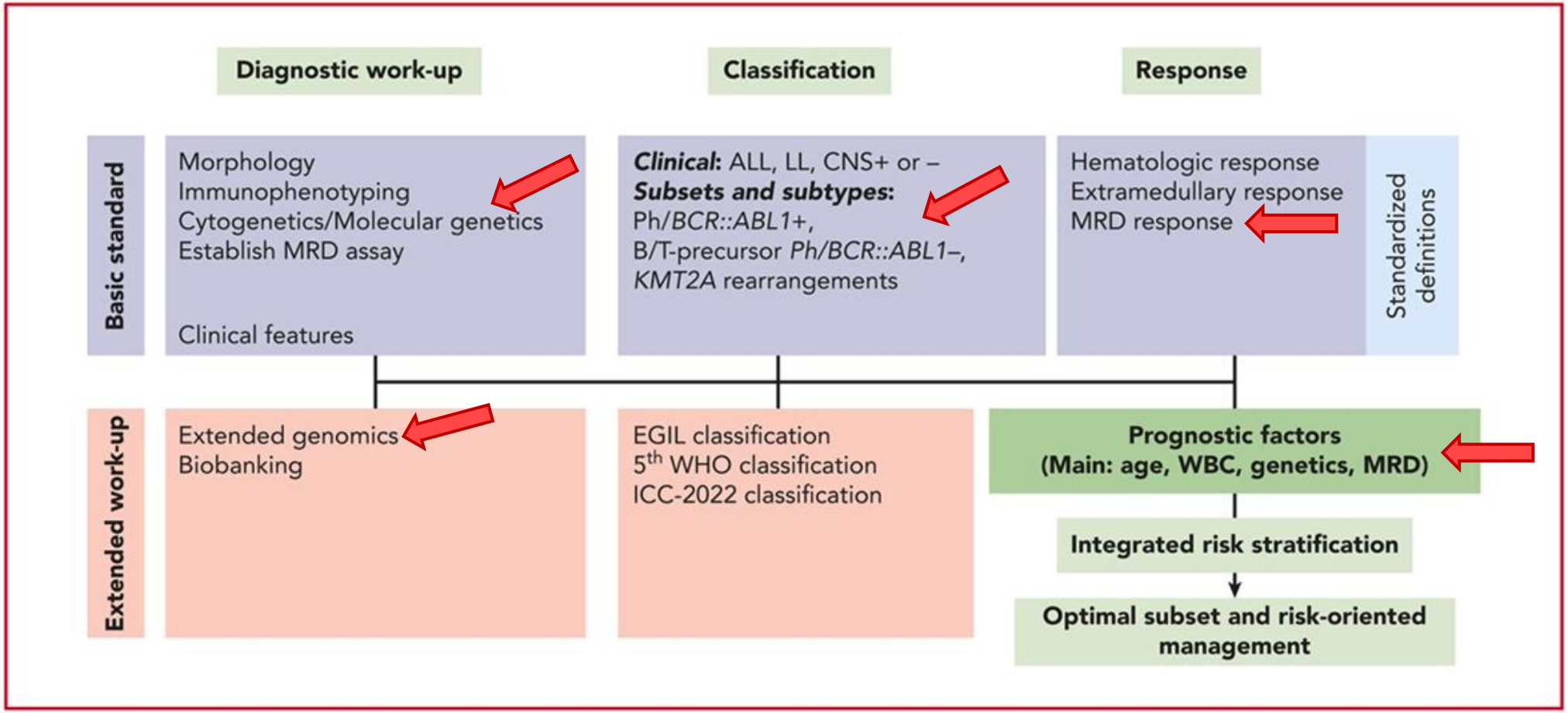
Postavljanje dijagnoze[1]. Molekularna dijagnostika.



PREPORUKA: Za B-ALL se preporuča učiniti kariogram/FISH prema gornjem algoritmu za procjenu rizika te PCR za definiciju markera praćenja minimalne ostatne bolesti. Za T-ALL se uz kariogram/FISH preporuča učiniti NGS ako je moguće te PCR za praćenje MRD-a. Kod bolesnika koji nisu podobni za intenzivnu kemoterapiju dovoljno je učiniti klasičan kariogram/FISH te PCR.

https://www.krohem.hr/wp-content/uploads/2022/02/ALL-smjernice-finalna-verzija-22022.pdf

Diagnosis, Prognostic Factors, and Risk Stratification of Acute Lymphoblastic Leukemia (ALL) in Adults: 2024 ELN Recommendations from a European Expert Panel



Gökbuget N, et al.. Blood. 2024 May 9;143(19):1891-1902.

NGS paneli za ALL

Fuzije kinaza koje se prepoznaju kao *BCR::ABL1*-like ALL

Gen	TKI	5′ Fuzijski partner
ABL1	Imatinib/dasatinib	*CENPC*, *ETV6*, *FOXP1*, *LSM14A*, *NUP153*, *NUP214*, *RANBP2*, *RCSD1*, *SFPQ*, *SNX1*, *SNX2*, *SPTNA1*, *ZMIZ1*
ABL2	Imatinib/dasatinib	*PAG1*, *RCSD1*, *ZC3HAV1*
CSF1R	Imatinib/dasatinib	*MEF2D*, *SSBP2*, *TBL1XR1*
PDGFRA	Imatinib/dasatinib	*FIP1L1*
PDGFRB	Imatinib/dasatinib	*ATF7IP*, *EBF1*, *ETV6*, *SNX29*, *SSBP2*, *TNIP1*, *ZEB2*, *ZMYND8*
LYN	Imatinib/dasatinib	*GATAD2A*, *NCOR1*
CRLF2	JAK2 inhibitor	*CSF2RA*, *IGH*, *P2RY8*
JAK2	JAK2 inhibitor	*ATF7IP*,*BCR*, *EBF1*, *ETV6*, *GOLGA5*, *HMBOX1*, *OFD1*, *PAX5*, *PCM1*, *PPFIBP1*, *RFX3*, *SMU1*, *SNX29*, *SSBP2*, *STRN3*, *TERF2*, *TPR*, *USP25*, *ZBTB46*, *ZNF274*, *ZNF340*
EPOR	JAK2 inhibitor	*IGH*, *IGK*, *LAIR1*, *THADA*
TSLP	JAK2 inhibitor	*IQGAP2*
TYK2	TYK2 inhibitor	*MYB*, *SMARCA4*, *ZNF340*
IL2RB	JAK1/JAK3 inhibitor	*MYH9*
NTRK3	TRK inhibitor	*ETV6*
PTK2B	FAK inhibitor	*KDM6A*, *STAG2*, *TMEM2*
FGFR1	Ponatinib	*BCR*
FLT3	FLT3 inhibitor	*ZMYM2*
DGKH		*ZFAND3*
BLNK		*DNTT*
CBL		*KANK1*

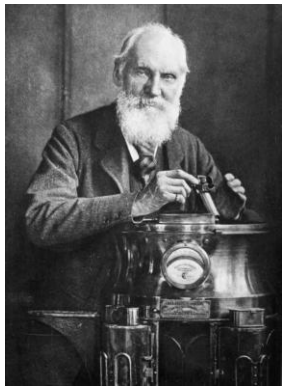
Fuzije
Prekrajanje
SNV
Indels
Ekspresija

B-ALL, *BCR::ABL1*–like, ABL-1 class rearranged
B-ALL, *BCR::ABL1*–like, JAK-STAT activated
B-ALL, *BCR::ABL1*–like, NOS
B-ALL with iAMP21
B-ALL with *MYC* rearrangement
B-ALL with *DUX4* rearrangement
B-ALL with *MEF2D* rearrangement
B-ALL with *ZNF384(362)* rearrangement
B-ALL with *NUTM1* rearrangement
B-ALL with *HLF* rearrangement
B-ALL with *UBTF::ATXN7L3/PAN3,CDX2* ("CDX2/UBTF")
B-ALL with mutated *IKZF1* N159Y
B-ALLwith mutated *PAX5* P80R
Provisional entity: B-ALL, *ETV6::RUNX1*-like
Provisional entity: B-ALL, with *PAX5* alteration
Provisional entity: B-ALL, with mutated *ZEB2* (p.H1038R)/ IGH::*CEBPE*
Provisional entity: B-ALL, *ZNF384* rearranged-like
Provisional entity: B-ALL, *KMT2A* rearranged-like

ABL1	ABL2	AICDA	BCL11B	BCL2	BCL6	BCR	BLNK	BRAF
CD274	CHD1	CREBBP	CRLF2	CSF1R	CTLA4	DNM2	DNTT	EBF1
EPOR	ETV6	EZH2	FBXW7	FGFR1	FLT3	HOXA10	HOXA9	IDH1
IDH2	IKZF1	IKZF2	IKZF3	IL7R	IRF4	IRF8	JAK1	JAK2
JAK3	KDM6A	KLF2	KMT2A	KRAS	LMO1	LYL1	MLLT4	MPL
MYC	NF1	NOTCH1	NRAS	NT5C2	NTRK3	NUP214	NUP98	P2RY8
PAG1	PAX5	PBX1	PDCD1	PDCD1LG2	PDGFRA	PDGFRB	PICALM	PTK2B
PTPN11	RAG1	RAG2	RUNX1	SEMA6A	SETD2	SH2B3	SOX11	STAT3
STAT5B	STIL	TAL1	TCF3	TLX1	TLX3	TYK2	WT1	ZCCHC7

MRD – engl. *mesurable residual disease*

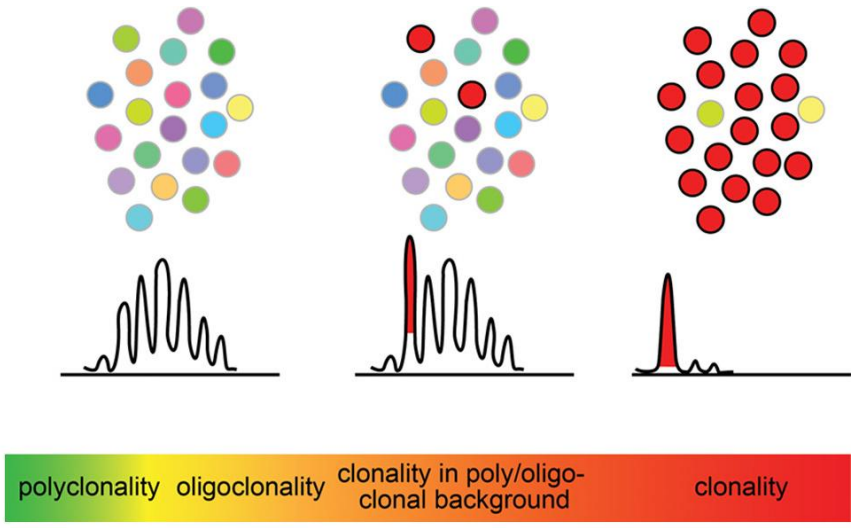
- ✓ ***Uzorak – periferna krv, koštana srž, slobodna tumorska DNA***
- ✓ ***Vremenske točke praćenja***
- ✓ ***Biljeg – fuzijski gen*** *(npr. BCR::ABL1)****, Ig/TCR klonalnost***
- ✓ ***Metoda***



"When you can measure what you are speaking about and express it in numbers, you know something about it."

Lord Kelvin (William Thompson), scientist

Chen, J., Gale, R.P., Hu, Y. *et al.* Measurable residual disease (MRD)-testing in haematological and solid cancers. *Leukemia* **38**, 1202–1212 (2024). https://doi.org/10.1038/s41375-024-02252-4



	Multi-color flow cytometry	qPCR for fusion genes	ASO-qPCR for IG/TR genes	High-throughput NGS
Sensitivity	10^{-4}	10^{-4} to 10^{-5}	10^{-4} to 10^{-5}	10^{-6}
Applicability	>90%	40-50%	90-95%	>90%
Advantages	- Rapid - Relatively inexpensive - DfN method does not require access to dia-gnostic specimen	- Sensitive - Standard primers used for specific fusions	- Sensitive - Applicable to most patients - Standardized guidelines in Europe	- Very sensitive - Applicable to almost all patients - Clone-unbiased (can track multiple clones and evolution) - Only US FDA-appro-ved assay (ClonoSEQ) - Data for MRD use in peripheral blood
Limitations	- Variable sensitivity - Requires technical expertise - Fresh cells required - Less standardized - Immunophenotypic shifts can lead to false negative results	- Not applicable to all patients	- Time-consuming - Expensive - Relies on pre-treat-ment sample - Requires extensive experience and labor	- Expensive - Longer turn-around time than MFC - Requires diagnostic pre-treatment sample

ALL: acute lymphoblastic leukemia; ASO: allele-specific oligonucleotide; DfN: different-from-normal; FDA: Food and Drug Administration; IG: immunoglobulin; MFC. multicolor flow cytometry; NGS, next-generation sequencing; qPCR, quantitative polymerase chain reaction; TCR, T-cell receptor.

Saygin C, et al. Haematologica. 2022 Dec 1;107(12):2783-2793.

Akutne mijeloične leukemije

Hrvatska komora medicinskih biokemičara

WHO 2022

Acute myeloid leukaemia.

Acute myeloid leukaemia with defining genetic abnormalities
Acute promyelocytic leukaemia with *PML*::*RARA* fusion
Acute myeloid leukaemia with *RUNX1*::*RUNX1T1* fusion
Acute myeloid leukaemia with *CBFB*::*MYH11* fusion
Acute myeloid leukaemia with *DEK*::*NUP214* fusion
Acute myeloid leukaemia with *RBM15*::*MRTFA* fusion
Acute myeloid leukaemia with *BCR*::*ABL1* fusion
Acute myeloid leukaemia with *KMT2A* rearrangement
Acute myeloid leukaemia with *MECOM* rearrangement
Acute myeloid leukaemia with *NUP98* rearrangement
Acute myeloid leukaemia with *NPM1* mutation
Acute myeloid leukaemia with *CEBPA* mutation
Acute myeloid leukaemia, myelodysplasia-related
Acute myeloid leukaemia with other defined genetic alterations

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
ASXL1
BCOR
EZH2
SF3B1
SRSF2
STAG2
U2AF1
ZRSR2

Myeloid neoplasms associated with germline predisposition:

- ✓ **introduced a novel scalable model in WHO 2022**
- ✓ **include AML, MDS, MPN, and MDS/MPN that arise in individuals with genetic conditions associated with increased risk of myeloid malignancies**

Khoury JD, et al. Leukemia. 2022;36(7): 1703-1719.

ICC 2022

AML and related neoplams

AML with recurrent genetic abnormalities (requiring ≥10% blasts in BM or PB)

- APL with t(15;17)(q24.1;q21.2)/*PML*::*RARA*[b]
- AML with t(8;21)(q22;q22.1)/*RUNX1*::*RUNX1T1*
- AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22)/*CBFB*::*MYH11*
- AML with t(9;11)(p21.3;q23.3)/*MLLT3*::*KMT2A*[b]
- AML with t(6;9)(p22.3;q34.1)/*DEK*::*NUP214*
- AML with inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2)/*GATA2, MECOM(EVI1)*[b]
- AML with other rare recurring translocations
- AML with mutated *NPM1*
- AML with in-frame bZIP mutated *CEBPA*[c]
- AML with t(9;22)(q34.1;q11.2)/*BCR*::*ABL1*[a]

Myeloid sarcoma

Acute leukemia of ambiguous lineage

- Acute undifferentiated leukemia
- MPAL with t(9;22)(q34.1;q11.2)/*BCR*::*ABL1*
- MPAL with t(v;11q23.3)/*KMT2A* rearranged
- MPAL, B/myeloid, not otherwise specified
- MPAL, T/myeloid, not otherwise specified

Categories designated AML (if ≥20% blasts in BM or PB) or MDS/AML (if 10-19% blasts)

- AML with mutated *TP53*[d]
- AML with myelodysplasia-related gene mutations
  Defined by mutations in *ASXL1*, *BCOR*, *EZH2*, *RUNX1*, *SF3B1*, *SRSF2*, *STAG2*, *U2AF1*, and/or *ZRSR2*
- AML with myelodysplasia-related cytogenetic abnormalities[e]
- AML not otherwise specified

Myeloid proliferations related to Down syndrome

- Transient abnormal myelopoiesis associated with Down syndrome
- Myeloid leukemia associated with Down syndrome

Blastic plasmacytoid dendritic cell neoplasm

Diagnostic qualifiers

Therapy-related; progression from MDS; progression from MDS/MPN; germline predisposition (specify type)

AML and MDS/AML with myelodysplasia-related gene mutations
10-19% (MDS/AML) and ≥ 20% (AML)

Defined by mutations in *ASXL1, BCOR, EZH2, RUNX1, SF3B1, SRSF2, STAG2, U2AF1, or ZRSR2*

Premalignant clonal cytopenias and myelodysplastic syndromes

- Clonal cytopenia of undetermined significance
- Myelodysplastic syndrome with mutated *SF3B1*
- Myelodysplastic syndrome with del(5q)
- Myelodysplastic syndrome with mutated *TP53*
- Myelodysplastic syndrome, not otherwise specified (MDS, NOS)
  - MDS, NOS without dysplasia
  - MDS, NOS with single lineage dysplasia
  - MDS, NOS with multilineage dysplasia
- Myelodysplastic syndrome with excess blasts
- Myelodysplastic syndrome/acute myeloid leukemia (MDS/AML)
  - MDS/AML with mutated *TP53*
  - MDS/AML with myelodysplasia-related gene mutations
  - MDS/AML with myelodysplasia-related cytogenetic abnormalities
  - MDS/AML, not otherwise specified

Arber, Daniel A et al. *Blood* vol. 140,11 (2022): 1200-1228.

Diagnosis and management of AML in adults: 2022 recommendations from an international expert panel on behalf of the ELN

blood® 22 SEPTEMBER 2022 | VOLUME 140, NUMBER 12

Tests and procedures	
Tests to establish the diagnosis	
Complete blood count and differential count*	
Bone marrow aspirate†	
Bone marrow trephine biopsy‡	
Immunophenotyping by flow cytometry (see Table 5)	
Genetic analyses	Results preferably available within
Cytogenetics§	• 5-7 d
Screening for gene mutations required for establishing the diagnosis and to identify actionable therapeutic targets#	
• *FLT3*,¶ *IDH1*, *IDH2*	• 3-5 d
• *NPM1*	• 3-5 d
• *CEBPA*,# *DDX41*, *TP53*; *ASXL1*, *BCOR*, *EZH2*, *RUNX1*, *SF3B1*, *SRSF2*, *STAG2*, *U2AF1*, *ZRSR2*	• 1st cycle
Screening for gene rearrangements**	
• *PML*::*RARA*, *CBFB*::*MYH11*, *RUNX1*::*RUNX1T1*, *KMT2A* rearrangements, *BCR*::*ABL1*, other fusion genes (if available)	• 3-5 d
Additional genes recommended to test at diagnosis††	
• *ANKRD26*, *BCORL1*, *BRAF*, *CBL*, *CSF3R*, *DNMT3A*, *ETV6*, *GATA2*, *JAK2*, *KIT*, *KRAS*, *NRAS*, *NF1*, *PHF6*, *PPM1D*, *PTPN11*, *RAD21*, *SETBP1*, *TET2*, *WT1*	

#Koristiti genske panele za dijagnostiku - NGS

¶*FLT3*: mutacijski status uključuje dokazivanje **interne tandemske duplikacije (ITD)** i mutacije u **tirozin kinaznoj domeni (TKD)** Kombinirati kapilarnu elektroforezu i NGS.

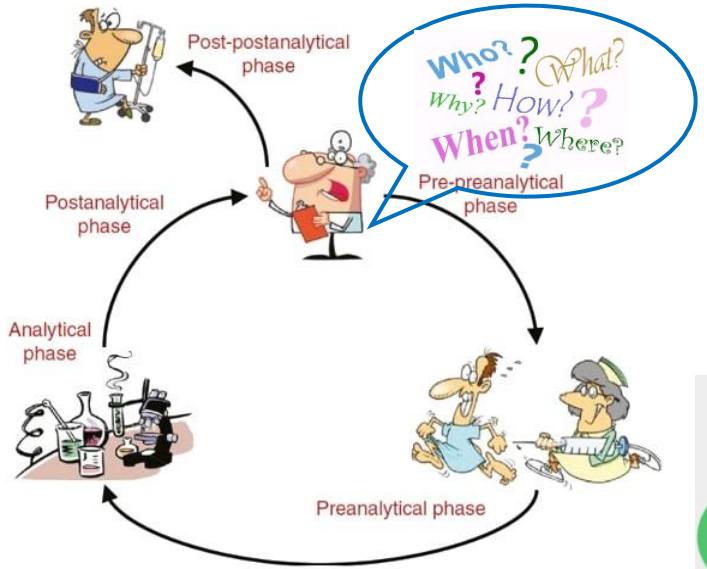
treba specificirati tip mutacije jer u ovu skupinu ulaze samo tzv. „in-frame" mutacije koje ne dovode do pomaka okvira čitanja u **bZIP regiji *CEBPA***, bez obzira zahvaćaju li jedan ili oba alela *CEBPA* gena.

†† rezultati nisu neophodni za postavljanje dijagnoze ili za odabir terapije već se koriste kao alati za praćenje MRD pomoću tehnika baziranih na NGSu (s iznimkom mutacija u genima *DNMT3A*, *TET2*, *ASXL1)*

Döhner H et al. Diagnosis and management of AML in adults: 2022 recommendations from an international expert panel on behalf of the ELN. Blood. 2022 Sep 22;140(12):1345-1377.

Algoritam molekularne dijagnostike AMLa

AML?



Uzorkovanje:
(kliničko osoblje, patolozi, citolozi, med.sestre, laboratorijsko osoblje)

Vrsta uzorka, količina?
Pohrana?
Transport?

Koštana srž

- ✓ **citologija**
- ✓ **imunofenotipizacija**
- ✓ **citogenetika (FISH - CBF, MLL, Ph)**
- ✓ **molekularna hematoonkološka dg**

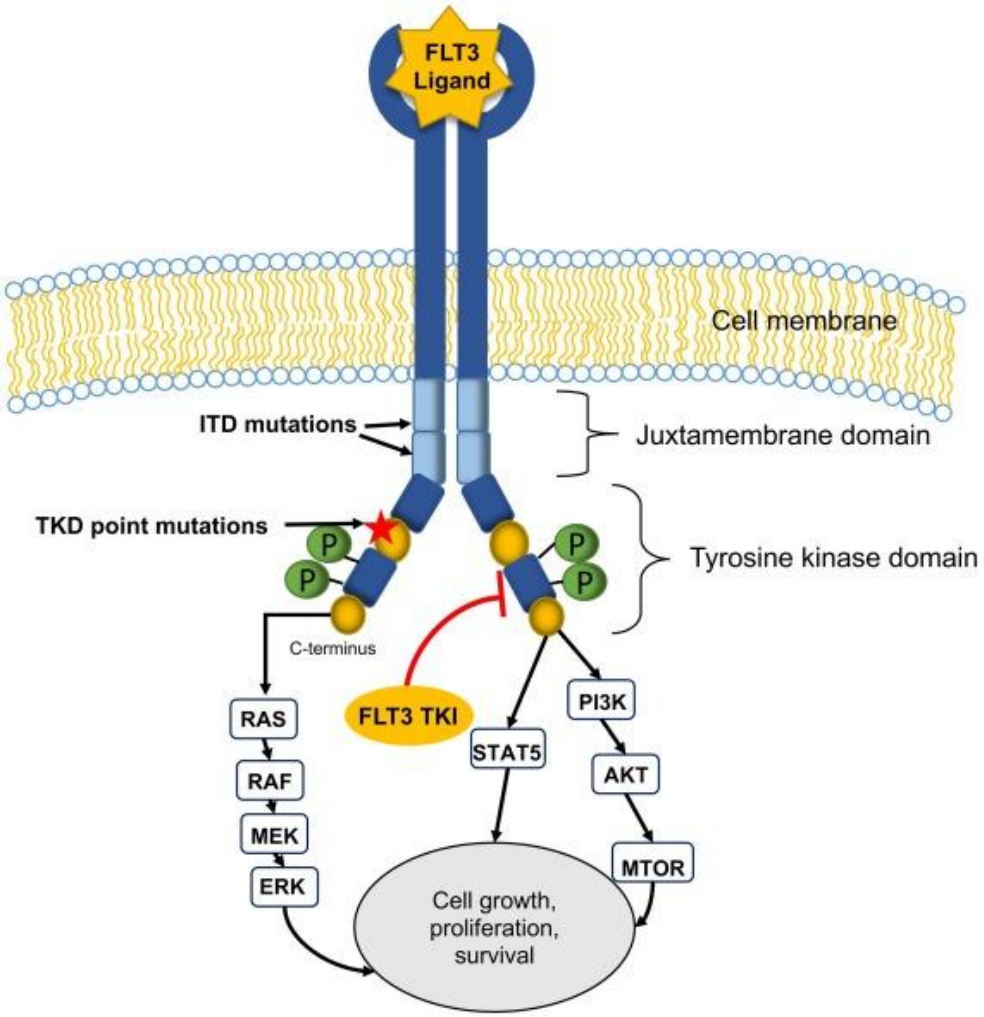
Multidisciplinarni tim - MIC

- ✓ ***FLT3 ITD/TKD***
- ✓ ***NPM1* – tip mutacije (obavezno)**

- ✓ **NGS – mijeloidni panel**

- ✓ **Fuzijski prijepisi kao potvrda CTG te određivanje tipa prijepisa**
  - ✓ **obavezno za *PML::RARA, RUNX1::RUNX1T1, CBFB::MYH11, BCR::ABL1***

FLT3 - engl. *FMS like tyrosine kinase*



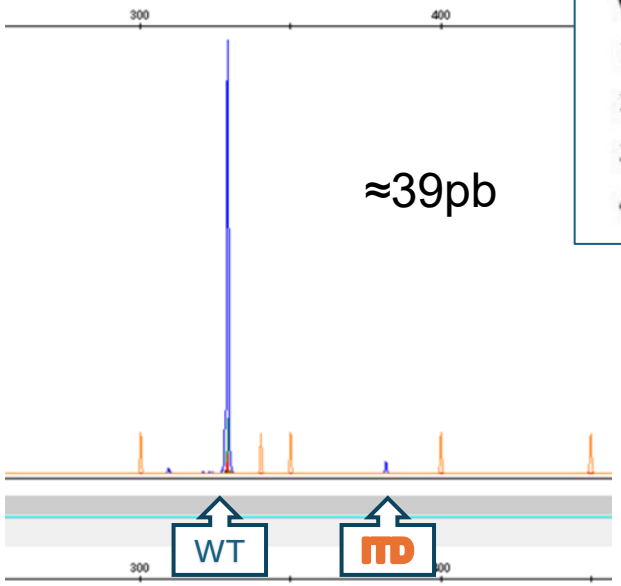
- ✓ **~30% *de novo* AML**

- ✓ **Visoka incidencija u AML s:**
  - ✓ **NPM1 mutacijom (40%)**
  - ✓ **t(15;17)(q21;q21)/PML-RARA (40-45%)**
  - ✓ **t(6;9)(p23;q34)/DEK-NUP214 (75%)**

- ✓ **Povezan s nepovoljnom prognozom**
  - ✓ **ITD mutacije (engl. *Internal tandem duplication*) (JMD, TD1)**
  - ✓ **TKD mutacije (engl. *Tyrosine kinase domain mutation*)**

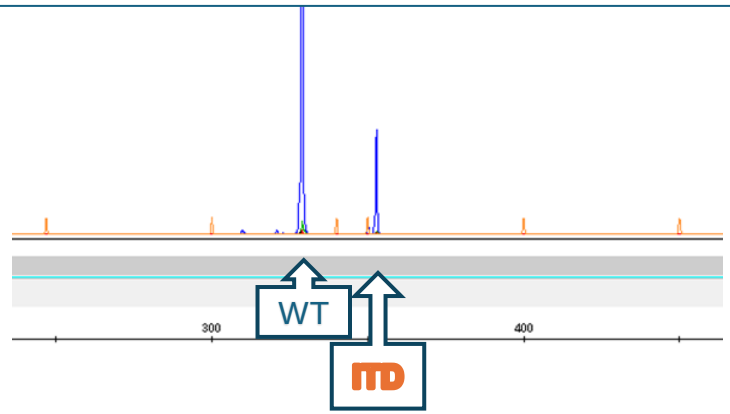
FLT3 ITD na kapilarnoj elektroforezi

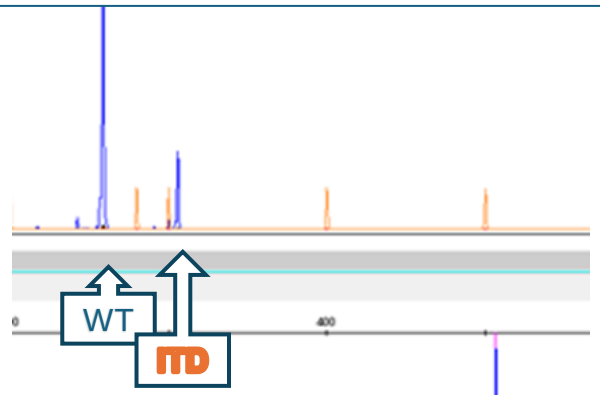
– podatak o **veličini duplikacije i udjelu** mutiranog klona



≈39pb

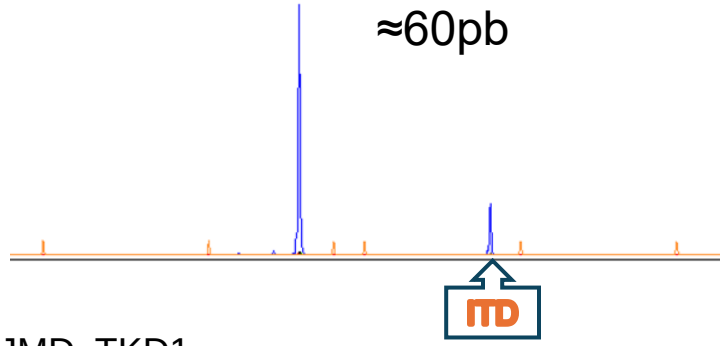
W	QFRYESQLQMVQVTGSSDNEYFYVDFREYEYDLKWEFPRENLEF
1	QFRYESQLQMVQVTGSSDNEYFYV**DFREYEYDL**DFREYEYDLKWEFPRENLEF
2	QFRYESQLQMVQVTGSSDNEYF**YVDFREYEY**YVDFREYEYDLKWEFPRENLEF
3	QFRYESQLQMVQVT**GSSDNEYFYVDFREY** GSSDNEYFYVDFREYEYDLKWEFPRENLEF
4	QFRYESQLQMV**QVTGSSDNEYFYVDFREYEYDLKWEFPRENLEF*GKNV*** QVTGSSDNEYFYVDFREYEYDLKWEFPRENLEF



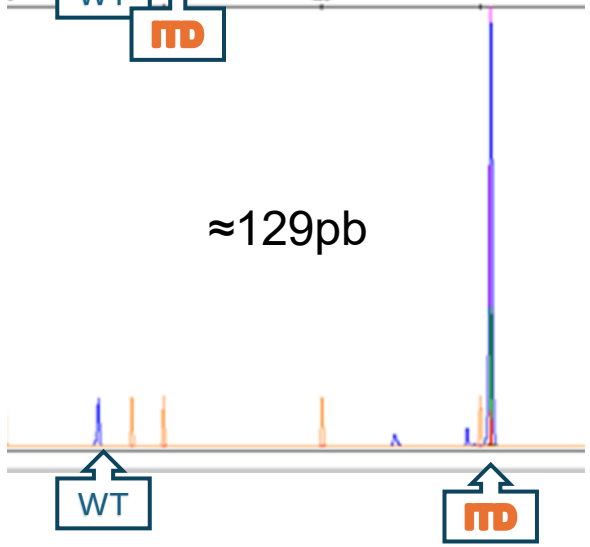


- **Klasifikacija rizika**
- **Ciljana terapija**
  - **FLT3 inhibitori**
- **Predviđanje relapsa**
  - **Molekularni MRD**

≈60pb



≈129pb

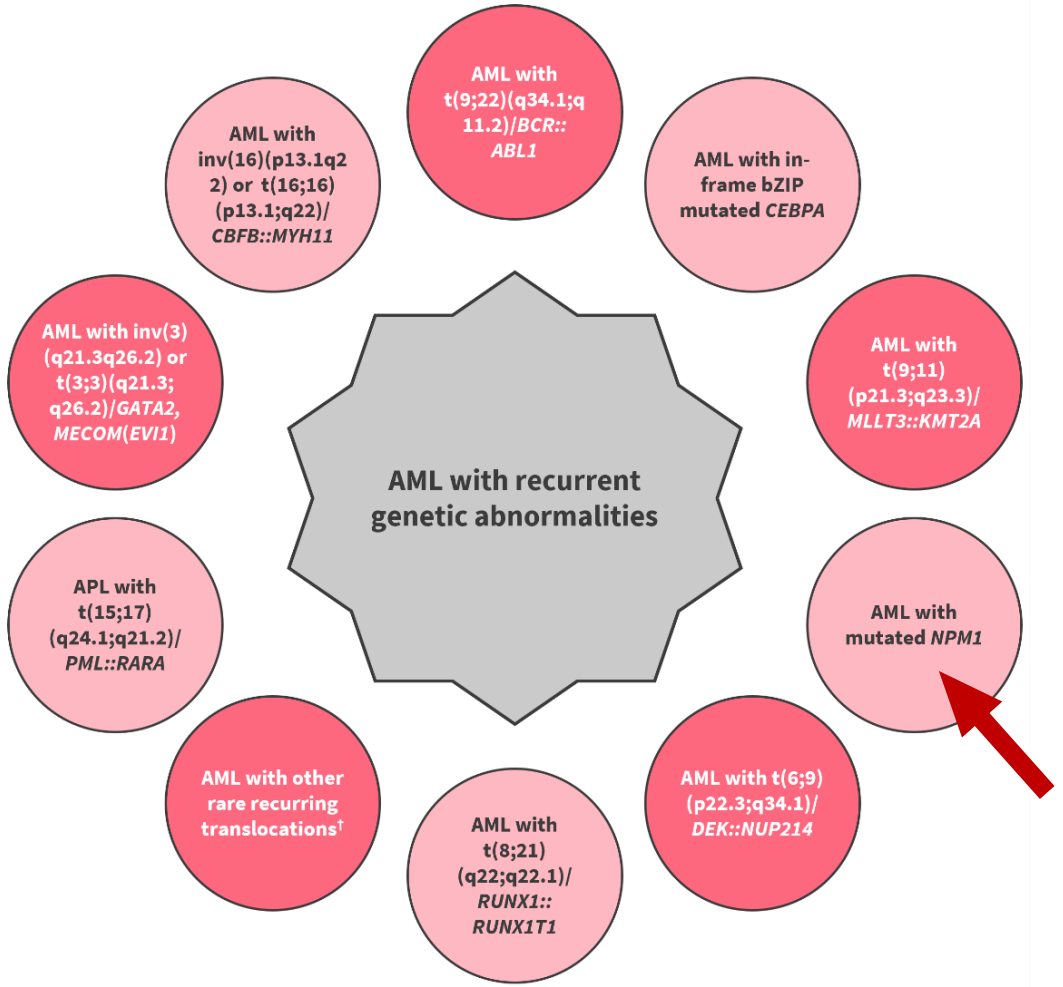


Ne daje informaciju o mjestu duplikacije – JMD, TKD1

NUKLEOFOZMIN – NPM1

- ✓ Potvrđuje dijagnozu neovisno o postotku blasta
  - ✓ ICC2022/WHO2022
- ✓ 30–35% odraslih s AML
- ✓ 50–60% AML normalni kariotip
- ✓ Mijelomonocitne ili monocitne AML
  - ✓ ali i druge AML (osim M7)
- ✓ Često u kombinaciji s *FLT3* i *DNMT3A* mutacijama

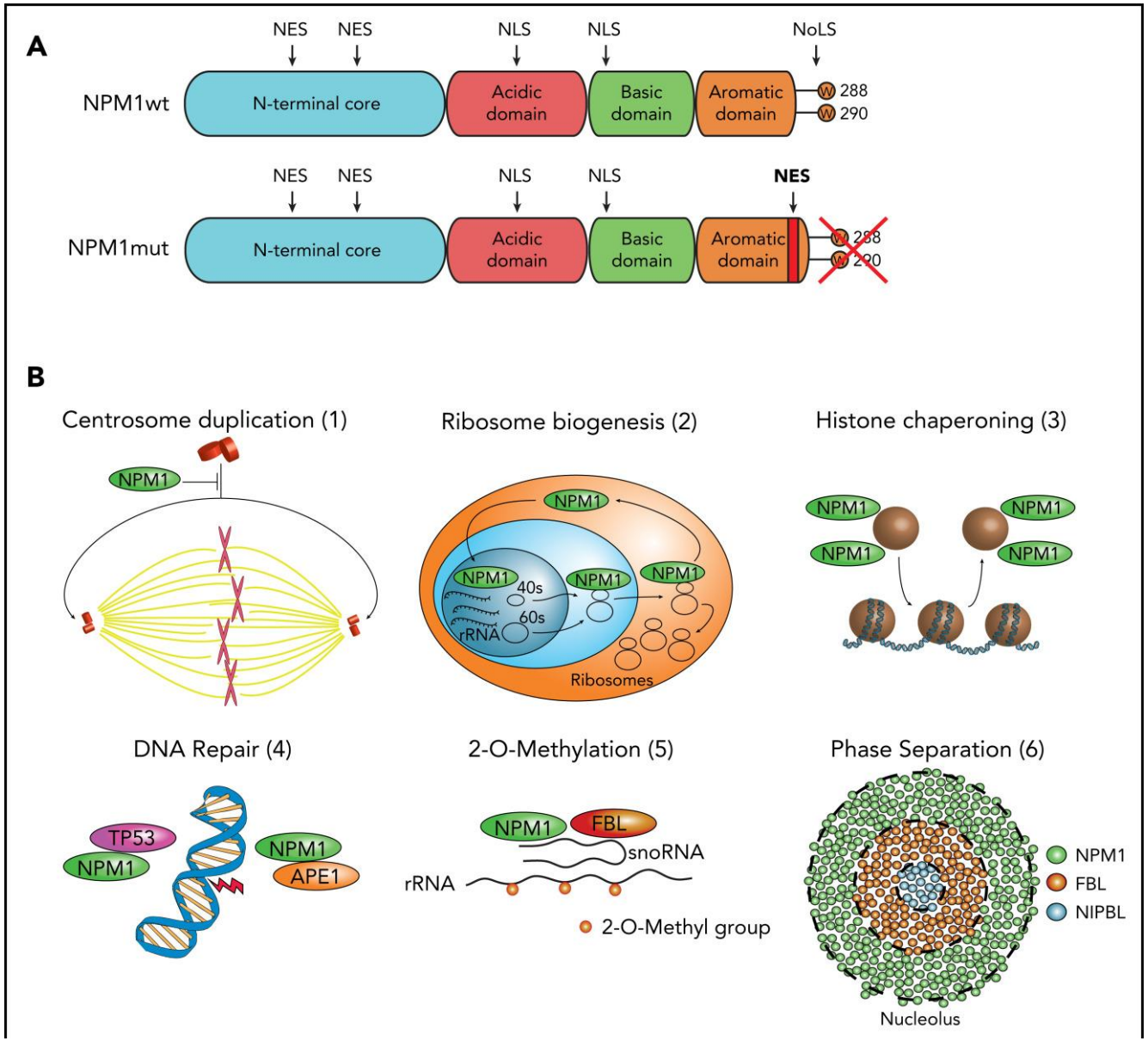
- ✓ MRD praćenje RQ-PCRom (A, B i D mutacija)



NUKLEOFOZMIN – NPM1

- ✓ **Duplikacija centrosoma**
- ✓ **Biogeneza ribosoma**
- ✓ **Vezanje histona te stvaranje heterokromatina**
- ✓ **Popravak DNA**
- ✓ **Metilacija rRNA – regulacija transkripcije**

- ✓ **Mutacije - prerana terminaciju proteina**
  - ✓ (gubi terminalne W288 i W290) koje mu omogućuju **lokalizaciju u jezgri** te vezanje na DNA, histone i *TP53* te samim time i **gubitak funkcije**



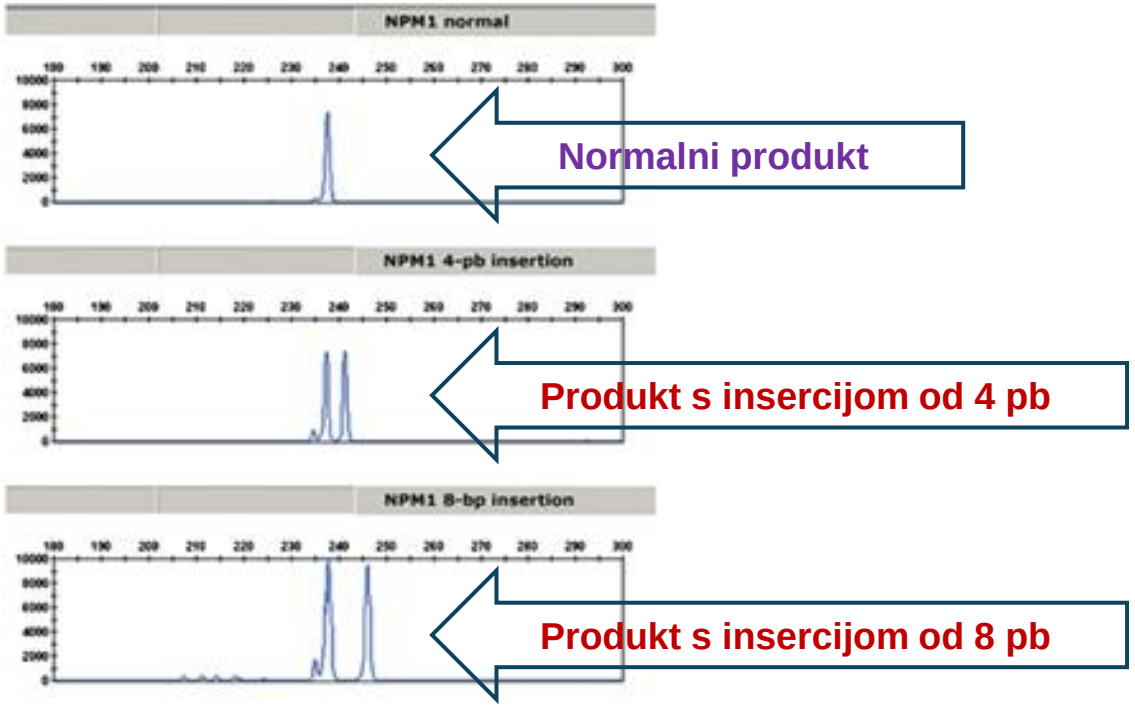
Falini B, Brunetti L, Sportoletti P, Martelli MP. NPM1-mutated acute myeloid leukemia: from bench to bedside. Blood. 2020 Oct 8;136(15):1707-1721.

Određivanje NPM1 mutacije kod dijagnoze

- ✓**Dokazivanje mutacija – duplikacija ili insercija**
- ✓**Određivanje tipa mutacije**
- ✓**Kvantifikacija**
  - **–** ***real time*** **PCR za A, B i D mutaciju**

Mutation	Freq. (%)	Nucleotide sequence	Protein
Wild Type		GATCTCTG----GCAGT----GGAGGAAGTCTCTTTAAGAAAATAG	DLWQWRKSL*
A	80	GATCTCTGTCTGGCAGT----GGAGGAAGTCTCTTTAAGAAAATAG	DLCLAVEEVSLRK*
B	6	GATCTCTGCATGGCAGT----GGAGGAAGTCTCTTTAAGAAAATAG	DLCMAVEEVSLRK*
C	<1	GATCTCTGCGTGGCAGT----GGAGGAAGTCTCTTTAAGAAAATAG	DLCVAVEEVSLRK*
D	6	GATCTCTGCCTGGCAGT----GGAGGAAGTCTCTTTAAGAAAATAG	DLCLAVEEVSLRK*
E	<1	GATCTCTG----GCAGTCTCTTGCCCAAGTCTCTTTAAGAAAATAG	DLWQSLAQVSLRK*
F	<1	GATCTCTG----GCAGTCCCTGGAGAAAGTCTCTTTAAGAAAATAG	DLWQSLEKVSLRK*
~45 more	7		

1. Analiza fragmenata

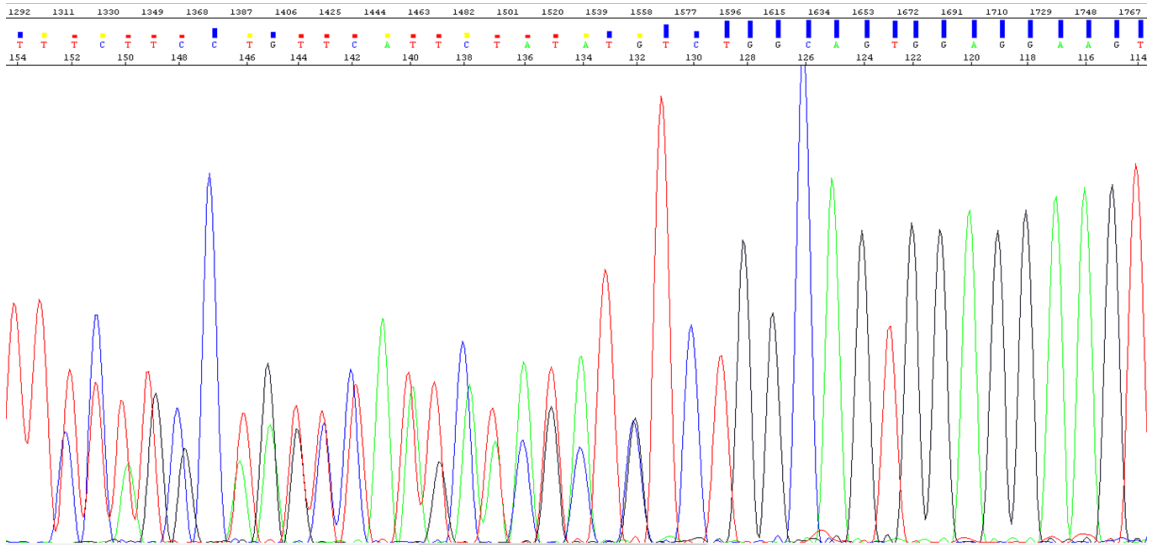


Normalni produkt

Produkt s insercijom od 4 pb

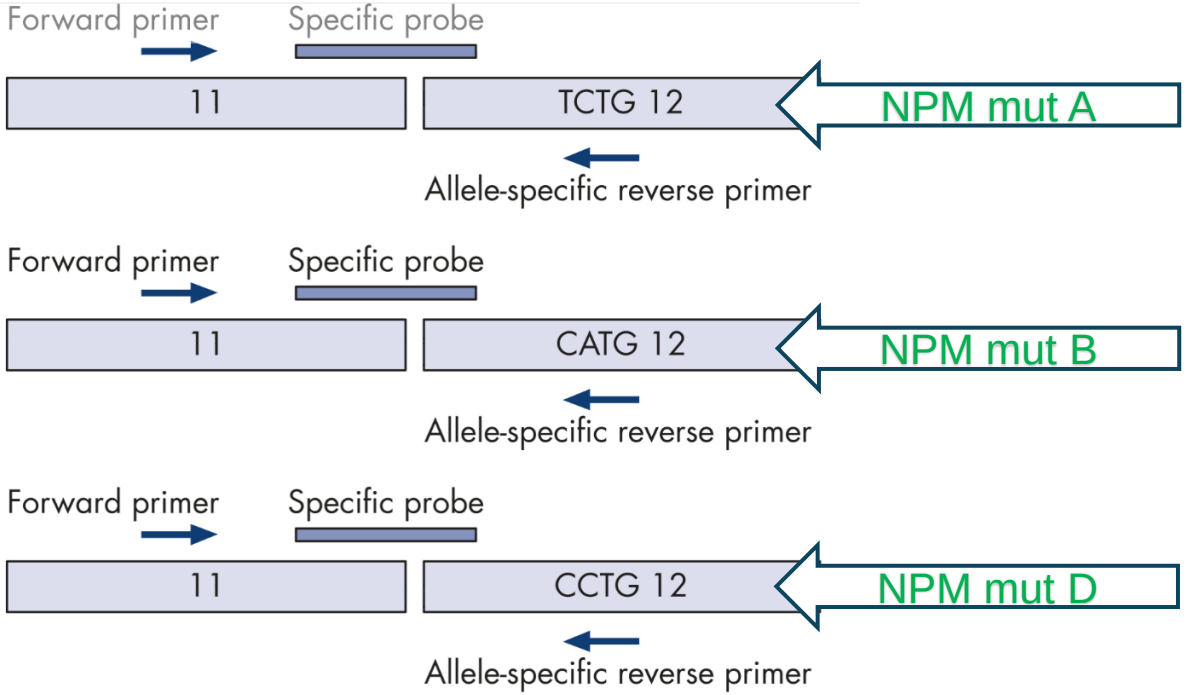
Produkt s insercijom od 8 pb

2. Sekvenciranje



- ➢ **Sekvenciranje po Sangeru ili NGS za utvrđivanje tipa mutacije**

Praćenje MRD pomoću „real time" PCRa kvantitativno



2021 Update on MRD in acute myeloid leukemia: a consensus document from the European LeukemiaNet MRD Working Party

Michael Heuser,[1] Sylvie D. Freeman,[2] Gert J. Ossenkoppele,[3] Francesco Buccisano,[4] Christopher S. Hourigan,[5] Lok Lam Ngai,[3]

blood® 30 DECEMBER 2021 | VOLUME 138, NUMBER 26

Table 7. Methods for detection of MRD in AML

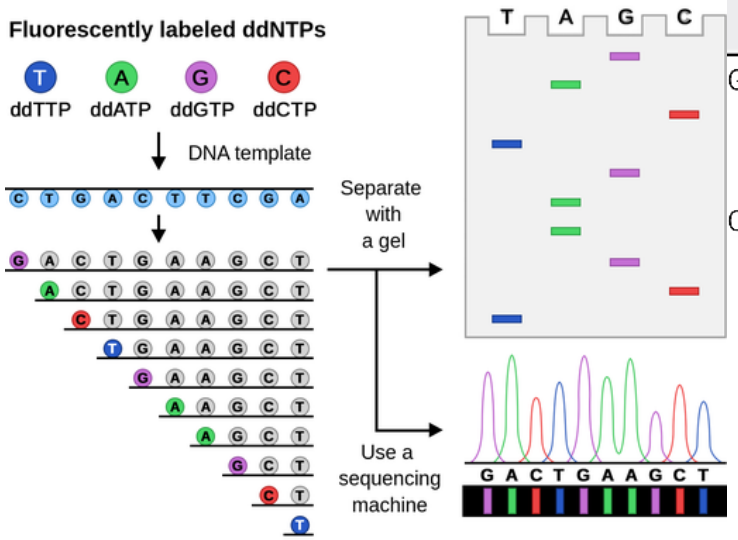
	Method	Target	Sensitivity	Applicable in % of AML	Turn-around time (days)	Limitations/ problems
Established	Multi-parameter flow cytometry (MFC)	Leukemia-associated immunophenotype (LAIP) or different from normal (DfN)	10^{-3} to 10^{-4}	85-90	2	Less sensitive, more subjective analysis
Established	Real-time quantitative PCR (RT-qPCR)	Robust data: *NPM1*, *CBFB::MYH11*, *RUNX1::RUNX1T1* Less validated: *KMT2A::MLLT3*, *DEK::NUP214*, *BCR::ABL1*, *WT1*	10^{-4} to 10^{-5}	40-50*	3-5	Limited applicability

Prednosti i ograničenja metoda

Važno je utvrditi tip mutacije jer u protivnom nije moguće kvantitativno pratiti MRD.

The Times, They Are A-Changing: The Impact of Next-Generation Sequencing on Diagnosis, Classification, and Prognostication of Myeloid Malignancies With Focus on Myelodysplastic Syndrome, AML, and Germline Predisposition

Sandeep Gurbuxani, MBBS, PhD[1]; Michael J. Hochman, MD[2]; Amy E. DeZern, MD, MHS[2]; and Akiko Shimamura, MD, PhD[3]

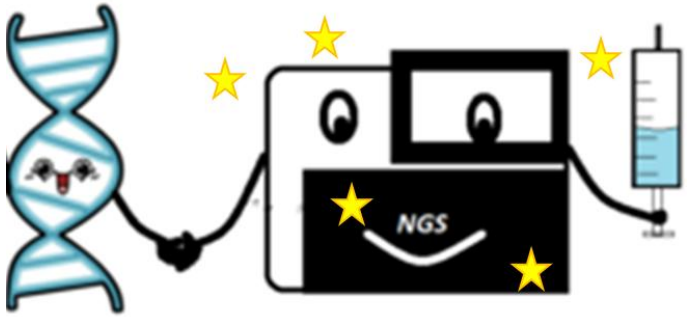


Genetic Variants Associated With Cytopenias, MDS, MDS/MPN, and AML

Indication	Single-Gene Mutations (NGS)	Structural Variants (karyotype, FISH, RNA-Seq)
MDS, MDS/MPN, cytopenia	*ASXL1, BCOR, BCORL1, CBL, CEBPA, CSF3R, DDX41, DMNT3A, ETV6, ETNK1, EZH2, FLT3-ITD, FLT3-TKD, GATA2, GNB1, IDH1, IDH2, JAK2, KIT, KRAS, KMT2A-PTD, NF1, NPM1, NRAS, PHF6, PPM1D, PRPF8, PTPN11, RAD21, RUNX1, SETBP1, SF3B1, SRSF2, STAG2, TET, TP53, U2AF1, UBA1, WT1, ZRSR2*	Genes required for diagnosis and risk stratification: *ASXL1, BCOR, CEBPA,[a] DDX41, EZH2, FLT3-ITD, FLT3-TKD, IDH1, IDH2, NPM1,[a] RUNX1, SF3B1, SRSF2, STAG2, TP53, U2AF1, ZRSR2* Other genes: *ANKRD26, BCORL1, BRAF, CBL, CSF3R, DNMT3A, ETV6, GATA2, JAK2, KIT, KRAS, NRAS, NF, PHF6, PPM1D, PTPN11, RAD21, SETBP1, TET, WT1* Common fusions: *BCR::ABL1 CBFB::MYH11 DEK::NUP214 MECOM::R KMT2A::R RUNX1::RUNX1T1 PML::RARA* Rare fusions: *PRDM16::RPN1 NPM1::MLF1 KAT6A::CREBBP RBM15::MRTF1 ETV6::MNX1 PICALM::MLLT10 FUS::ERG RUNX CBFA2T3 CBFA2T3::GLIS2 NUP98::NSD1 NUP98::KMD5A*

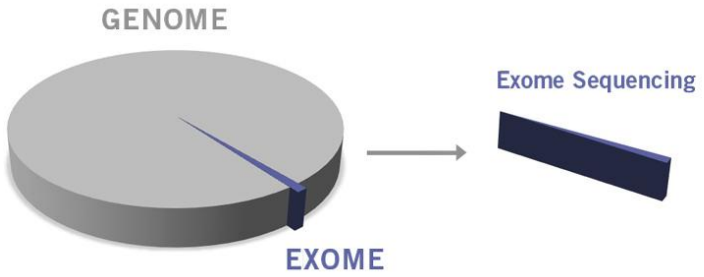
Abbreviations: FISH, fluorescent in situ hybridization; ICC, International Consensus Classification; MDS, myelodysplastic syndrome; MPN, myeloproliferative neoplasm; NGS, next-generation sequencing.

Gurbuxani, Sandeep et al. *American Society of Clinical Oncology educational book. American Society of Clinical Oncology. Annual Meeting* vol. 43 (2023): e390026.

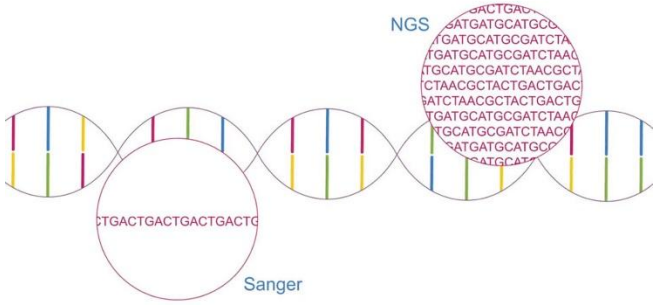


„...established methodology in the clinical laboratory for screening and diagnosis of germline (inherited) and somatic (acquired) genomic mutations...”

Ascierto PA, et al. Mol Diagn. 2019 Sep;21(5):756-767.



https://the-dna-universe.com/2022/04/07/ngs-a-powerful-tool-for-disease-diagnostics/



https://www.lubio.ch/blog/ngs-adapters

Types of NGS based on sequencing based on outcomes

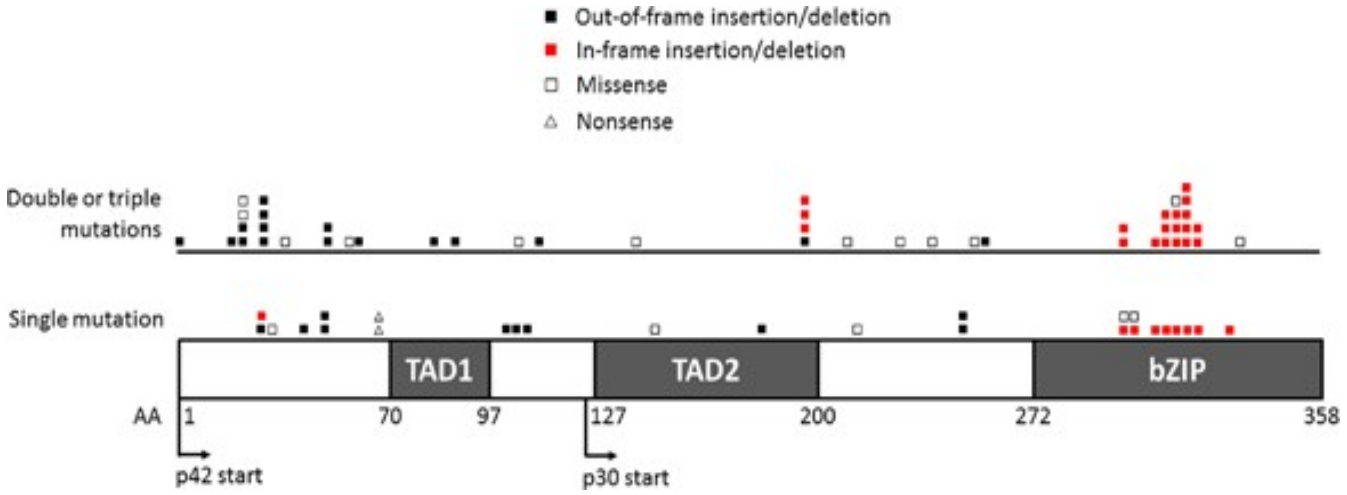
	Whole genome sequencing	Whole Exome sequencing	Targeted sequencing
Target	Sequences complete genome of an organism (Human, Plant, Microbes)	Sequences only exome (codes for protein)	Specific region of interest is sequenced
Detection	Can detect all possible DNA variations of coding & non-coding region	Can detect variations in coding regions	Detects genes already associated with one or more diseases
Cost	Higher cost	Relatively cost effective	Cost effective
Sequencing depth	Low	Moderate	High
Used for	Identification of all kinds of variant	Identification of rare variant/possible disease causing variant within exons	Identification of known & novel variant in selected sets of genes

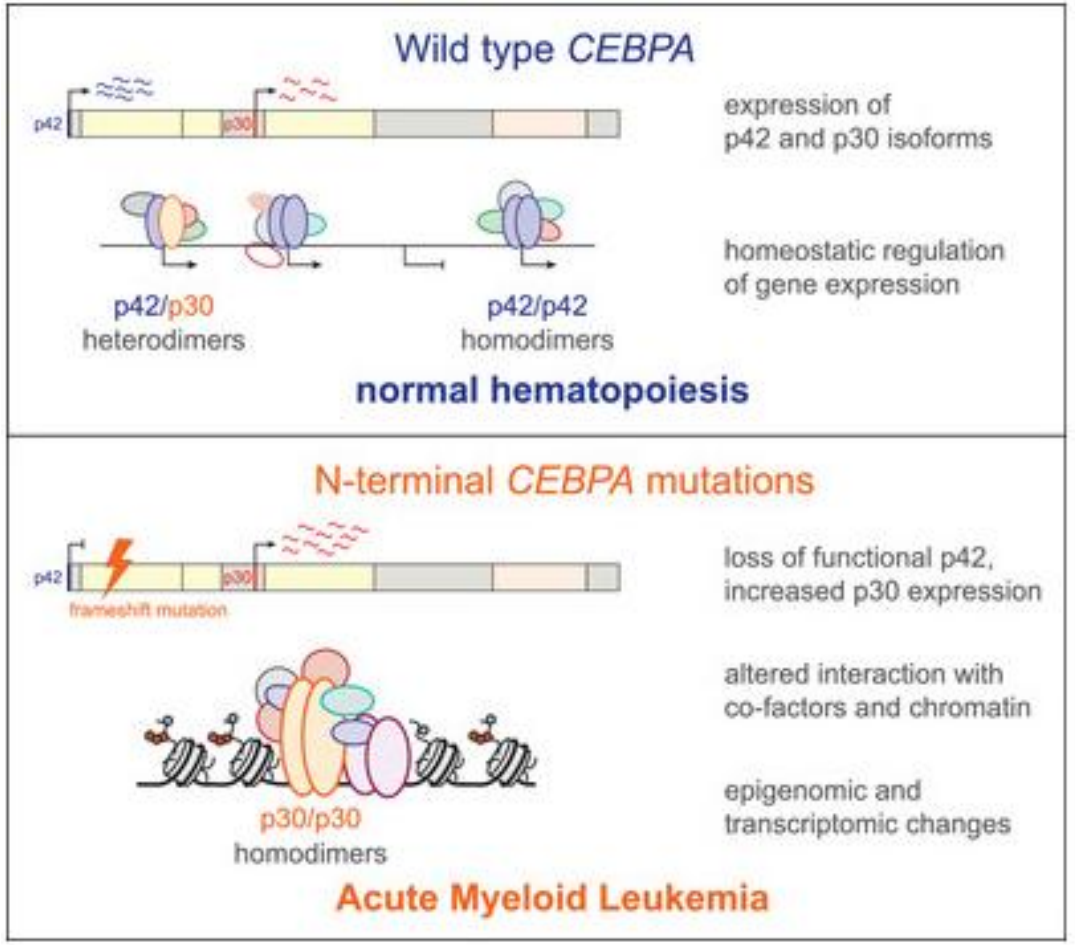
https://www.dssimage.com/blog/next-generation-sequencing-in-clinical-genomics-need-of-the-hour/

CEBPA mutacije

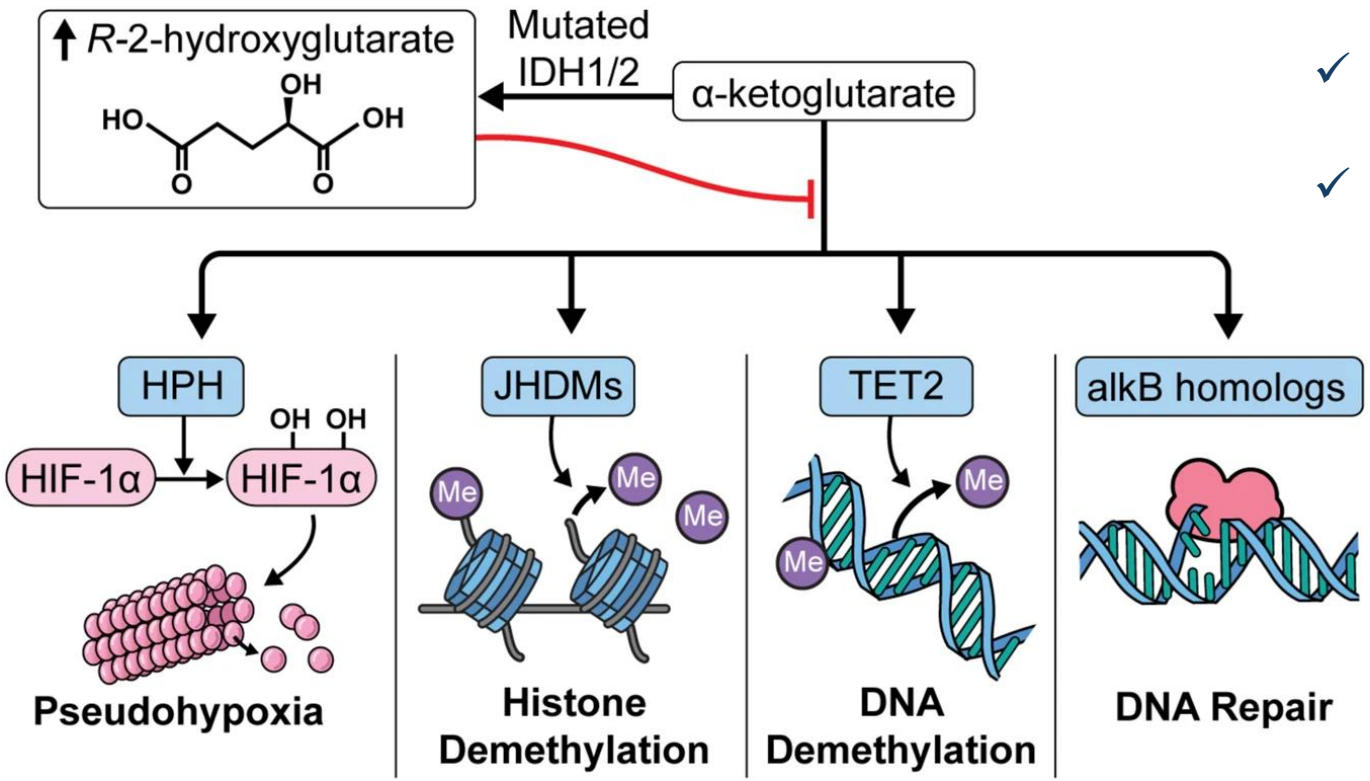
- ✓ **Potvrđuje dijagnozu neovisno o postotku blasta - ICC2022/WHO2022**
- ✓ **ključan transkripcijski čimbenik u mijeloidnoj diferencijaciji**

- ✓ **Mutacije na N-terminusu - p30 homodimer**
  - **promjenjena interakcija kofaktora i kromatina**
  - **regulacija transkripcije i epigenetičke promjene**
  - **nekontrolirana proliferacija mijeloidne loze**
- ✓ **Povoljna prognoza bolesti - „*in frame*" mutacija u bZIP regiji**

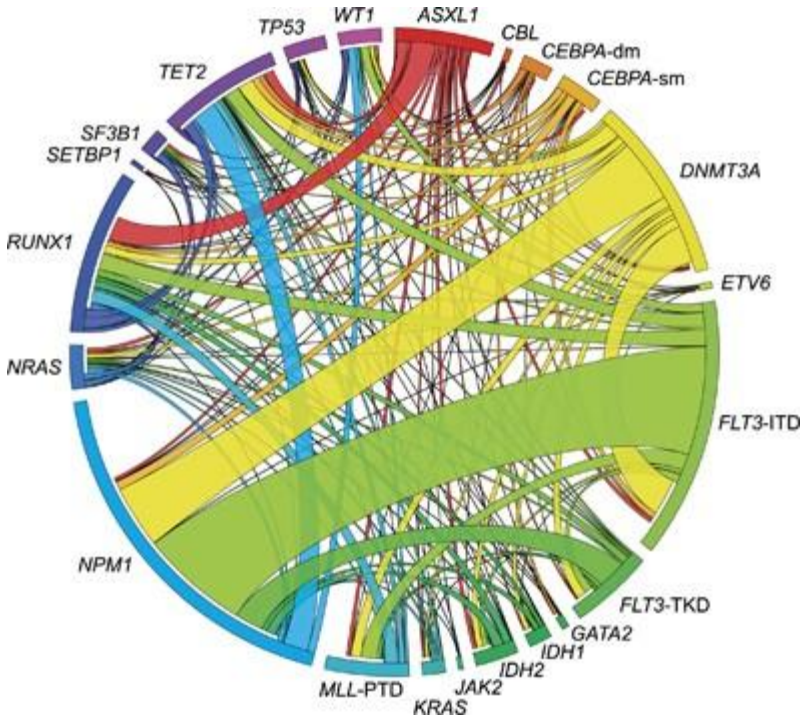




IDH mutacije



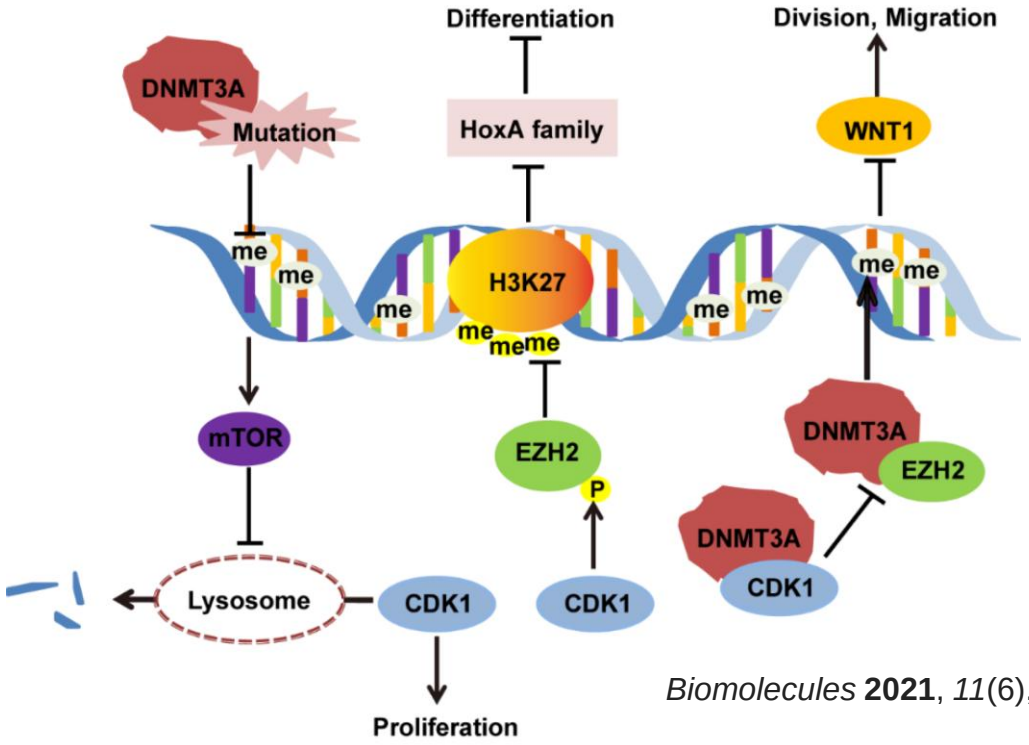
- ✓ **8-12% AML**
- ✓ **Starija dob i lošija prognoza bolesti**
- ✓ **Najčešće mutacije su**
  - ✓ **na kodonu 132 *IDH1***
  - ✓ **na kodonima 140 i 172 u *IDH2***



- ✓ **Mutirani *IDH1/2* pretvara α-ketoglutarat u *R*-2-hidroksiglutarat**
  - **– inhibira enzime ovisne o α-ketoglutaratu**
- ✓ **Oko 30% AML s mutacijom *NPM1***
- ✓ **Komutacije s genima *DNMT3A, SRSF2, i RUNX1* te *TP53***

DNA metilacija

- Metilacija je neophodna za normalnu diferencijaciju i proliferaciju matične hematopoetske stanice
- *TET2, DNMT3A, IDH1 i IDH2*
  - *„on/off swich" genes*
- Mutacije u ovim genima dovode do poremećaja metilacije DNA – poremećaj ekspresije gena
  - Tumor supresori
  - Geni za popravak DNA
- Javljaju se u ranom stadiju bolesti
- *DNMT3A* R882 - 50% AML → 20-30% MDS
- *TET2, IDH1 i IDH2* – međusobno se isključuju
- *IDH1 i IDH2* – terapija - inhibitori



Biomolecules **2021**, *11*(6), 781

RNA splicing

- **Male molekule**
  - **prekursorska mRNA → zrela mRNA**
  - **stvaranje izoformi proteina**

- ***SF3B1, SRSF2, U2AF1, ZRZR2***
  - **mutacije se međusobno se isključuju**

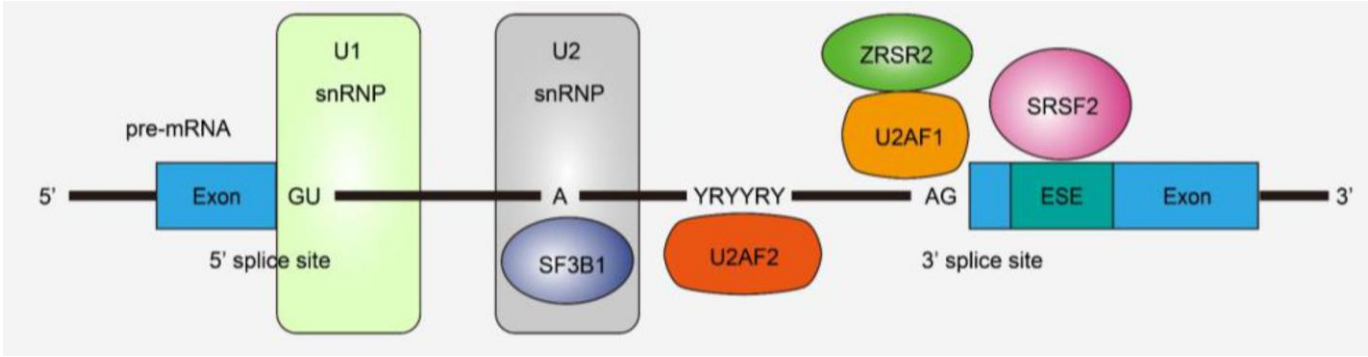
- **Mutacije u ovim genima dovode do**
  - aberantnog mjesta cijepanja na 3' kraju
  - preskakanja eksona pri transkripciji
  - inkluzije dijelova introna u mRNA

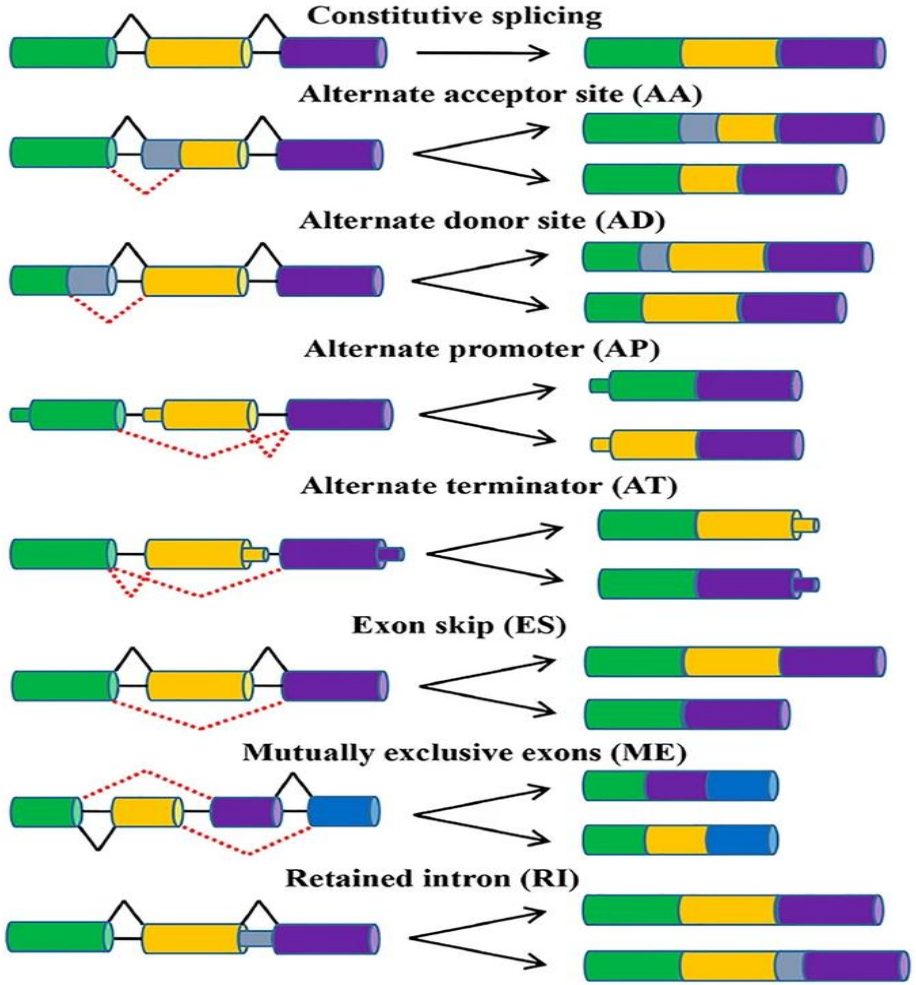
- **>60% MDSa**

- ***SF3B1***
  - >80% MDS s prstenastim sideroblastima
  - *RUNX1* komutacija – rana progresija u AML

- ***SRSF2 , U2AF1 -*** 10-15% MDS – brza progresija u AML

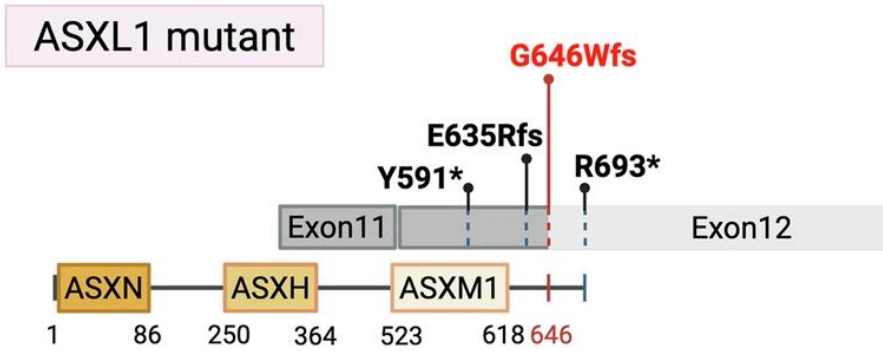
- ***ZRZR2* – često komutiran TET2**
  - Abnormalnosti u funkciji ribosoma, upali i staničnoj mobilnosti
  - Poremećaj regulacije MAPK signalnog puta



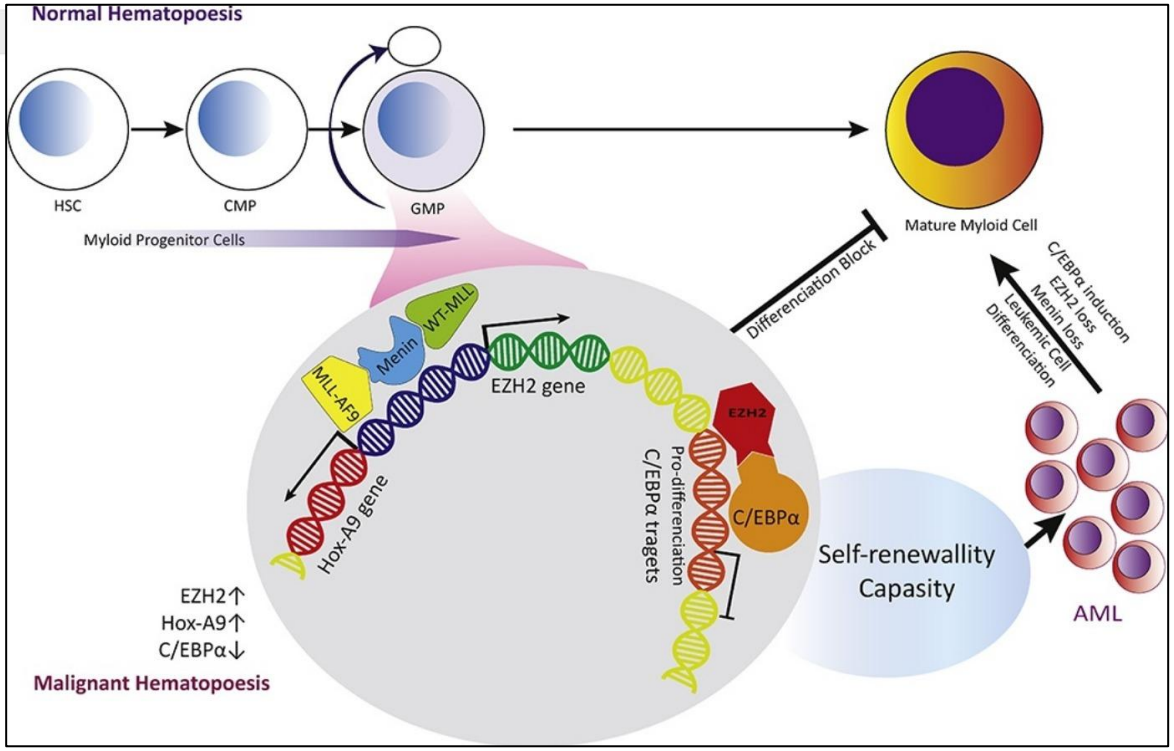


Kromatin modificirajući geni

- **Modifikacija strukture kromatina i histona – poremećaj transkripcije**
- ***ASXL1, EZH2***

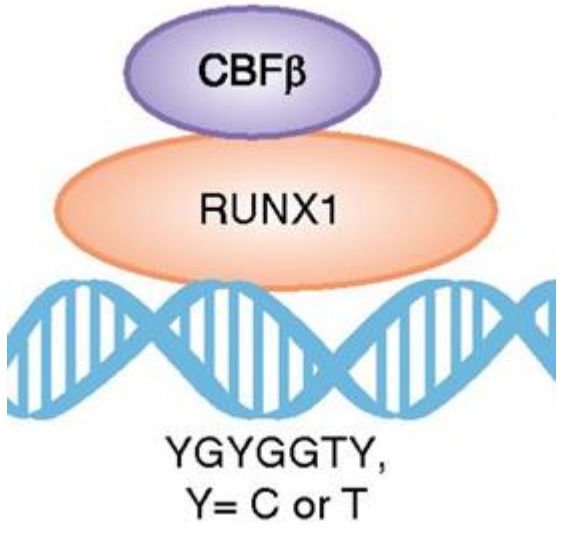


- ***ASXL1* –10% u AML**
  - **Komutacije česte**
  - **osim s genima *SF3B1, DNMT3A***
- ***EZH2* - 5–10% MDS**
  - komutacije *TET2, RUNX1 i ASXL1*
  - zahvaćen kod -7/del7q
  - blokira diferencijaciju i omogućava samoobnavljanje tumorskih stanica
  - loš prognostički biljeg

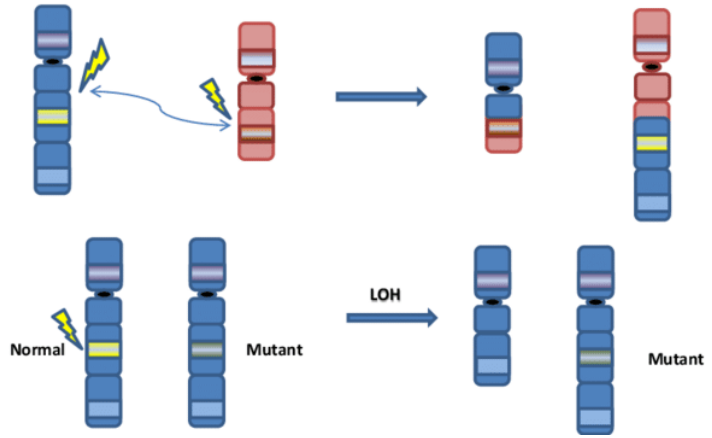


Transkripcijski faktori

- ***RUNX1, BCOR, ETV6, GATA2***
- **somatske mutacije i/ili mutacije zametnih stanica (*germline*)**
- ***RUNX1* – regulator hematopeze**
  - **CBF kompleks – alteracije u transkripciji gena**
  - komutacije u *ASXL1, SRSF2, TET2, SF3B1* i *EZH2* te -7/del(7q)
- **Transkripcija ciljanih gena (aktivacija ili supresija)**
  - **Diferencijacija hematopoetskih stanica**
  - **Regulacija staničnog ciklusa**
  - **Biogeneza ribosoma**
  - ***TP53 i TGFβ* signalni putevi**

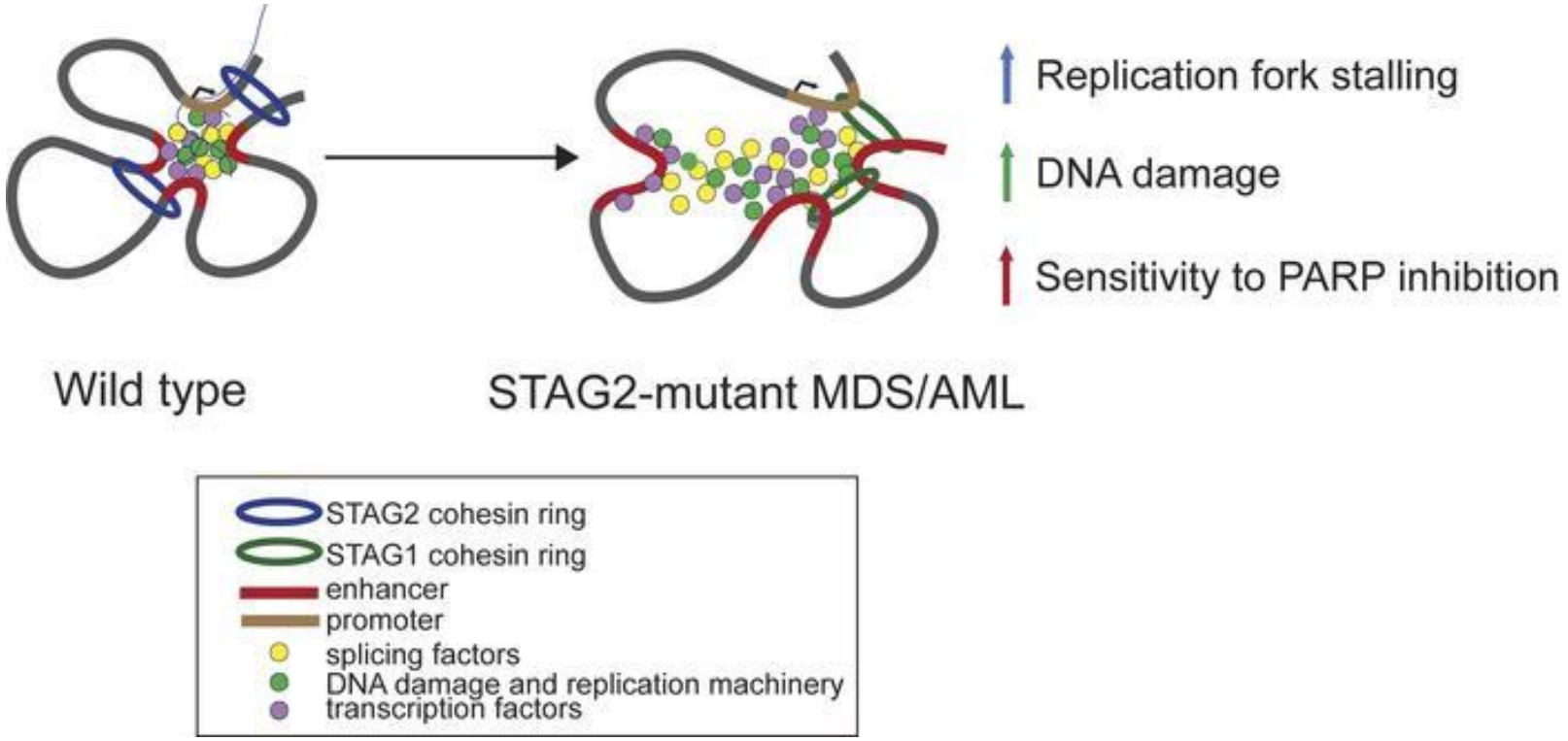


Blood (2017) 129 (15): 2070–2082.



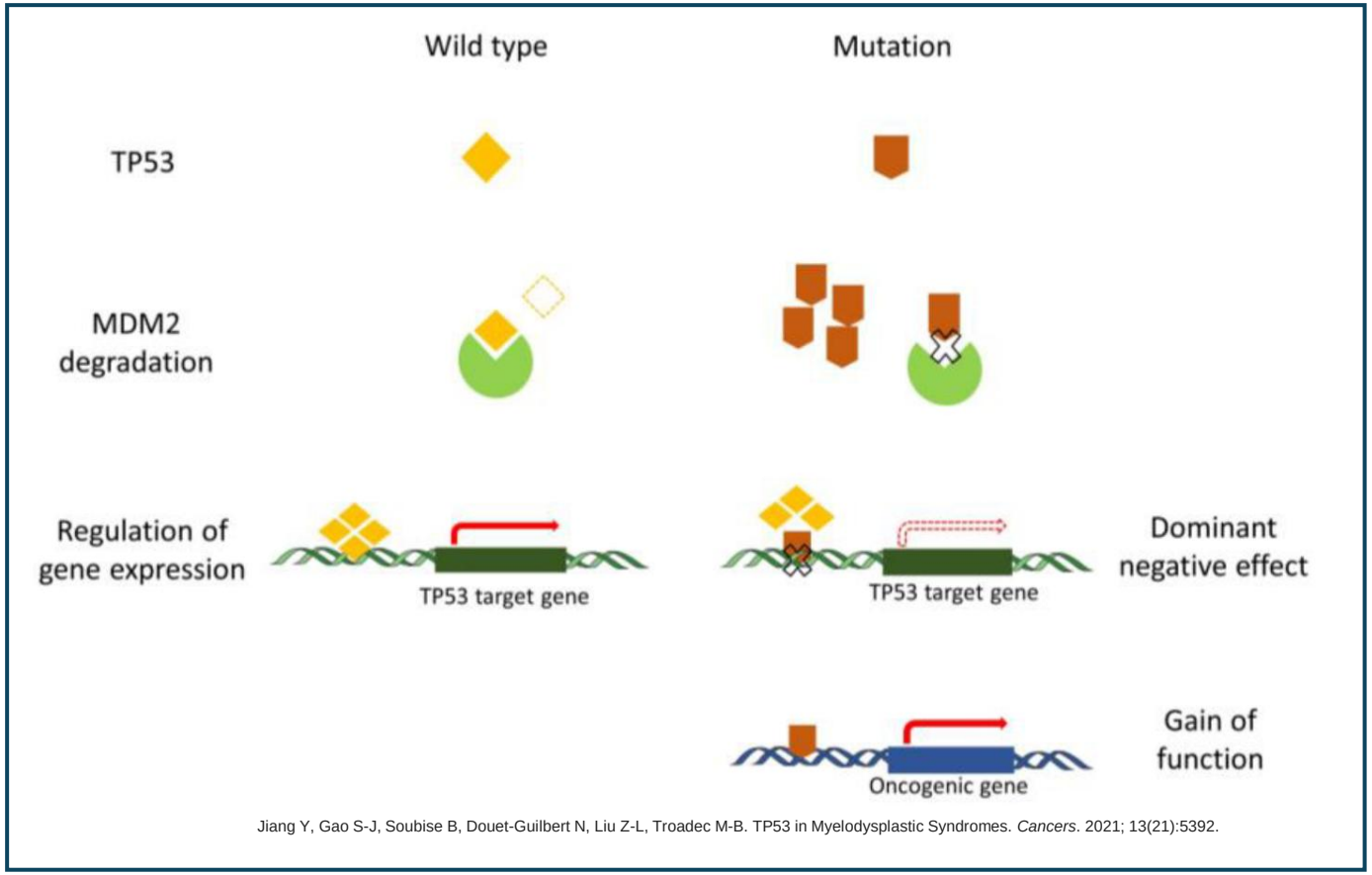
Kohezin kompleks

- ***STAG2* – 5% MDS, loša prognoza**
  - Ključna komponenta kohezin kompleksa, globalne protektivne strukture DNA molekule
  - Mutacije – *loss of function*



TP53

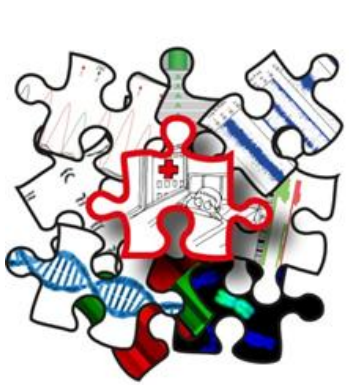
- **Tumor supresor**
- **Kompleksni kariotip ili del17p**
- **Mono/bialelna *TP53* inaktivacija**
- ***loss of function* vs *gain of function***
- ***TP53* mutacije**
  - Neovisno o drugim genetskim čimbenicima - visoki rizik
  - Rezistencija na standardnu kemoterapiju
  - Brza progresija i transformacija bolesti
  - Loš ishod
- **Terapija?!**

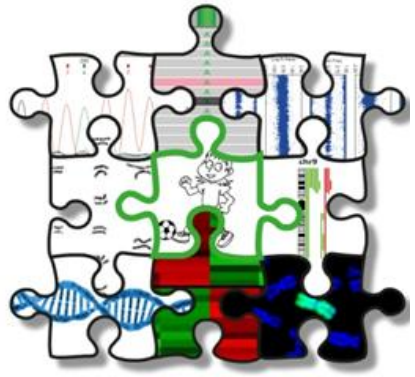


Jiang Y, Gao S-J, Soubise B, Douet-Guilbert N, Liu Z-L, Troadec M-B. TP53 in Myelodysplastic Syndromes. *Cancers*. 2021; 13(21):5392.

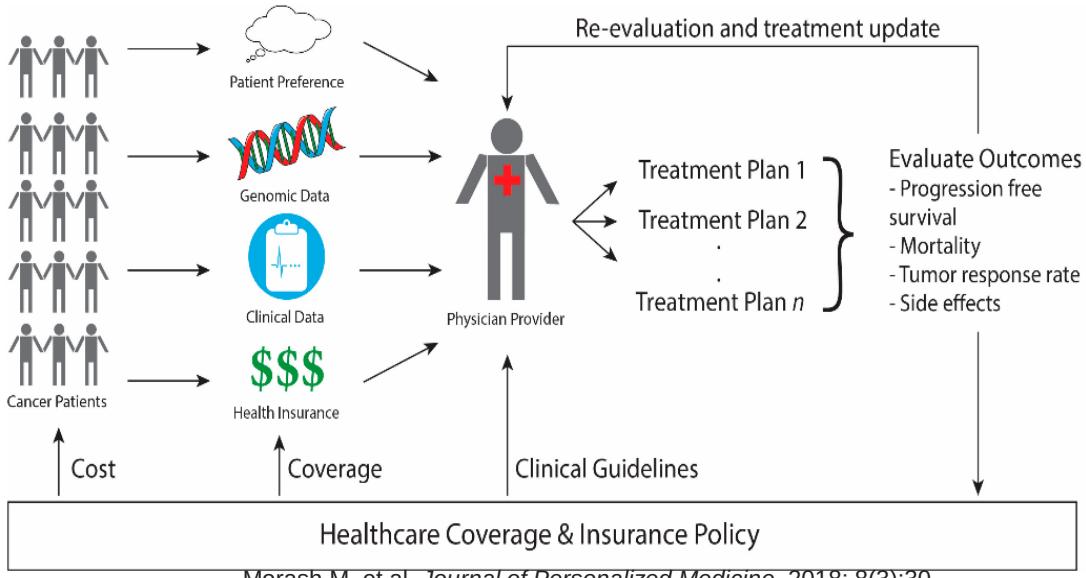
- razgradnjom normalnog p53 pomoću MDM2 kontolirana je razina aktivacije ciljanih *TP53* ovisnih gena
- mutirani „***loss of function"*** *TP53* stvara „oštećene" p53 koji ne mogu biti propisno degradirani te se natječu s nemutiranim za vezna mjesta na DNA i time **sprječavaju regulaciju ekspresije** *TP53* ovisnih gena
- mutirani „***gain-of-function"*** *TP53* omogućuje pojačanu ulogu p53 u regulaciji ekspresije, najčešće onkogena

Snaga NGSa



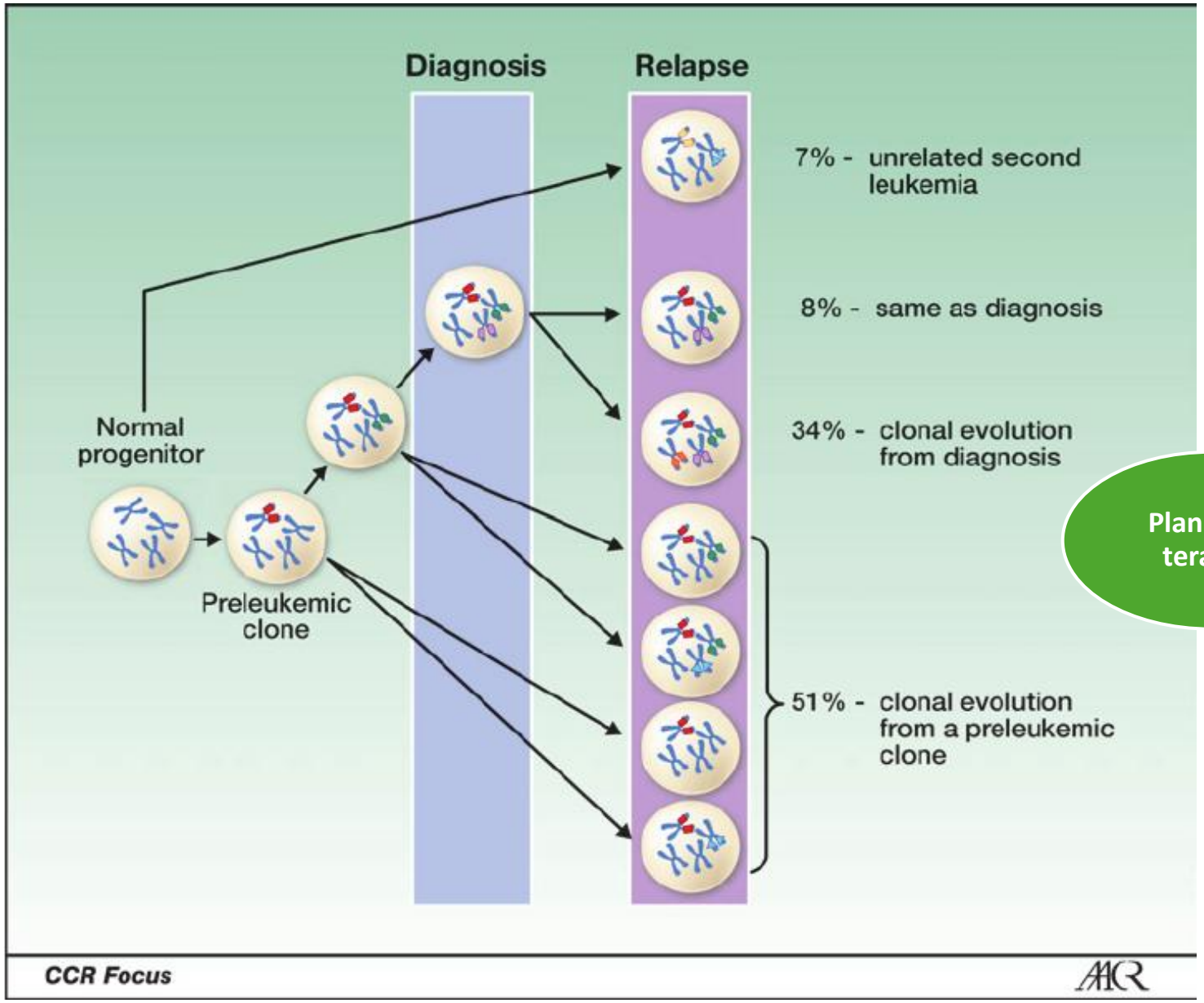


- ✓ Sveobuhvatna i istovremena analiza svih mutacija u odabranim genima
- ✓ Otkrivanje mogućih koperativnih učinaka mutacija
- ✓ Somatske i nasljedne (germline) mutacije
- ✓ Izvještavanje patogenih i vjerojatno patogenih varijanti
- ✓ VUS – varijante nepoznatog značaja
- ✓ VAF – predstavlja udio mutacije
  - ✓ ovisi o broju neoplastičnih stanica
  - ✓ subkonalna hematopoeza
  - ✓ stadij i raširenost bolesti
  - ✓ vrsta analiziranog uzorka
- ✓ Interpretacija rezultata u kliničkom kontekstu bolesti
- ✓ Obavezne kliničke i laboratorijske smjernice



Morash M, et al. *Journal of Personalized Medicine*. 2018; 8(3):30.

MRD – moćni alat za kontrolu bolesti



https://www.researchgate.net/figure/Clonal-relationship-of-diagnosis-and-relapse-samples-in-ALL-Most-relapse-samples-have-a_fig3_224966691