

Citogenetika u akutnim leukemijama

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- ▶ Zagreb, 06.12.2025



Akutna leukemija-definicija

- ▶ maligna klonalna preuredba progenitorskih hematopoetskih stanica
- ▶ klonalna preuredba dovodi do narušene diferencijacije i proliferacije hematopoetskih stanica
- ▶ višestruke somatske mutacije (~13 po slučaju) doprinose leukemogenezi



Uloga citogenetike u akutnim leukemijama

- ▶ citogenetska analiza = esencijalna, uz druge dijagnostičke metode, za postavljanje dijagnoze AML i ALL
- ▶ otkriva strukturne i numeričke promjene kromosoma
- ▶ ključna za klasifikaciju i stratifikaciju rizika



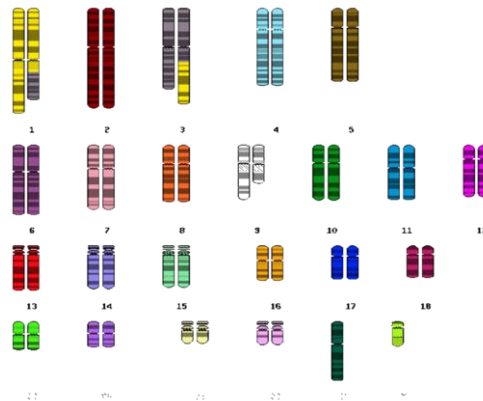
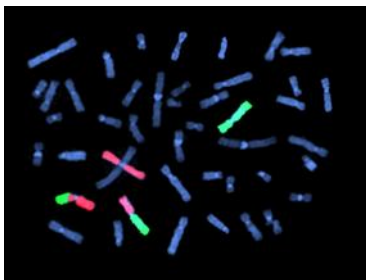
Povijest

- ▶ 1972: de la Chapelle – trisomija 8
- ▶ 1973: Janet Rowley – t(8;21)(q22;q22)
- ▶ danas poznajemo preko 300 rekurentnih kromosomskih promjena u akutnim leukemijama

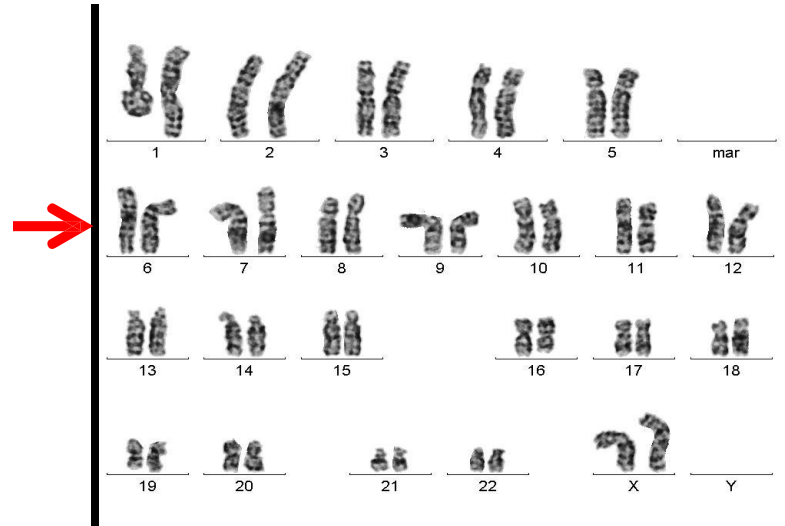


Citogenetske metode

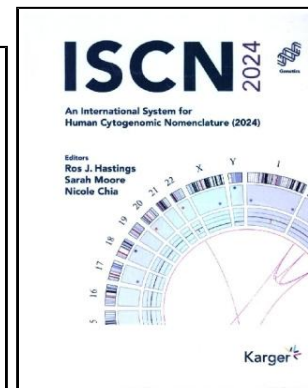
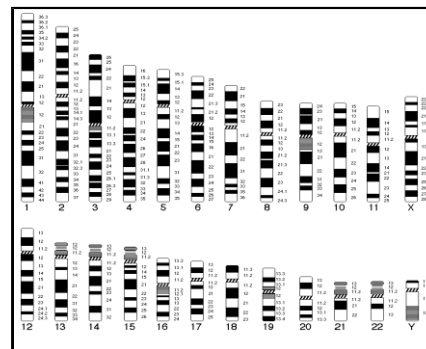
- ▶ klasična citogenetika (G-pruganje)
- ▶ fluorescentna in situ hibridizacija (FISH)
- ▶ komparativna genomska hibridizacija (CGH), array, molekularna kariotipizacija...

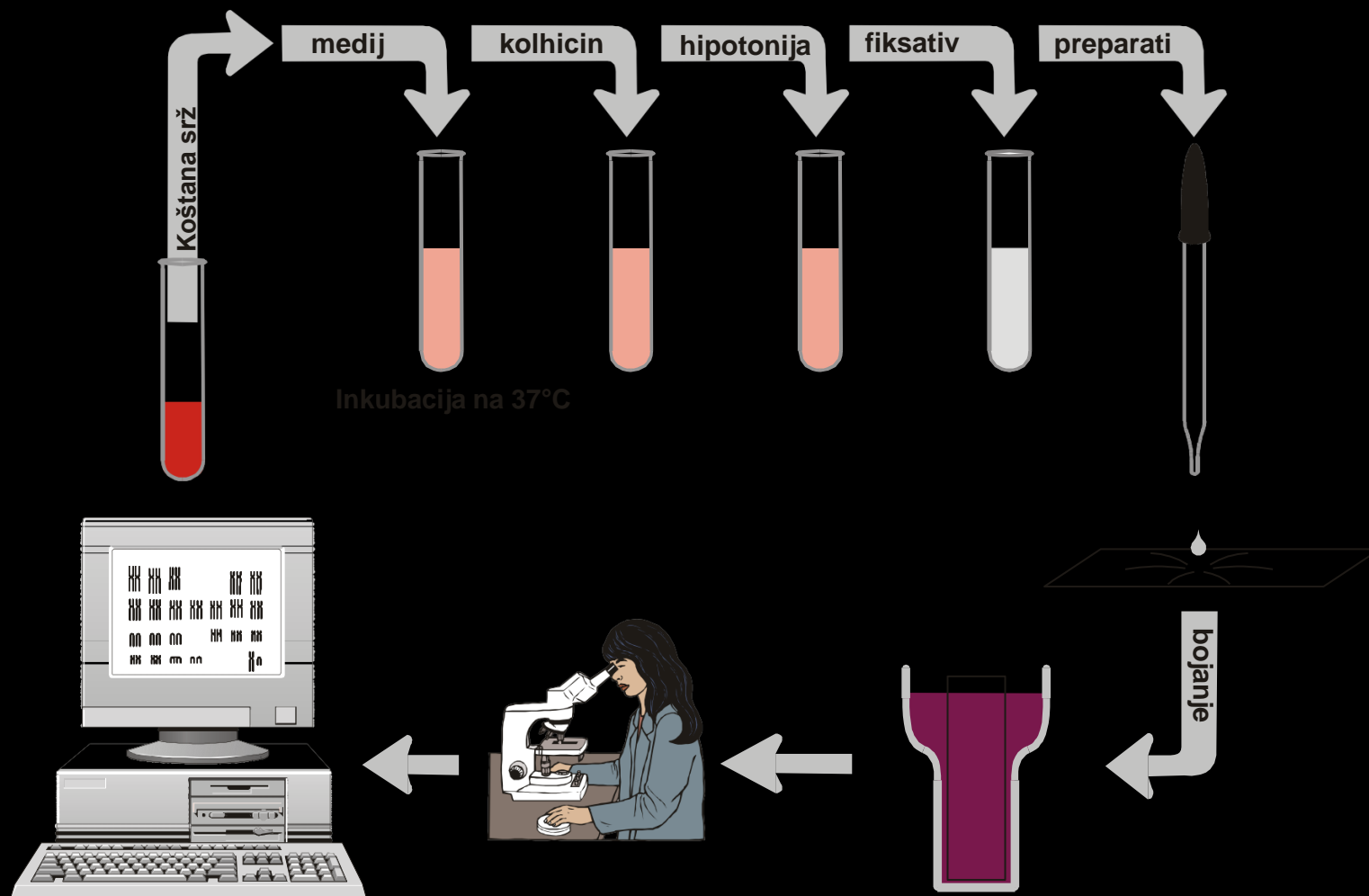


KLASIČNA CITOGENETIKA



- ➔ Analiza kariotipa
- ➔ Otkrivanje promjena kromosoma



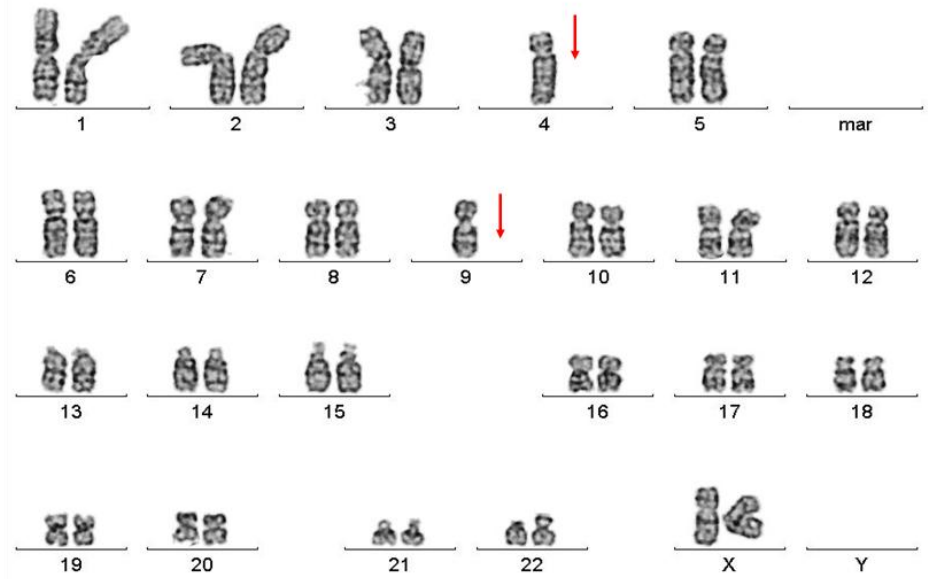


Postupak kultiviranja stanica koštane srži

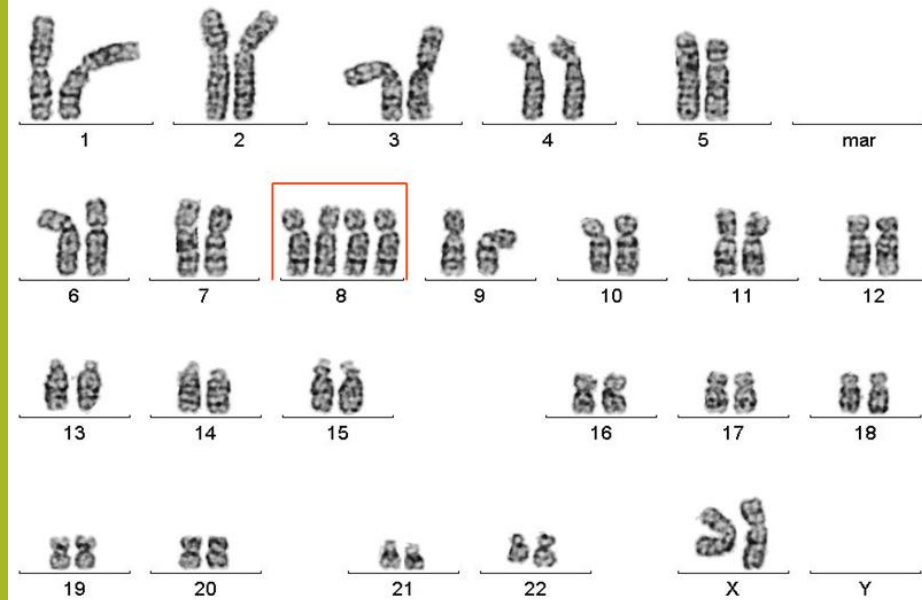
hiperdiploidija



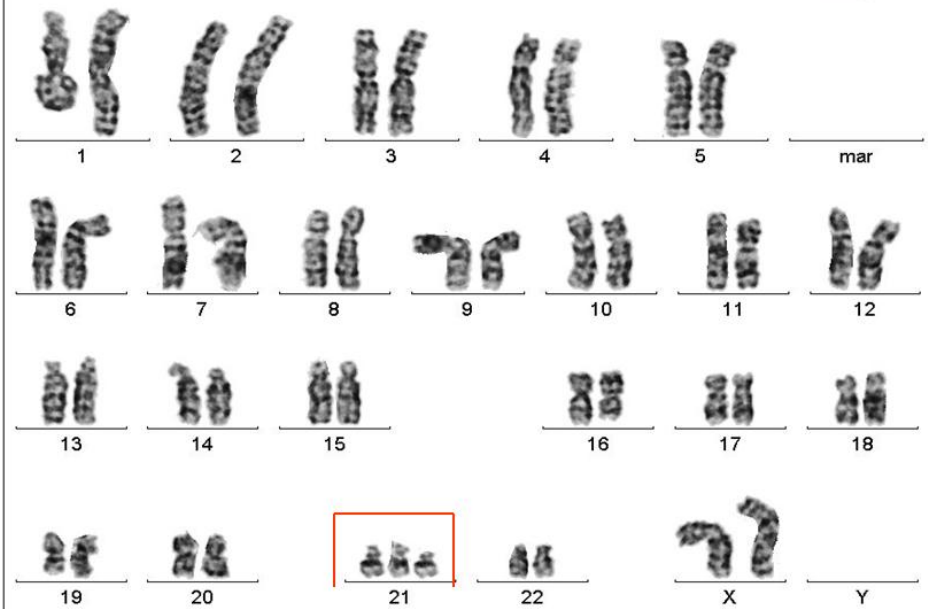
monosomija



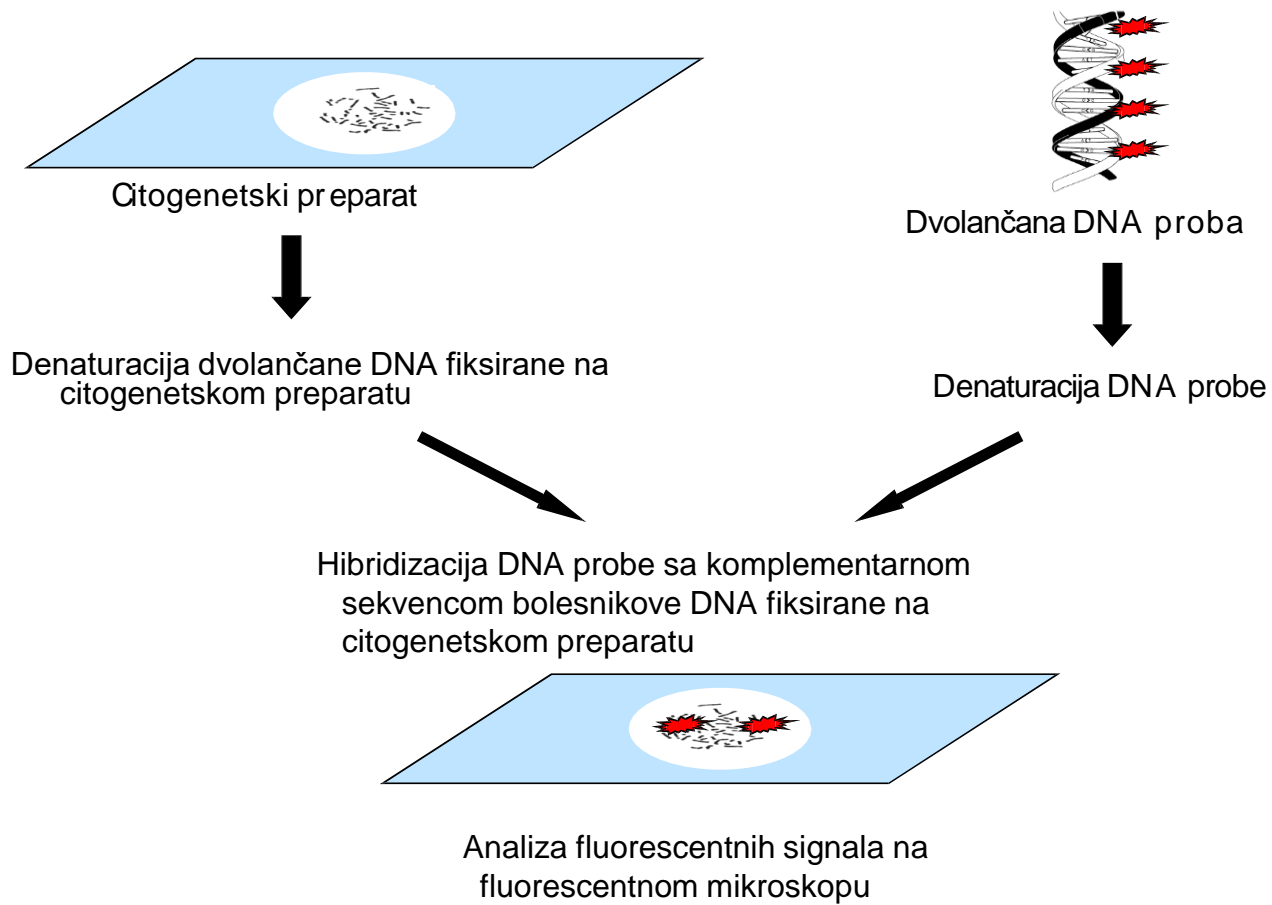
tetrasomija

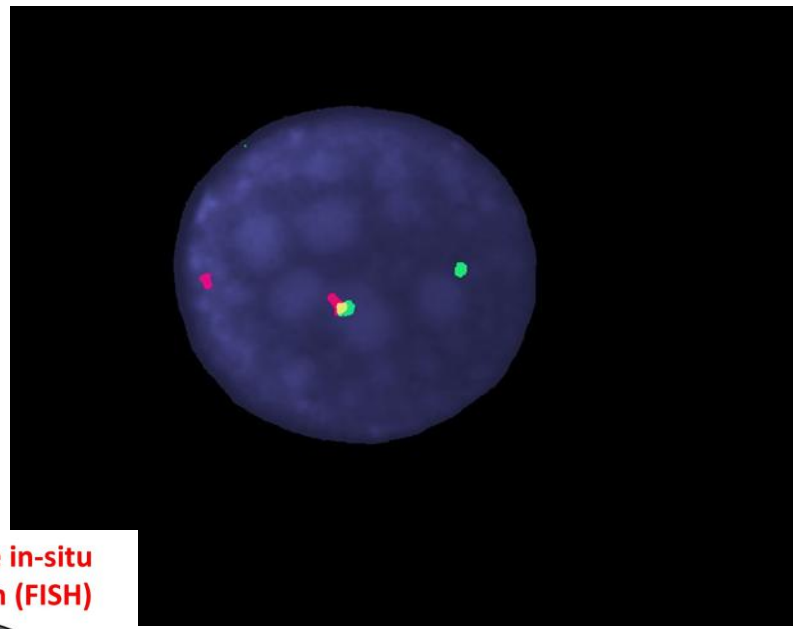
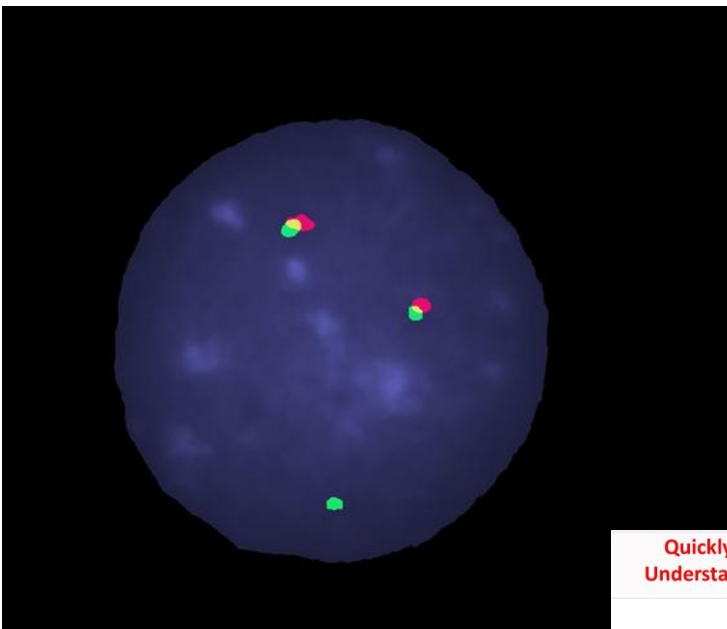


trisomija

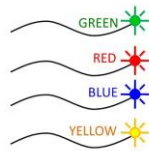


FLUORESCENTNA IN SITU HIBRIDIZACIJA - FISH

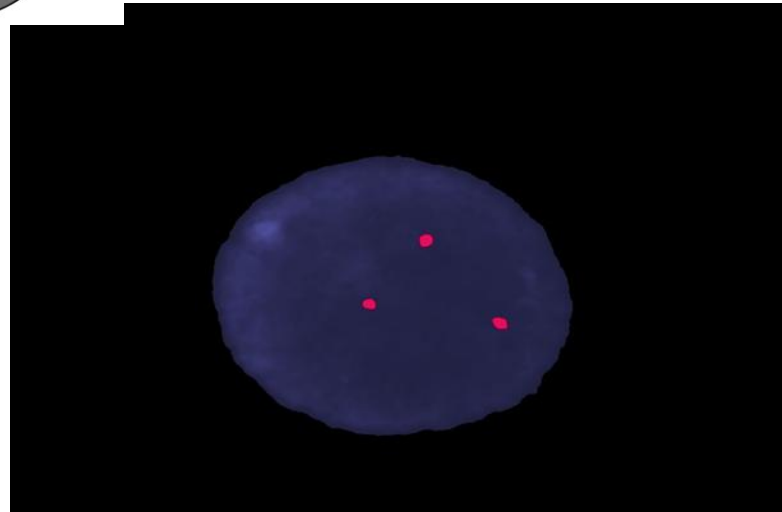
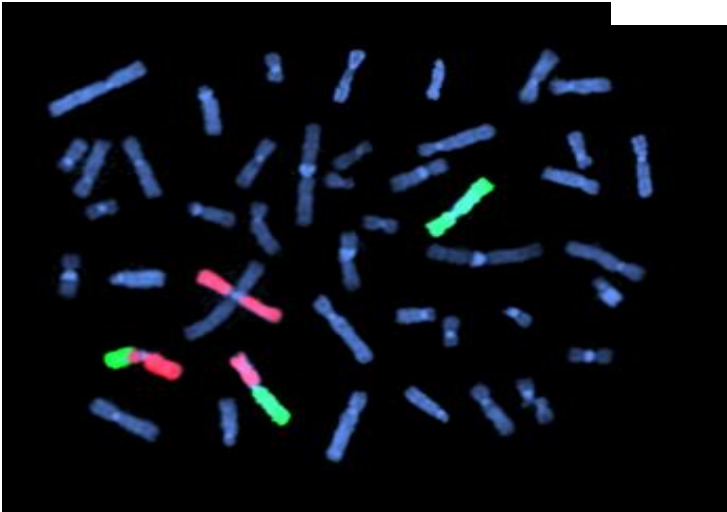
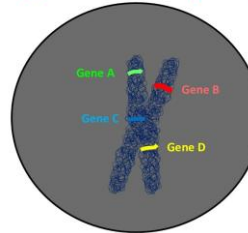




Quickly Understand

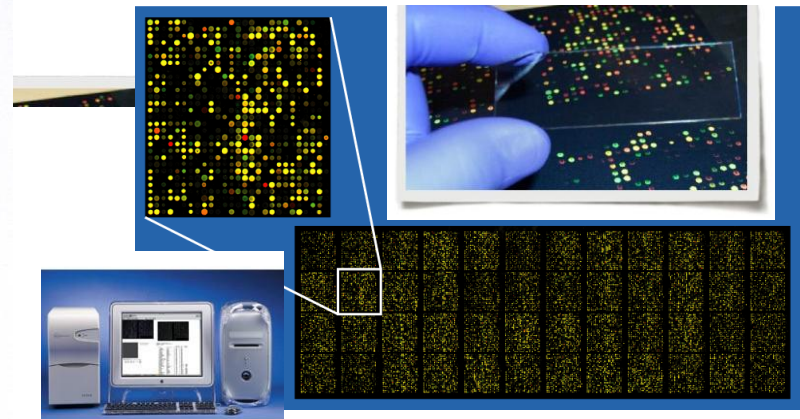
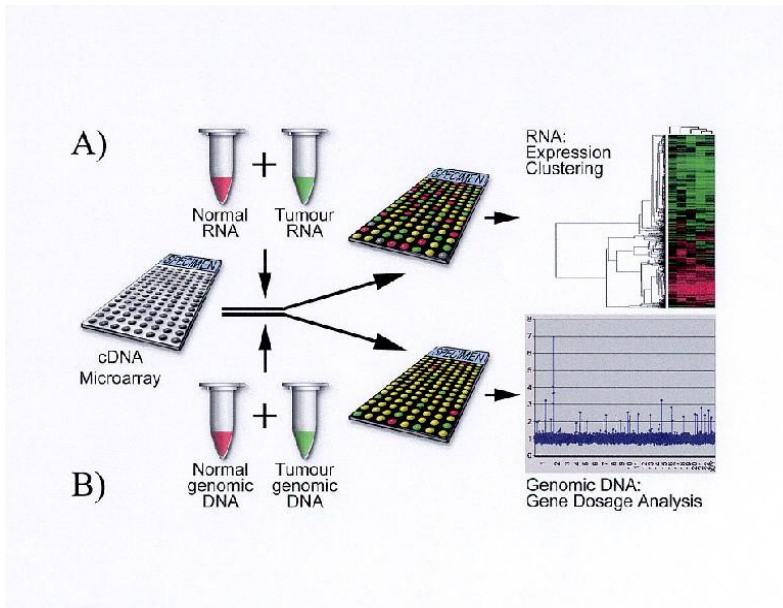


Fluorescence in-situ Hybridization (FISH)



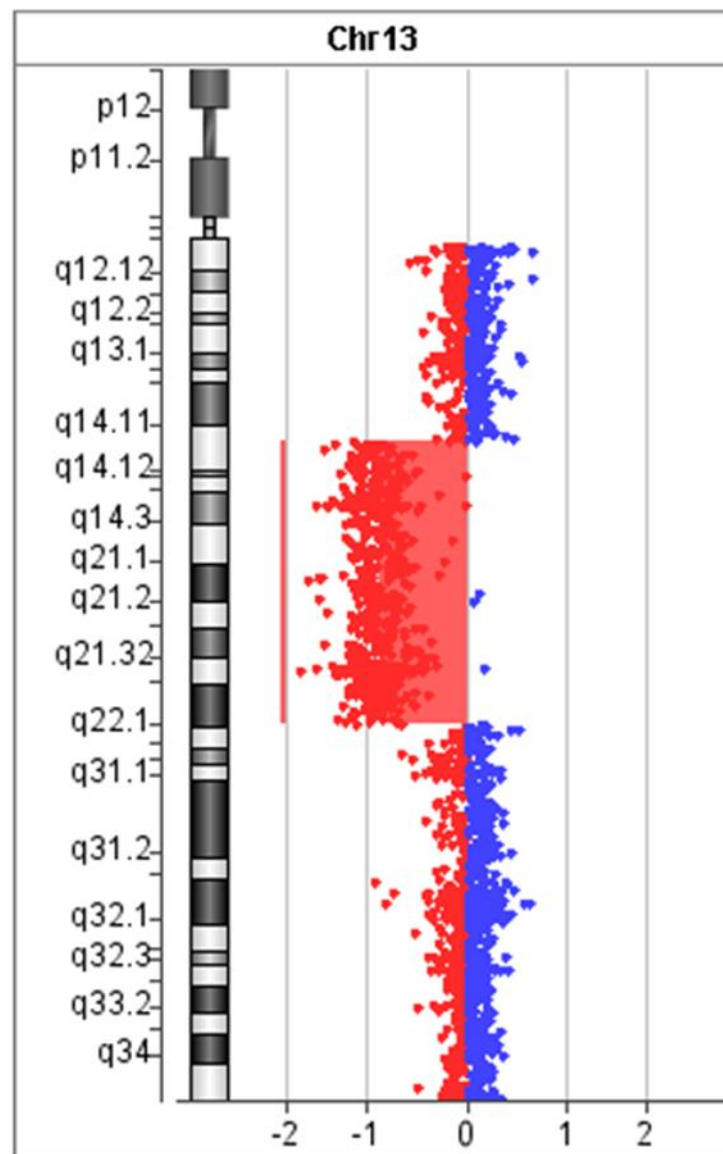
Komparativna genomna hibridizacija na čipu (aCGH)

- novi alat za nalaženje rekurentnih viškova ili manjkova kromosomskih regija kroz čitav genom - vrlo visoka rezolucija (copy number changes) na nivou DNA
- prije kratkog vremena aCGH je uspješno uveden u dijagnostiku leukemija što je dovelo do otkrivanja velikog broja tzv. genomskih nestabilnosti, uključujući i nove rekurentne delecije i amplifikacije



BAC arrays ~1MB

Oligo arrays ~100 kb (maks. rezolucija ~ 35 kb)



AML

- heterogena bolest matične hematopoetske stanice
- nakupljanje nezrelih stanica
- broj rekurentnih genetskih promjene varira ovisno o podtipu
- danas je poznato oko 160 različitih strukturnih rekurentnih promjena
- postoji značajno preklapanje između promjena u MDSu i AMLu
- česte su translokacije

WHO 2022 AML klasifikacija

- ▶ koristi citogenetske i molekularne biljega za definiranje podtipova AML
- ▶ prepoznaje 11 glavnih AML entiteta sa definiranim genetičkim promjenama
- ▶ primjeri: PML::RARA, RUNX1::RUNX1T1, CBFB::MYH11, DEK::NUP214

Category	Subtypes / Features
AML with Defining Genetic Abnormalities	t(8;21) RUNX1::RUNX1T1 inv(16) CBFB::MYH11 t(15;17) PML::RARA (APL) KMT2A rearranged MECOM rearranged NUP98 rearranged
Mutationally Defined AML	Mutated NPM1 Biallelic CEBPA TP53-mutated AML
AML, Myelodysplasia-Related (AML-MR)	MDS-related mutations/cytogenetics Prior MDS/MDS-MPN
AML with Germline Predisposition	Germline CEBPA, DDX41, RUNX1, ANKRD26, ETV6, GATA2
AML, Not Otherwise Specified (AML-NOS)	Minimal differentiation Without maturation With maturation Myelomonocytic Monoblastic/Monocytic Pure erythroid Megakaryoblastic Basophilic Panmyelosis w/ fibrosis
Myeloid Sarcoma	Extramedullary myeloblast tumor

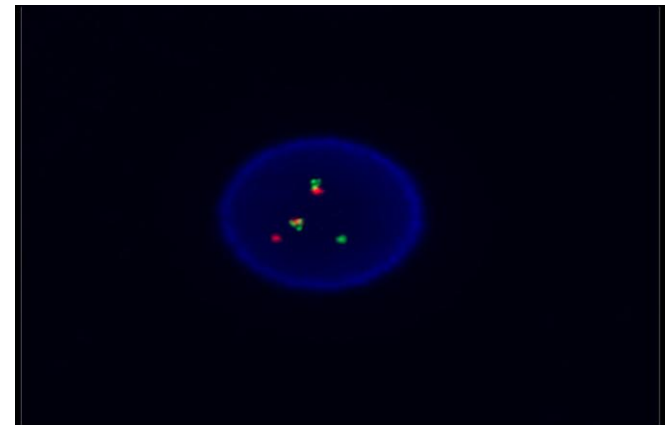
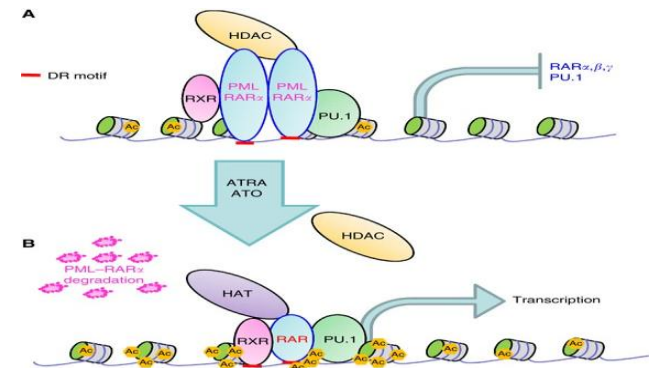
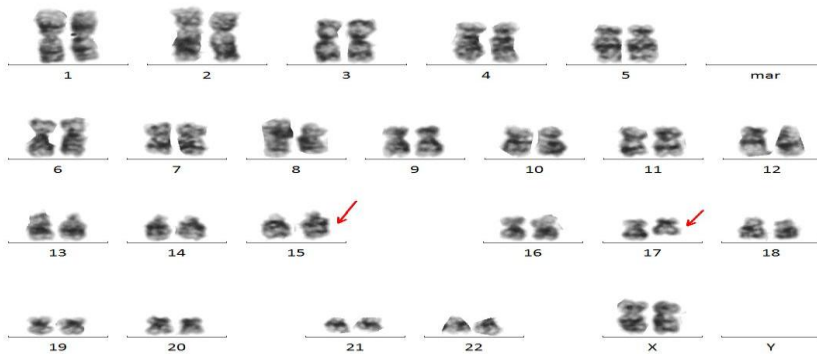
ELN 2022 Genetic Risk Classification

- ▶ spaja citogenetske i molekularne nalaze
- ▶ povoljna prognoza: t(8;21), inv(16), NPM1-mutiran (bez FLT3-ITD)
- ▶ srednja: t(9;11), NPM1 + FLT3-ITD
- ▶ loša prognoza: inv(3), t(6;9), kompleksni kariotip, TP53 delecije/ mutacije

AML and related neoplasms	
<p>AML with recurrent genetic abnormalities (requiring ≥10% blasts in BM or PB)*</p> <ul style="list-style-type: none"> • APL with t(15;17)(q24.1;q21.2)/PML::RARA† • AML with t(8;21)(q22;q22.1)/RUNX1::RUNX1T1 • AML with inv(16)(p13.1;q22) or t(16;16)(p13.1;q22)/CBFB::MYH11 • AML with t(9;11)(p21.3;q23.3)/MLL3::KMT2A‡ • AML with t(6;9)(p22.3;q34.1)/DEK::NUP214 • AML with inv(3)(q21.3;q26.2) or t(3;3)(q21.3;q26.2)/GATA2, MECOM(EV1)§ • AML with other rare recurring translocations • AML with mutated NPM1 • AML with in-frame bZIP mutated CEBPA¶ • AML with t(9;22)(q34.1;q11.2)/BCR::ABL1* 	<p>Myeloid sarcoma</p> <p>Acute leukemia of ambiguous lineage</p> <ul style="list-style-type: none"> • 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
<p>Categories designated AML (if ≥20% blasts in BM or PB) or MDS/AML (if 10-19% blasts in BM or PB)</p> <ul style="list-style-type: none"> • AML with mutated TP53# • AML with myelodysplasia-related gene mutations Defined by mutations in ASXL1, BCOR, EZH2, RUNX1, SF3B1, SRSF2, STAG2, UZF1, and/or ZRSR2 • AML with myelodysplasia-related cytogenetic abnormalities** • AML not otherwise specified 	<p>Myeloid proliferations related to Down syndrome</p> <ul style="list-style-type: none"> • Transient abnormal myelopoiesis associated with Down syndrome • Myeloid leukemia associated with Down syndrome <p>Blastic plasmacytoid dendritic cell neoplasm</p>
<p>Diagnostic qualifiers††</p> <p>Therapy-related‡‡</p> <ul style="list-style-type: none"> • Prior chemotherapy, radiotherapy, immune interventions <p>Progressed from MDS</p> <ul style="list-style-type: none"> • MDS should be confirmed by standard diagnostics and >3 mo prior to AML diagnosis <p>Progressed from MDS/MPN (specify type)</p> <ul style="list-style-type: none"> • MDS/MPN should be confirmed by standard diagnostics and >3 mo prior to AML diagnosis <p>Germline predisposition (specify type)</p>	

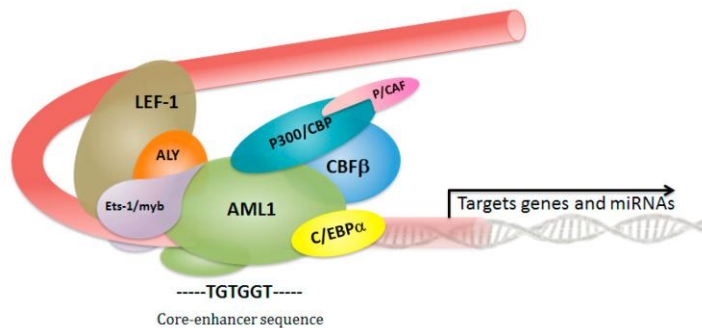
Akutna Promijelocitna Leukemija (PML::RARA)

- ▶ t(15;17)(q22;q12) → PML::RARA fuzija
- ▶ javlja se u oko 8–12% odraslih AML
- ▶ vrlo se uspješno liječi kombinacijom ATRA ± arsenic trioxide
- ▶ sekundarne promjene koje možemo naći su: +8, del(7q), del(9q)...



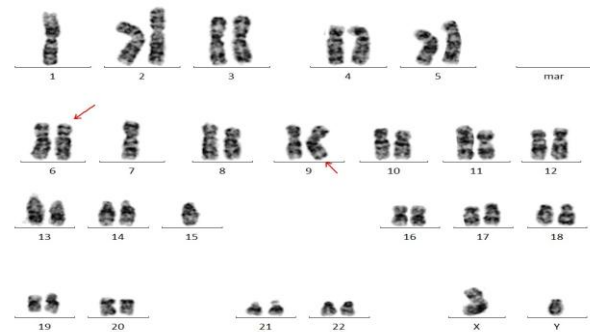
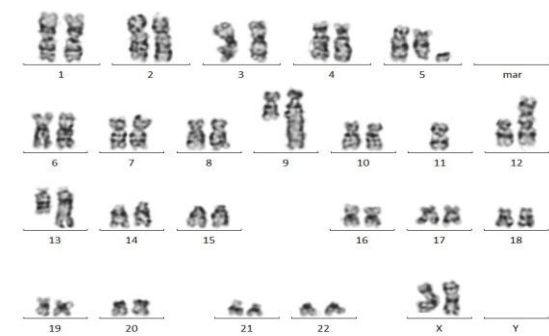
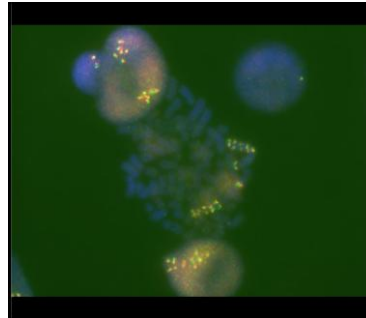
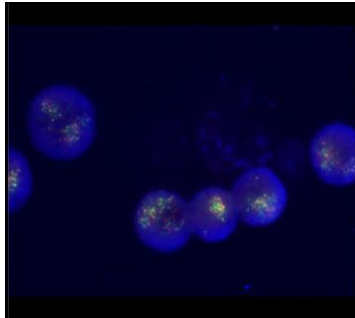
Core-Binding Factor AML

- ▶ uključuje $t(8;21)(q22;q22.1)/RUNX1::RUNX1T1$ i $inv(16)/t(16;16)/CBFB::MYH11$
- ▶ povoljna prognoza – terapija visokim dozama Cytarabin-a
- ▶ KIT i FLT3 mutacije pogoršavaju ishod bolesti



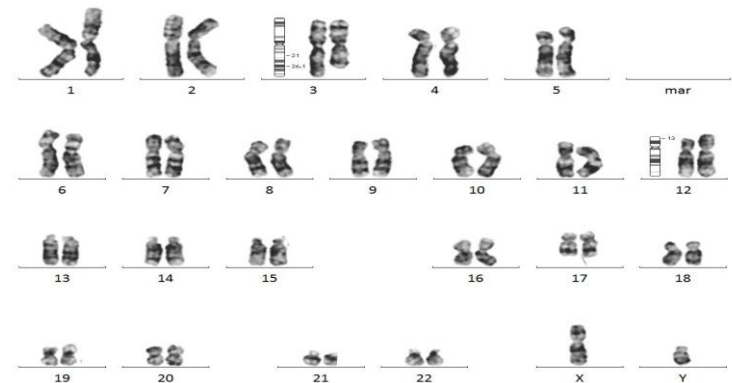
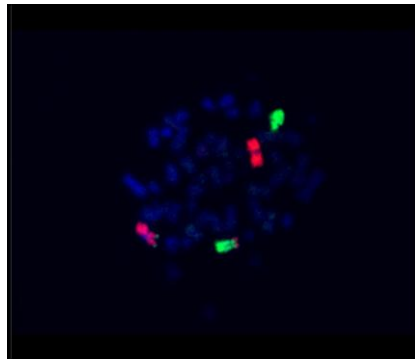
AML sa DEK::NUP214 i KMT2A preuredbom

- ▶ $t(6;9)(p23;q34.1)/DEK::NUP214$ – rijetka, loša prognoza, povezana s MDS i bazofilijom
- ▶ $11q23/KMT2A$ preuredba – >80 fuzijskih partnera, javlja se i u ALL
- ▶ $t(9;11)$ najčešća, intermedijarna prognoza



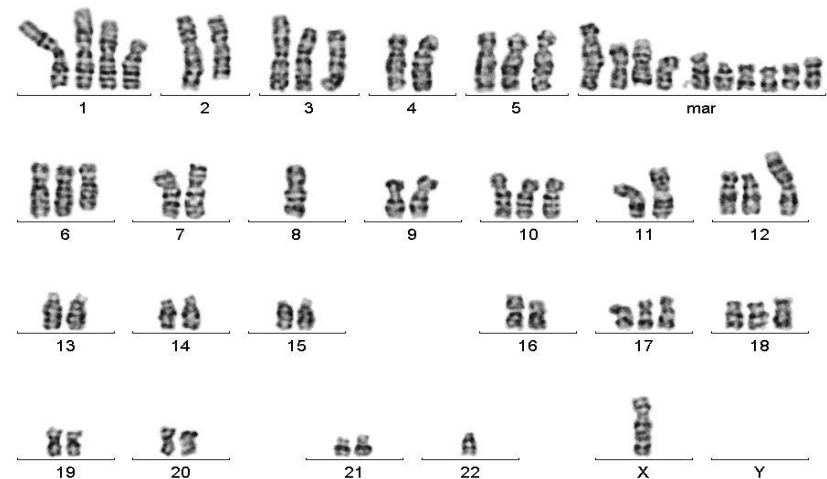
AML s MECOM ili NUP98 preuredbom

- ▶ $inv(3)/t(3;3)$ → uzrokuje aktivaciju MECOM (EV11), loš ishod, često uz monosomiju 7
- ▶ NUP98 preuredba - više od >30 fuzijskih partner gena (e.g., HOXA9, NSD1), javlja se i u ALL, češće T ALL
- ▶ češće u pedijatrijskoj AML



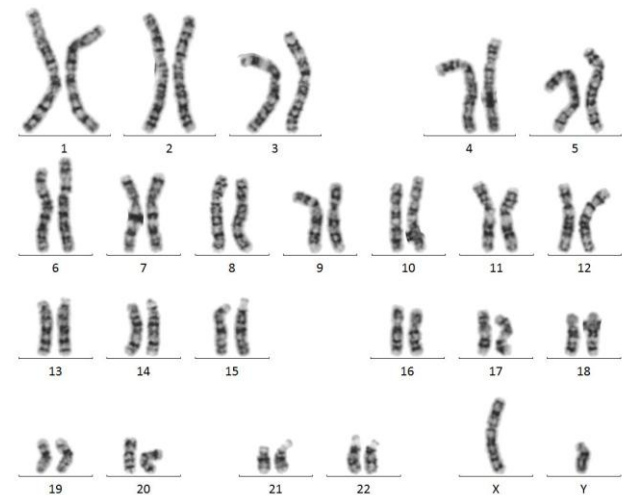
Kompleksni karioptip u AML

- ▶ ≥ 3 kromosomske promjene strukturne i numeričke (balansirane translokacije broje se kao jedna promjena)
- ▶ vidamo ih kod $\sim 10\text{--}12\%$ odraslih AML
- ▶ česti su gubici: 5q, 7q, 17p \rightarrow TP53 mutacije
- ▶ loša prognoza- posebno kod visoko kompleksnih kariotipova (više od 5 promjena)



Citogenetski normalna AML (CN-AML)

- ▶ ~40–45% of AML pacijenata imaju normalan kariotip (G-pruganje)
- ▶ najčešće submikroskopske mutacije su: NPM1, FLT3, CEBPA, DNMT3A, IDH1/2
- ▶ prognoza ovisi o molekularnom profilu



ALL

- neoplazma limfocita koja ima za posljedicu nakupljanje nezrelih limfocita u koštanoj srži
- B i T subtipovi
- karakteriziraju je genetske abnormalnosti koje dovode do blokade u diferencijaciji ili ubrzavaju proliferaciju limfatičkih prekursorskih stanica
- genetske abnormalnosti važne su za stratifikaciju bolesnika
- čini 30% svih karcinoma kod djece i 80% svih dječjih leukemija

ALL WHO 2022 klasifikacija (odrasli)

B-lymphoblastic leukemia/lymphoma (B-ALL):

- B-ALL with BCR::ABL1
- B-ALL with KMT2A rearrangements
- B-ALL with ETV6::RUNX1
- B-ALL with hyperdiploidy
- B-ALL with hypodiploidy
- B-ALL, BCR::ABL1-like

T-lymphoblastic leukemia/lymphoma (T-ALL):

- Early T-precursor ALL (ETP-ALL)
- T-ALL with TLX1, TLX3, HOX11L2

ALL ELN stratifikacija rizika (odrasli)

Visoki rizik:

- kompleksni kariotip
- niska hipodiploidija (30-35 kromosoma)/near triploidija (60-78 kromosoma)
- KMT2A preuredba
- **BCR::ABL1 pozitivni ALL (pre-TKI era)**
- starija dob (>60)

Srednji rizik:

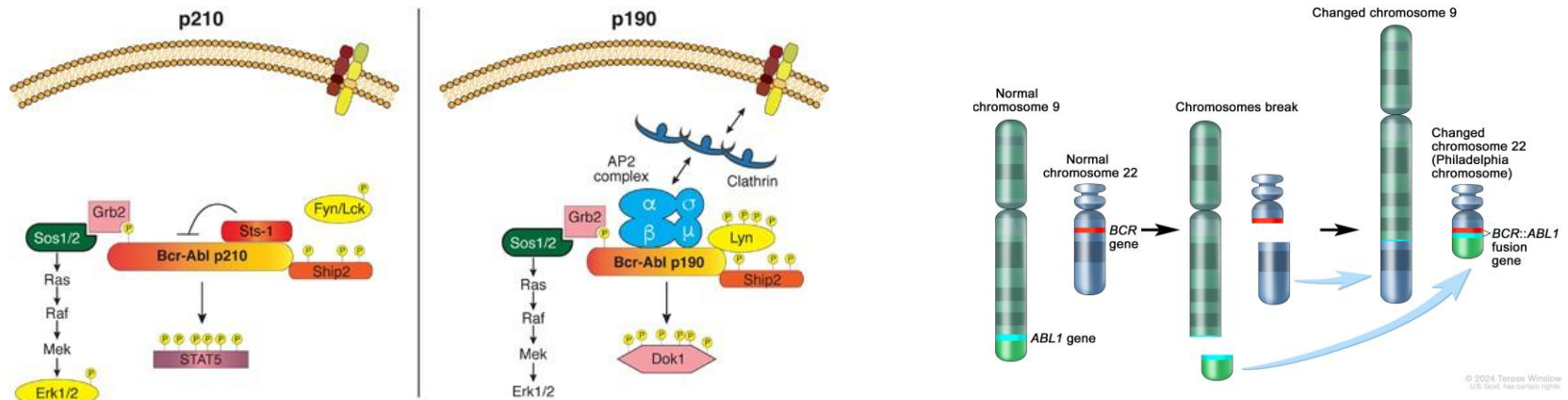
- ostale B-ALL
- T-ALL bez ETP fenotipa

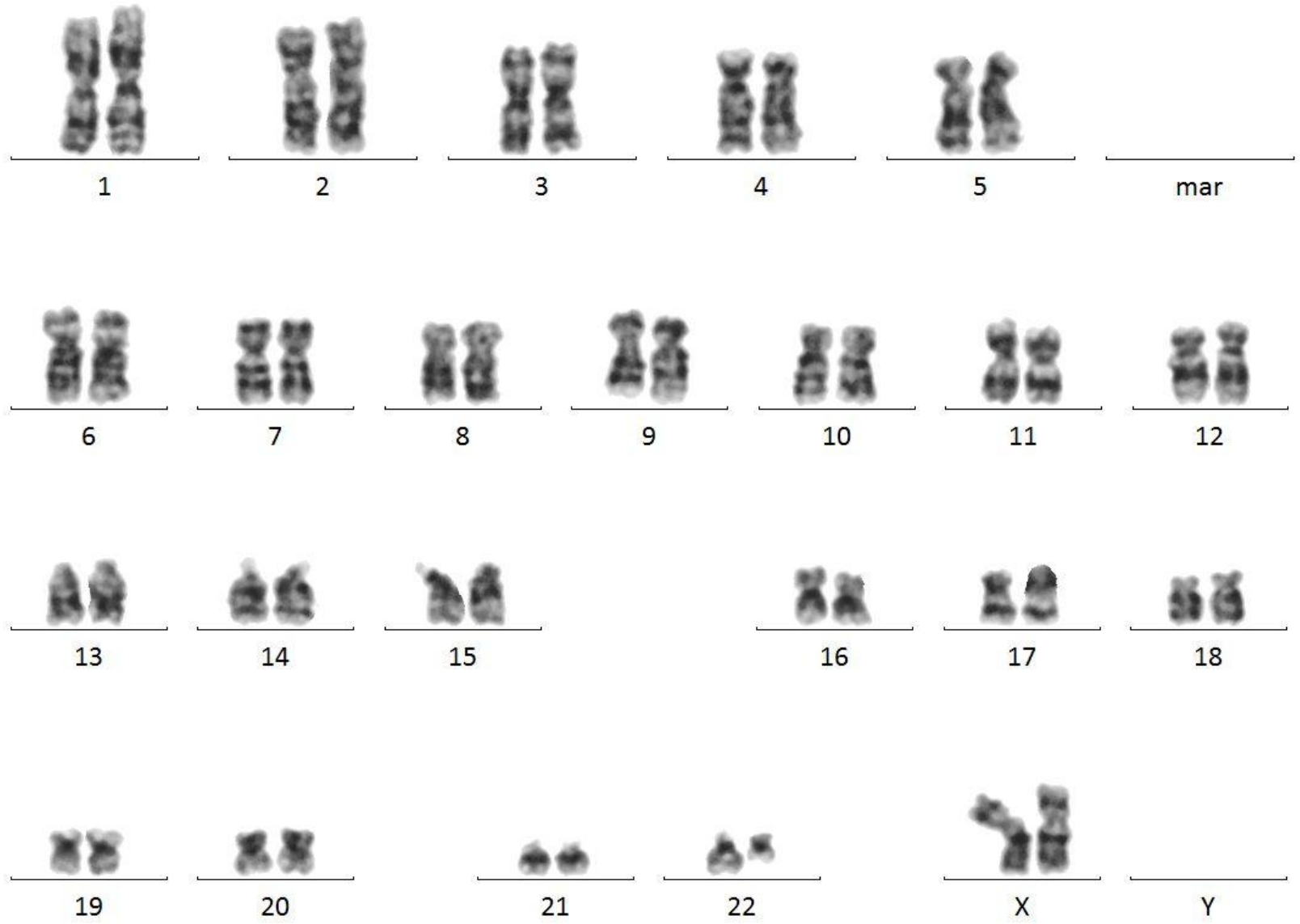
Povoljan rizik:

- nije jasno definiran kod odraslih pacijenta

B-ALL BCR::ABL

- najčešća promjena
- javlja se u oko 25-30% ALL
- p190 transkript, rjeđe p210
- dodatne citogenetske promjene nalazimo u oko 60% slučajeva
- najčešće su: +der(22), -7, +8, del(3p)





ALL ELN stratifikacija rizika (odrasli)

Visoki rizik:

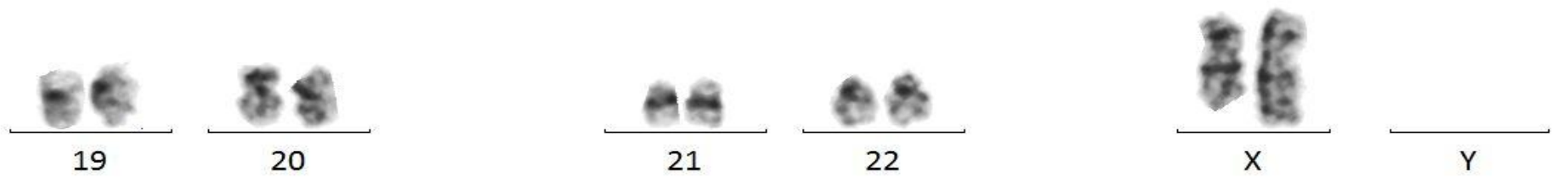
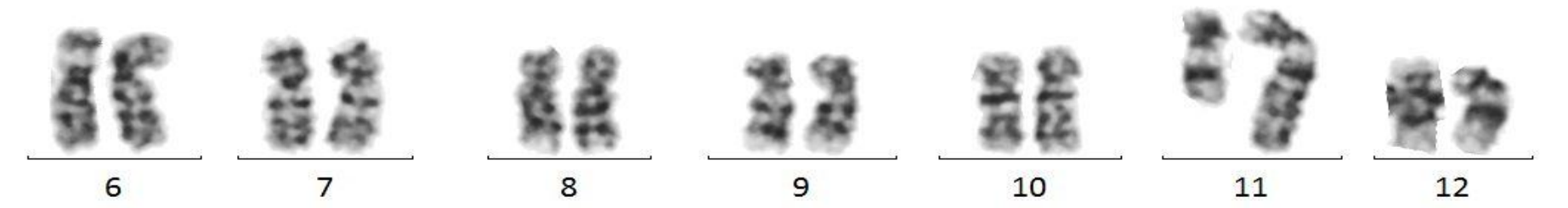
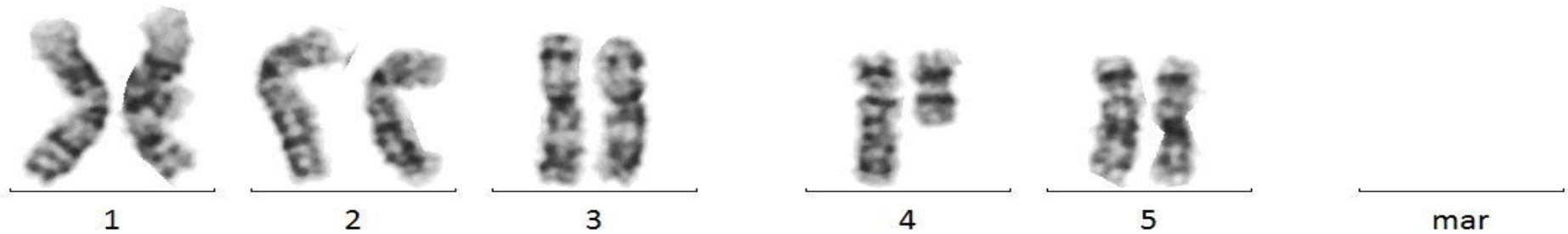
- kompleksni kariotip
- niska hipodiploidija (30-35 kromosoma)/near triploidija (60-78 kromosoma)
- **KMT2A preuredba (MLL)**
- BCR::ABL1 pozitivni ALL (pre-TKI era)
- starija dob (>60)

Srednji rizik:

- ostale B-ALL
- T-ALL bez ETP fenotipa

Povoljni rizik:

- nije jasno definiran kod odraslih pacijenta



Ph like

➤ poseban entitet

- 10% djece
- 20-25% odraslih

➤ podtipovi:

- JAK2(JAK STAT path) -JAK2,CRLF2,EPOR 50%
- ABL - ABL,PDGFRB,CSF1R,PDGFRA 10%
- RAS KRAS,NRAS,BRAF

T-ALL

Gene	Cytogenetic Abnormality	Frequency	Prognosis
TAL1	t(1;14), SIL-TAL1	15–20%	Intermediate
TLX1	t(10;14)	10–15%	Relatively good
TLX3	t(5;14)	20–25%	Poor
LMO1/2	t(11;14), t(7;11)	~10%	Variable
NOTCH1	Mutations	>50%	Good early response
PTEN	Deletion/mutation	10–15%	Poor
ETP-ALL lesions	FLT3, JAK1/3, IL7R	10–15%	High risk

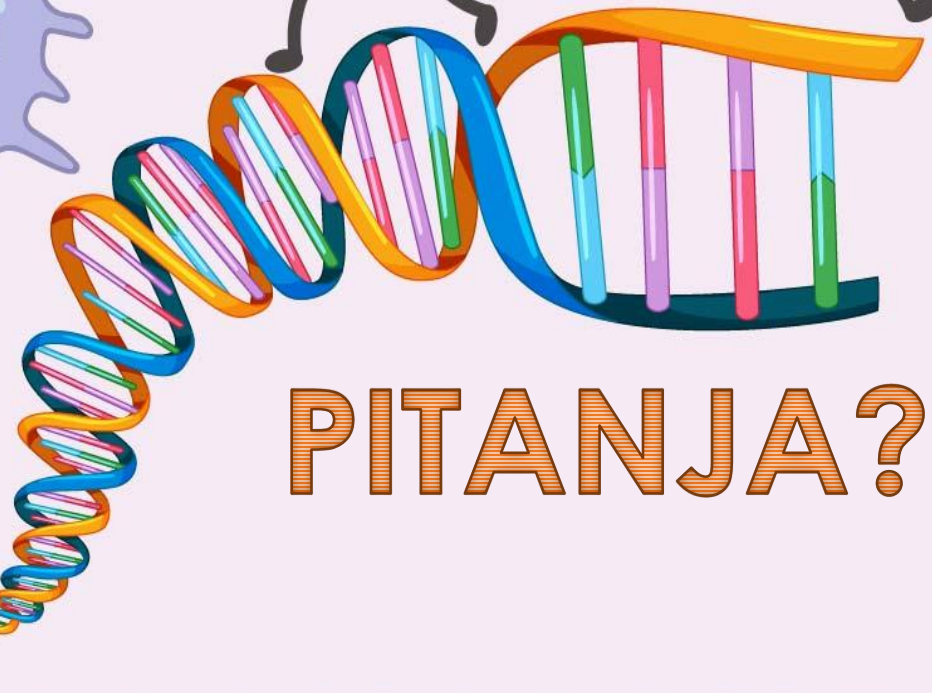
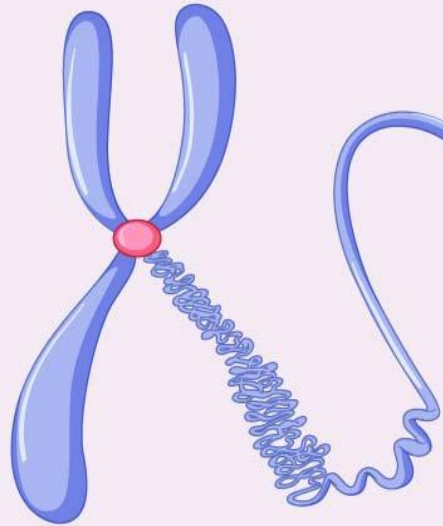
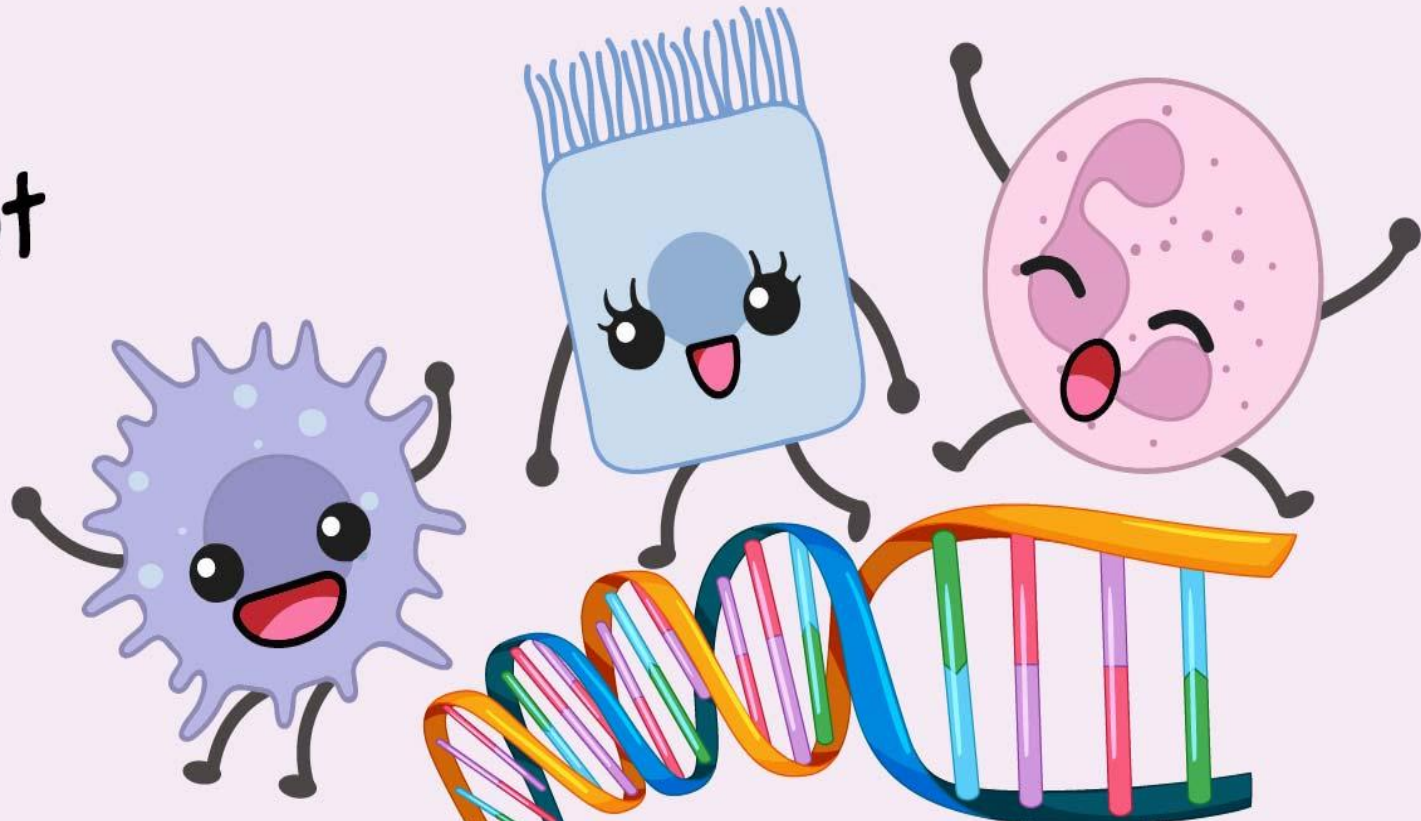
Klinički značaj citogenetike

- ▶ bitna za odluku o terapiji (npr. odluka o HSCT)
- ▶ može predvidjeti ishod liječenja
- ▶ unapređuje individualizirani pristup pacijentu

Umjesto zaključka

- ▶ citogenetske metode su jedan od stupova dijagnostike akutnih leukemija
- ▶ definira različite molekularne subtipove i prognostičke grupe
- ▶ zajedno s molekularnom genetikom stratificira pacijente u različite skupine rizika i time utječe na odluku o liječenju pacijenta

Let's talk
about



PITANJA?

CHROMOSOMES