

Sıvı bazlı (Hematopatoloji) FISH uygulaması deęerlendirmelerine temel bakış

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Patoloji AD

- Metafaz fazında hücrelere uygulanan sitogenetik analiz “altın standarttır”
- Çünkü kromozom morfolojisi ve sinyalin pozisyonu direk olarak görülür.
- FISH’in major avantajı bölünmeyen interfaz hücreye uygulanmasıdır.

- Moleküler testler
 - Hastalığın erken ve doğru teşhisini
 - En etkin tedaviyi belirlemeyi ve tedavinin etkisini değerlendirmeyi
 - Hastalığın prognozu ve rekürrensini tespit etmeyi sağlar

 - FISH bu testlerden birisidir

Tanı ve prognoz

Tanının doğru yapılması için:

- Akut promyelositik lösemide (APL) t(15;17) bakılması
- KML; t(9;22)
- Folliküler lenfoma: t(14;18)
- Mantle hücreli lenfoma: t(11;14)
- AML hastalarda, AML1/ETO ve inv(16) olanlar diğer anomalileri taşıyanlara göre daha iyi prognoza sahiptir

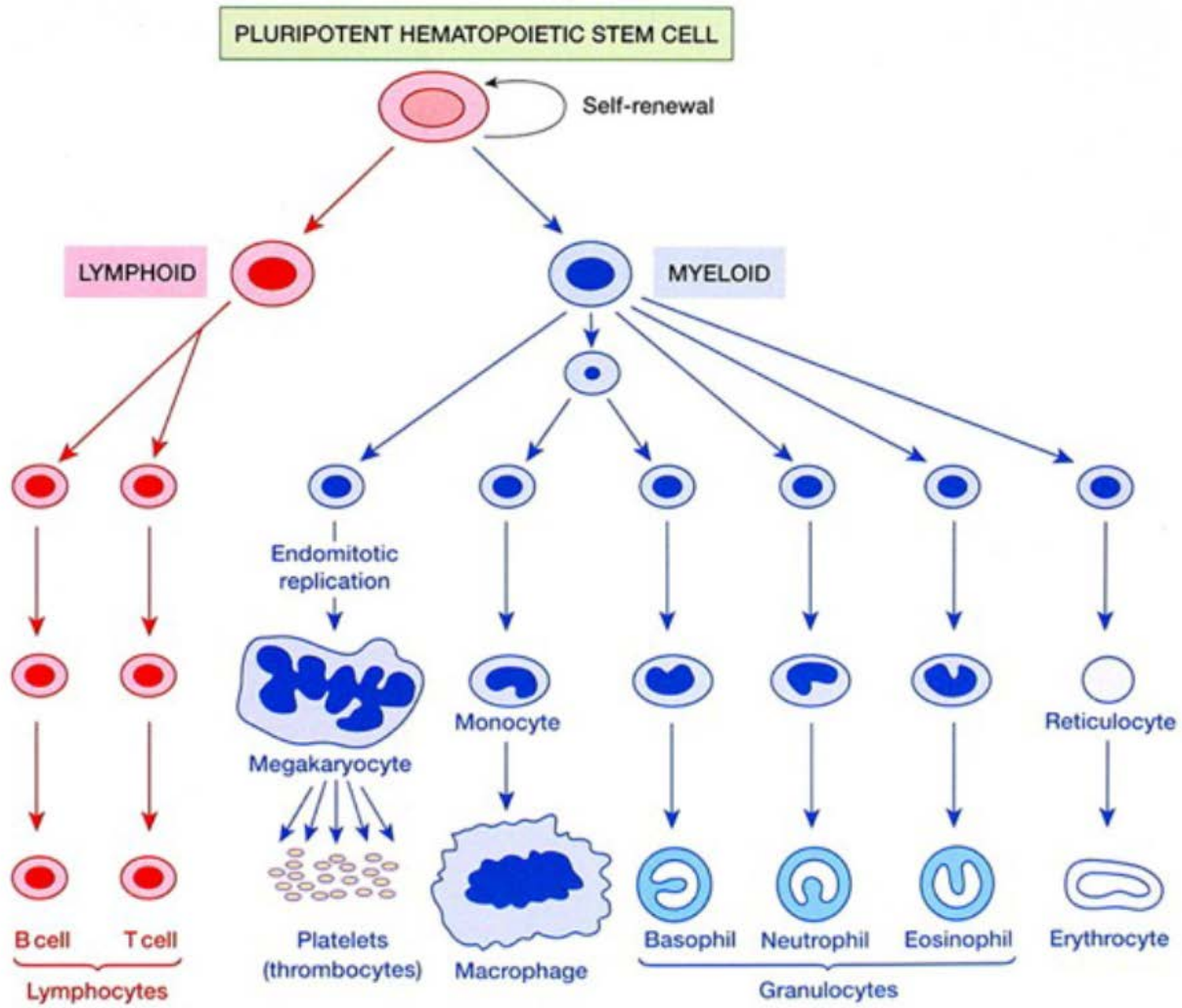
Tedavi seçimi ve tedavinin gidişini tahmin etme

- APL; t(15;17)
- KML; t(9;22) ve imatinib
- KLL; p53ve Rituximab

Minimal rezidüel hastalığın tespiti ve takibi

Hastalığa spesifik bir anomalinin takibi ile yapılabilir

- APL; t(15;17)
- KML; t(9;22)

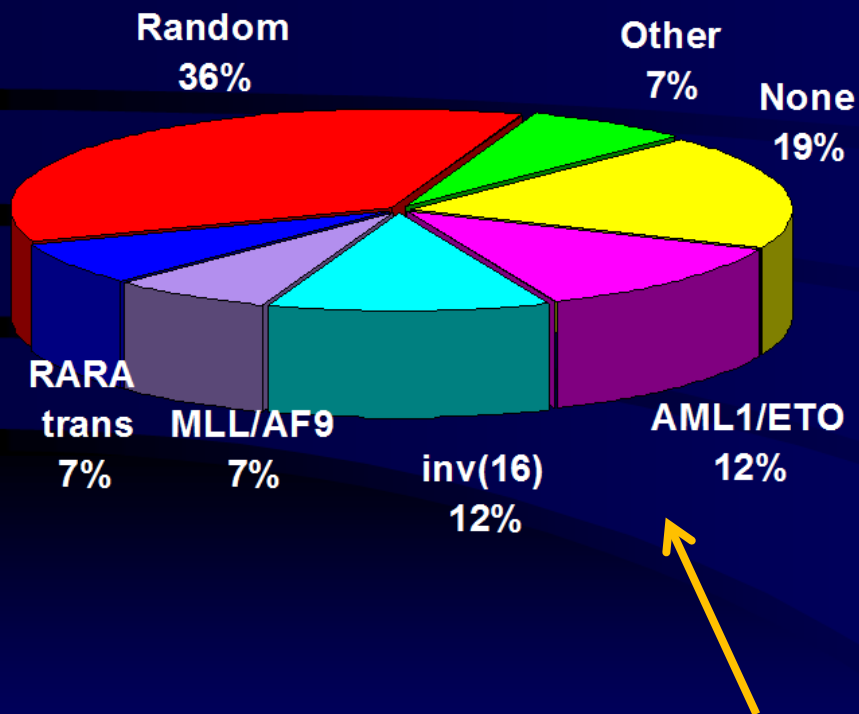


DSÖ sınıflamasındaki hemopoetik ve lenfoid tümörlerin sınıflamasında kullanılan genetik anomaliler

Sitogenetik alt gruplar

- Akut Myeloid Lösemi
 - AML with AML1/ETO
 - AML with PML/RARA ve türevleri
 - AML with inv(16) veya CFBF/MYH11X
 - AML with 11q23 anomalileri
- Kronik Myeloid Lösemi
 - BCR/ABL
- Myelodisplastik Sendrom
 - 5q- sendromu

Acute Myelogenous Leukemia



- LSI EGR-1 -5/5q-
- LSI CSF1R -5/5q-
- LSI D7S486/CEP 7 -7/7q-
- LSI D7S522/CEP7 -7/7q-
- LSI AML1/ETO t(8;21)
- LSI BCR/ABL t(9;22)
- LSI BCR/ABL ES t(9;22)
- LSI MLL t(11q23)
- LSI RARA t(11;17), t(5;17)
- LSI PML/RARA t(15;17)
- LSI CBFB t(16;16), inv(16)
- LSI D20S108 20q-

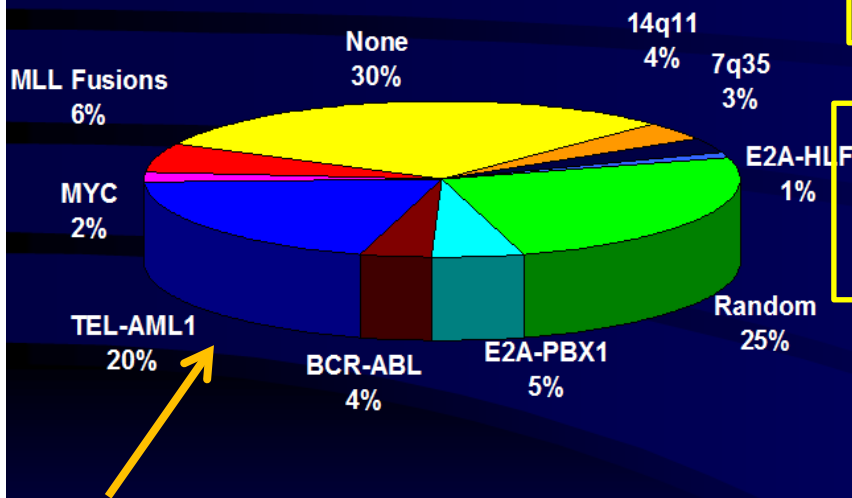
Source: Look, T. Science Vol.278



DSÖ hemopoetik ve lenfoid tümörlerin sınıflamasında kullanılan genetik anomaliler sitogenetik alt gruplar

- Akut lenfoid Lösemi
 - BCR/ABL
 - 11q23 (MLL) rearrangement
 - E2A/PBX1 t(1;19)
 - TEL/AML1

Leukemia:ALL



- LSI IGH/MYC t(8;14)

- BCR/ABL t(9;22)

- BCR/ABL ES t(9;22)

- LSI MLL t(11q23)

- TEL/AML1 t(12;21)

- LSI MYC t(MYC)

Source: Look, T. Science Vol.278



Malign myeloid neoplazilerde görülen sitogenetik anomaliler

Disease*	Chromosome Abnormality	Frequency	Involved Genes ^b		Consequence
MPN CML	t(9;22)(q34;q11.2)	~99% ^c	<i>ABL1</i>	<i>BCR</i>	Fusion protein—Altered cytokine signaling pathways, genomic instability
CML blast phase	t(9;22) with +8, i(17q), +19, or +der(22)t(9;22)	~70%			
PV	+8 +9 del(20q) del(13c) partial trisomy 1q	20% (all abnormalities combined)			
MF	+8 +9 -7/del(7q) del(5q)/t(5q) del(20q) del(13c) partial trisomy 1q	30% (all abnormalities combined)			
AML	t(8;21)(q22;q22)	10%	<i>RUNX1/ETO</i>	<i>RUNX1/AML1</i>	Fusion protein—Altered transcriptional regulation
	t(15;17)(q24.1;q21.1)	9%	<i>PML</i>	<i>RARA</i>	Fusion protein—Altered transcriptional regulation
	inv(16)(p13.1q22) or t(16;16)(p13.1,q22)	5%	<i>MYH11</i>	<i>CBFB</i>	Fusion protein—Altered transcriptional regulation
	t(9;11)(p22;q23)	5%–8% for all t(11q23)	<i>MLL3/AF9</i>	<i>MLL</i>	MLL histone methyltransferase fusion proteins—Altered chromatin structure and transcriptional regulation
	t(10;11)(p12;q23)		<i>MLL10/AF10</i>	<i>MLL</i>	
	t(11;17)(q23;q25)		<i>MLL</i>	<i>MLL7/AF17</i>	
	t(11;19)(q23;p13.3)		<i>MLL</i>	<i>MLL1/ENL</i>	
	t(11;19)(q23;p13.1)		<i>MLL</i>	<i>ELL</i>	
	t(6;11)(q27;q23)		<i>MLL14/AF6</i>	<i>MLL</i>	
	Other t(11q23)		<i>MLL</i>		
	del(11)(q23)	8%			
	+8	1%–2%			
	+11	14%	<i>MLL</i>		ITD
-7 or del(7q)	12%				
del(5q)/t(5q)	1%	<i>DEK</i>	<i>NUP214/CAN</i>		
t(6;9)(p23;q34)	2%	<i>RPW1</i>	<i>MECOM/EVI1</i>		
inv(3)(q21q26.2) or t(3;3)	5%				
del(20q)	2%				
t(12p) or del(12p)					
Therapy-related MN	-7 or del(7q)	45%			
	del(5q)/t(5q)	40%			
	der(1;7)(q10;p10)	2%			
	dic(5;17)(q11.1-13;p11.1-13)	5%		<i>TP53</i>	Loss of function—DNA damage response
	t(9;11)(p22;q23)/t(11q23)	3%	<i>MLL3/AF9</i>	<i>MLL</i>	MLL histone methyltransferase fusion proteins—Altered chromatin structure and transcriptional regulation
t(11;16)(q23;p13.3)	2% (t-MDS)	<i>MLL</i>	<i>CREBBP</i>	Fusion protein—Altered transcriptional regulation	
MDS (Unbalanced)	t(21q22)	2%	<i>RUNX1/AML1</i>		
	t(3;21)(q26.2;q22)	3%	<i>RPL22L1</i>	<i>RUNX1</i>	
	+8	10%			
	-7/del(7q) ^d	12%			
	del(5q)/t(5q) ^d	15%			
	del(20q)	5%–8%			
	-Y	5%			
	i(17q)/t(17p) ^d	3%–5%	<i>TP53</i>		Loss of function, DNA damage response
	-13/del(13q) ^d	3%			
	del(11q) ^d	3%			
	del(12p)/t(12p) ^d	3%			
	del(9q) ^d	1%–2%			
	idic(X)(q13) ^d	1%–2%			
t(1;3)(p35.3;q21.2) ^d	1%	<i>MME11</i>	<i>RPW1</i>	Deregulation of <i>MME11</i> —Transcriptional activation?	
(Balanced)	t(2;11)(p21;q23)/t(11q23) ^d	1%		<i>MLL</i>	MLL fusion protein—Altered transcriptional regulation
	inv(3)(q21q26.2)/t(3;3) ^d	1%	<i>RPW1</i>	<i>MECOM/EVI1</i>	Altered transcriptional regulation by <i>MECOM</i>
	t(6;9)(p23;q34) ^d	1%	<i>DEK</i>	<i>NUP214</i>	Fusion protein—Nuclear pore protein
	t(5;12)(q33.1;p13)	~2%	<i>PDGFRB</i>	<i>ETV6/TEL</i>	Fusion protein—Altered signaling pathways

Malign lenfoid neoplazilerde görülen sitogenetik anomaliler

Disease ^a	Chromosome Abnormality	Frequency ^b	Involved Genes ^c	Consequence		
ALL						
Precursor B	t(12;21)(p13;q22)	25%	<i>ETV6/TEL</i>	<i>RUNX1/AML1</i>	Fusion protein—TF	
	t(9;22)(q34;q11.2)	10% ^d	<i>ABL1</i>	<i>BCR</i>	Fusion protein—Altered cytokine signaling pathways	
	t(4;11)(q21;q23)	5%	<i>AFF14</i>	<i>MLL</i>	Fusion protein—TF	
	t(17;19)(q22;p13.3)	1%	<i>HLF</i>	<i>TCF3 (E2A)</i>	Fusion protein—TF	
Pre-B	t(11;19)(q23;p13.3)	1%	<i>MLL</i>	<i>MLL1/ENL</i>	Fusion protein—TF	
	t(1;19)(q23;p13.3)	6% (30%)	<i>PBX1</i>	<i>TCF3 (E2A)</i>	Fusion protein—TF	
	t(8;14)(q24.2;q32)	5% (95%)	<i>MYC</i>	<i>IGH</i>	Deregulated expression—TF	
	t(2;8)(p12;q24.2)	<1% (1%)	<i>IGK</i>	<i>MYC</i>	Deregulated expression—TF	
	t(8;22)(q24.2;q11.2)	<1% (4%)	<i>MYC</i>	<i>IGL</i>	Deregulated expression—TF	
Other	Hyperdiploidy (50–60)	10%				
	del(12p), t(12p)	10%				
T	t(11;14)(p15;q11.2)	1%	<i>LMO1</i>	<i>TRA</i>	Deregulated expression—TF	
	t(11;14)(p13;q11.2)	3%	<i>LMO2</i>	<i>TRA</i>	Deregulated expression—TF	
	t(8;14)(q24.2;q11.2)	<1%	<i>MYC</i>	<i>TRA</i>	Deregulated expression—TF	
	inv(14)(q11.2;q32)	<1%	<i>TRA</i>	<i>TCL1A</i>	Deregulated expression—TF	
	t(10;14)(q24;q11.2)	3%	<i>TLX1</i>	<i>TRA</i>	Deregulated expression—TF	
	t(1;14)(p32;q11.2)	1%	<i>TAL1</i>	<i>TRD</i>	Deregulated expression—TF	
	t(7;9)(q34;q34)		<i>TRB</i>	<i>NOTCH1</i>	Deregulated expression—TF	
	t(7;19)(q34;p13.2)	2%	<i>TRB</i>	<i>LYL1</i>		
	del(9p), t(9p)	<1%	<i>CDKN2A</i>		TSG—cell cycle regulation	
		<1% (10%)	<i>CDKN2B</i>			
NHL						
B-cell NHL						
	Burkitt					
Burkitt	t(8;14)(q24.2;q32)	95%	<i>MYC</i>	<i>IGH</i>	Deregulated expression—TF	
	t(2;8)(p12;q24.2)	1%	<i>IGK</i>	<i>MYC</i>	Deregulated expression—TF	
	t(8;22)(q24.2;q11.2)	4%	<i>MYC</i>	<i>IGL</i>	Deregulated expression—TF	
Follicular	t(14;18)(q32;q21.3)	80%	<i>IGH</i>	<i>BCL2</i>	Deregulated expression—Antiapoptosis protein	
DLBCL		20%				
	t(3;22)(q27;q11.2)	45% for all	<i>BCL6</i>	<i>IGL</i>	Deregulated expression—TF	
DLBCL	t(3;14)(q27;q32)	t(3q27)	<i>BCL6</i>	<i>IGH</i>	Deregulated expression—TF	
	t(11;14)(p13;q32)	~100%	<i>CCND1</i>	<i>IGH</i>	Deregulated expression—TF	
MCL	t(19;14)(p13;q32)		<i>PAK5</i>	<i>IGH</i>	Deregulated expression—TF	
LPL	t(14;19)(q32;q13.3)		<i>IGH</i>	<i>BCL3</i>	Deregulated expression—TF	
SLL	t(11;18)(q21;q21.3)	40%–50%	<i>BIRC3/API2</i>	<i>MALT1</i>	Fusion Protein—Increased NF- κ B activation	
MALT	t(1;14)(p22;q32)	10%	<i>BCL10</i>	<i>IGH</i>	Deregulated expression—Increased NF- κ B activation	
	t(14;18)(q32;q21.3)	10%–20%	<i>IGH</i>	<i>MALT1</i>	Deregulated expression—Increased NF- κ B activation	
	t(3;14)(p13;q32)	10%	<i>FOXP1</i>	<i>IGH</i>	Deregulated expression—TF	
	t(X;14)(p12;q32)	Rare	<i>GPR34</i>	<i>IGH</i>	Deregulated expression—G protein-coupled receptor	
					Deregulated expression—Increased NF κ B activation	
PCMZL	t(14;18)(q32;q21.3)	Rare	<i>IGH</i>	<i>MALT1</i>	Deregulated expression—Increased NF κ B activation	
PCFCL	t(14;18)(q32;q21.3)	40%	<i>IGH</i>	<i>BCL2</i>	Deregulated expression—Antiapoptosis protein	
T-cell NHL						
ALK ⁺ ALCL	t(2;5)(p23;q35.1)	75%	<i>ALK</i>	<i>NPM1</i>	Deregulated expression—TK	
	t(6;7)(p25.3;q32.3)	10%–15%	<i>IRF4</i> , <i>DUSP22</i>		Deregulated expression of TF (IRF4) and phosphatase (DUSP22)	
ALK ⁻ ALCL						
Nasal/NK cell Hepatosplenic Peripheral	t(1q), t(7q), t(17q)	>95%				
	t(7q)	15%	<i>ITK</i>	<i>SYK</i>	Constitutively active TK (SYK)	
CLL						
	B	t(11;14)(q13;q32)	10%	<i>CCND1</i>	<i>IGH</i>	Deregulated expression—Cell cycle regulation
		t(14;19)(q32;q13.2)	5%	<i>IGH</i>	<i>BCL3</i>	Deregulated expression—Increased NF- κ B activation
		t(2;14)(p16;q32)	5%	<i>BCL11A</i>	<i>IGH</i>	
		t(14q32)	15%	<i>IGH</i>		
	del(13q)	30%				
	+12	25%				

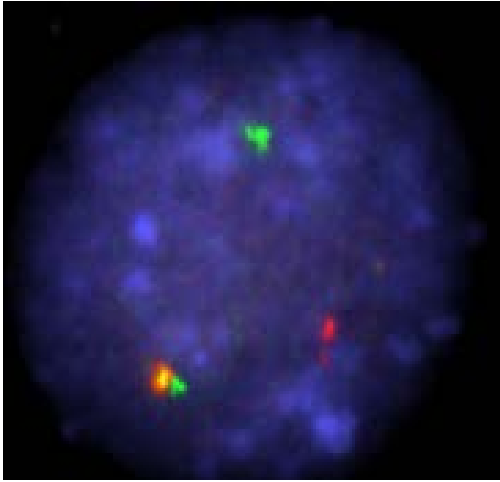
Plazma hücre neoplazisi

MM	Genetik bulgular	%	Gen	Gen	Deregüle edilmiş ekspresyon
B	-13/del(13q)	40%			
	t(4;14)(p16;q32)	15%	<i>WHSC1/FGFR3</i>	<i>IGH</i>	Deregüle edilmiş ekspresyon—Growth factor receptor
			<i>MMSET</i>	<i>IGH</i>	Deregüle edilmiş kromatin modifikasyonu ve gen ekspresyonu—Histone metiltransferaz
	t(14;16)(q32;q23)	5%	<i>IGH</i>	<i>MAF</i>	Deregüle edilmiş ekspresyon—TF
	t(6;14)(p21;q32)	4%	<i>CCND3</i>	<i>IGH</i>	Deregüle edilmiş ekspresyon—Cell cycle regulation
	t(11;14)(q13;q32)	15%	<i>CCND1</i>	<i>IGH</i>	Deregüle edilmiş ekspresyon—Cell cycle regulation
	t(14q32) del(17p)/t(17p)	50% 30%	<i>IGH</i> <i>TP53</i>		Loss of function—DNA damage response
Gain of 1q Hyperdiploidy: +3, +5, +7, +9, +11	20%				

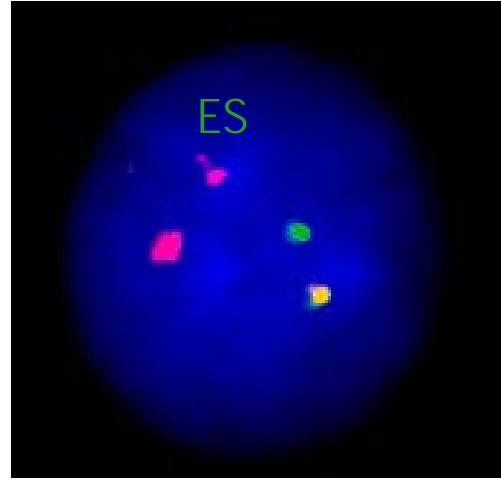
KML- Tanı, Prognoz ve tedavi

KML: Philadelphia kromozomunun tespiti(bcr/abl translokasyonu), t(9;22)

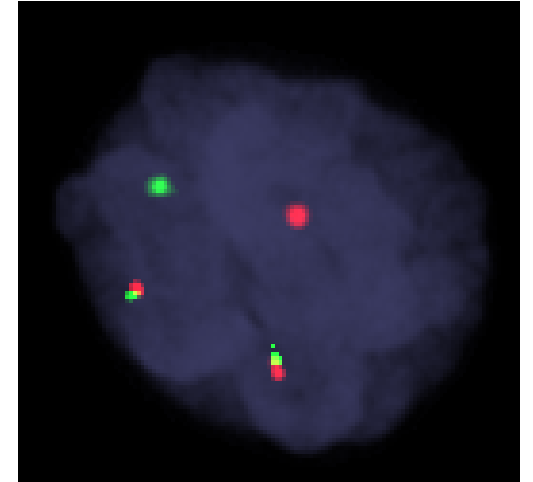
Tedavi seçimi: tyrosine kinase inhibitor (Imatinib)



Dual Colour, Single Fusion



Dual Colour, Extra signal



Dual Colour, Dual Fusion

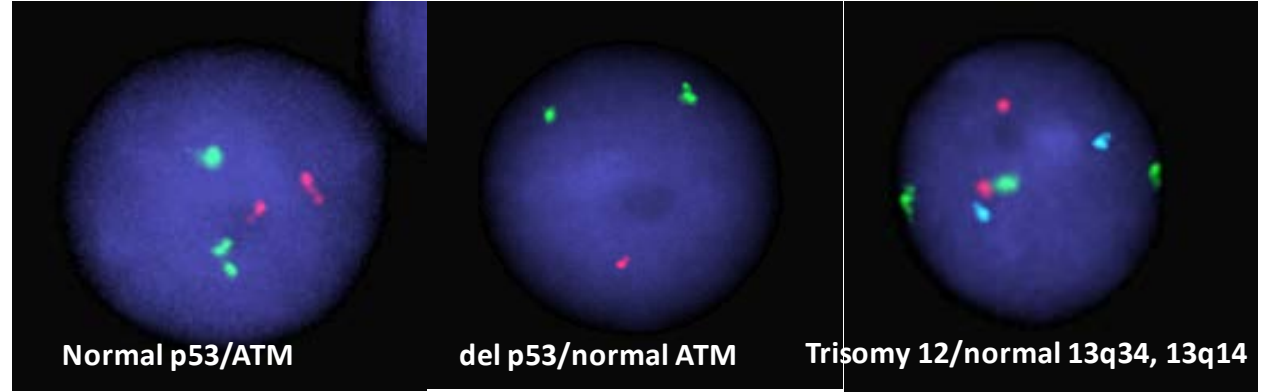
Kronik lenfositik lösemi (KLL)

- CEP 12 +12
- D13S25 del(13q14)
- D13S319 del(13q34)
- P53 del(17p13)
- ATM del(11q22.3)

KLL FISH

Prognoz&tedavi
seçimi

p53
ATM
13q14
13q34
CEP 12



Konvansiyonel sitogenetik ile KLL hastalarının sadece %40-50'sinde klonal anomali saptanır
İnterphase FISH ile KLL hastalarının %90 fazlasında anomali saptanır

Myelodisplastik sendrom

- 5/del(5q31) EGR-1
- 7/del(7q31) ve i(7q) CEP7
- +8 CEP8
- t(4;11q23) (9;11) (11;19) MLL
- p53

Multiple myelom

- Del(17p13.1) p53
- Del(13q14) Rb1
- T(11;14) IGH/CCND1
- T(4;14) IGH/FGFR3

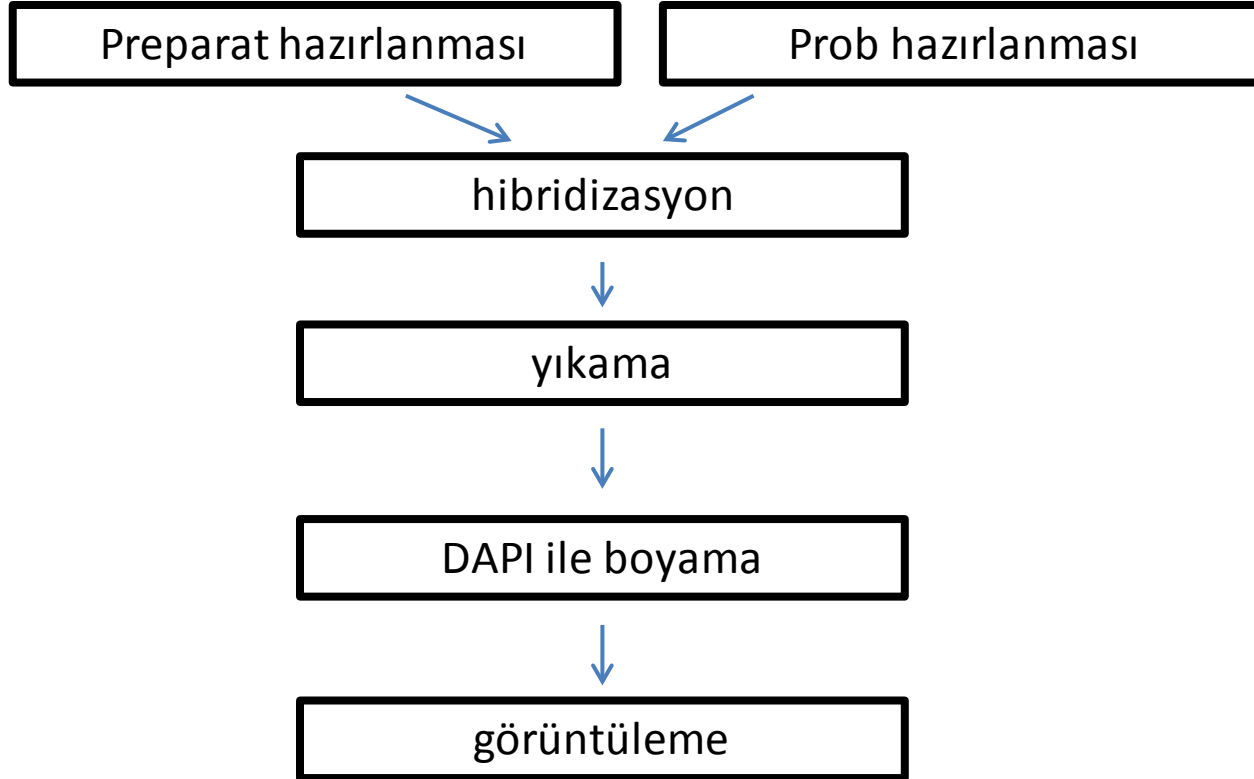
NHL'lara spesifik anomaliler

- ALK t(2;5) ABHL
- IGH/CCND1 t(11;14) MHL
- IGH/BCL2 t(14;18) FL
- MYC t(8;22) BL ve diğ erleri,
- BCL6 t(3q27) DBBHL



Sodyum sitrat

Temel FISH işlemi

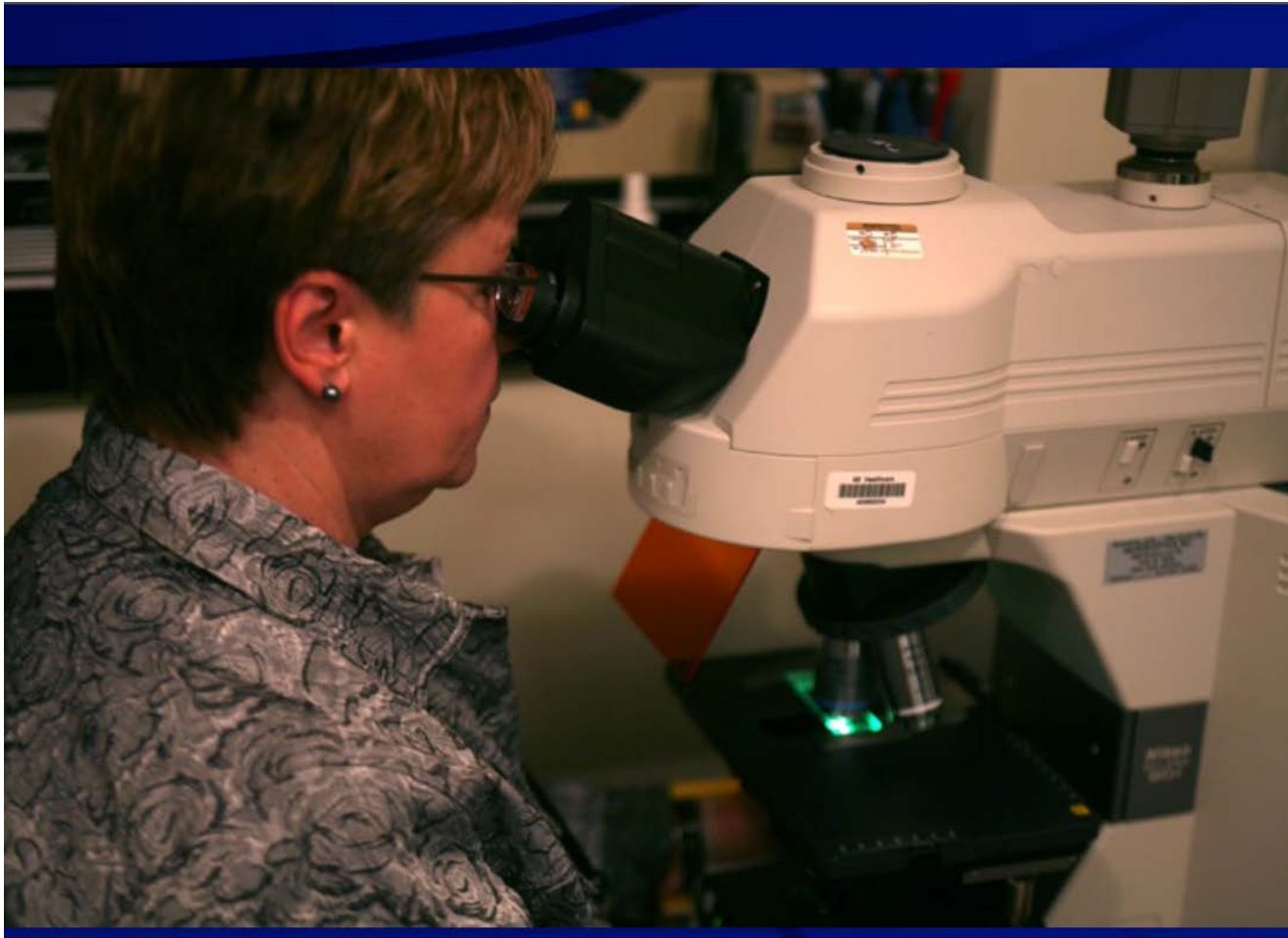


Hibridizasyon cihazı





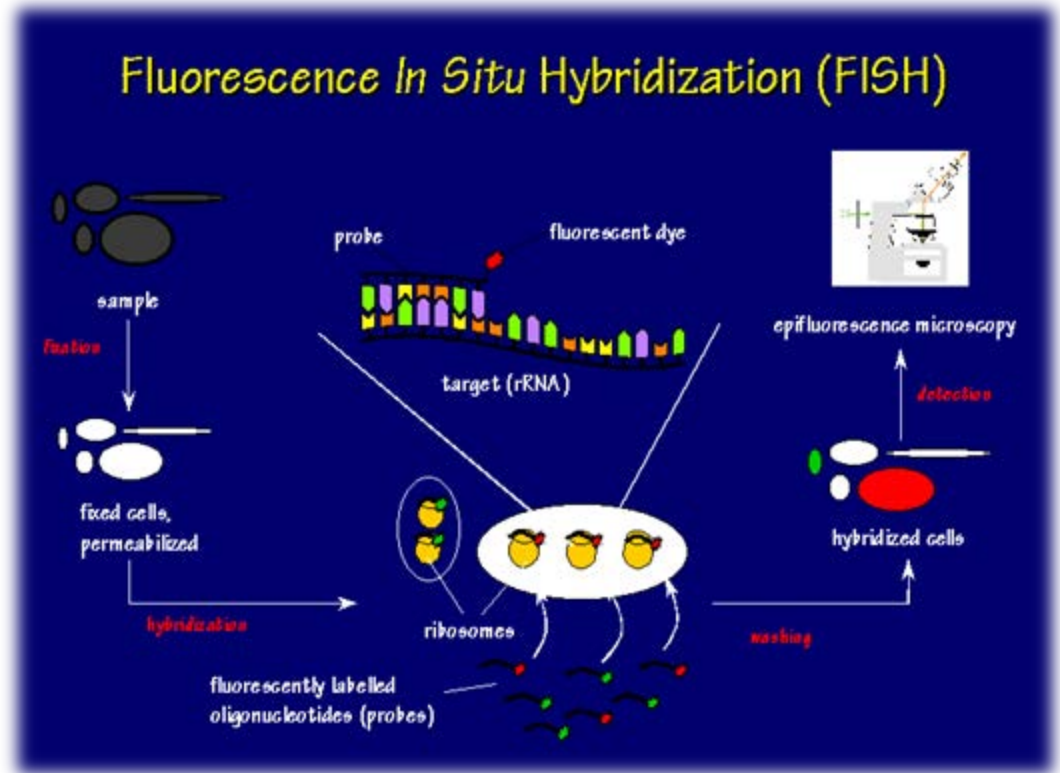
-Hedefe yönelik tasarlanmış floresan işaretli probun mikroskopik slaytın üzerindeki örnekte çalışılmasına imkan sağlar

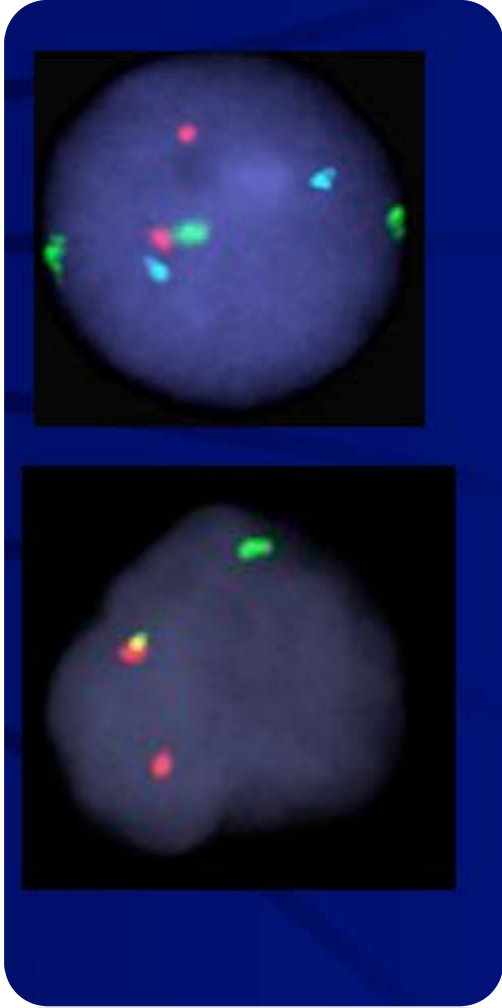


Prob

Prob bir nükleik asittir:

- Bir marker ile işaretlenmiştir.
- Hibridizasyonla hücredeki diğer complementer (tamamlayıcı) karşılığına bağlanır.

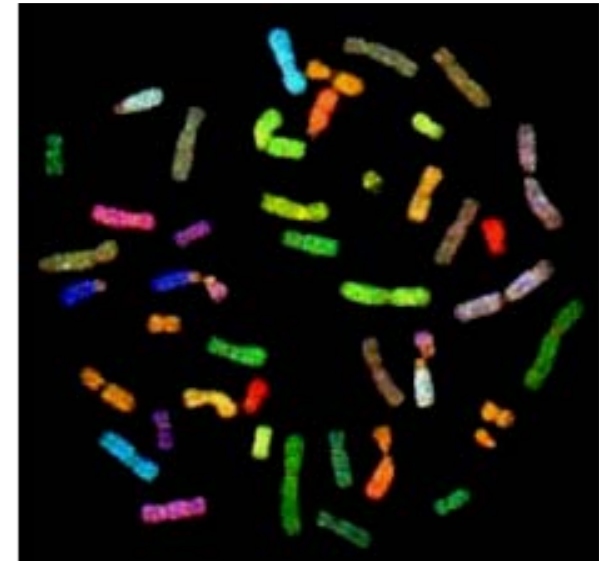
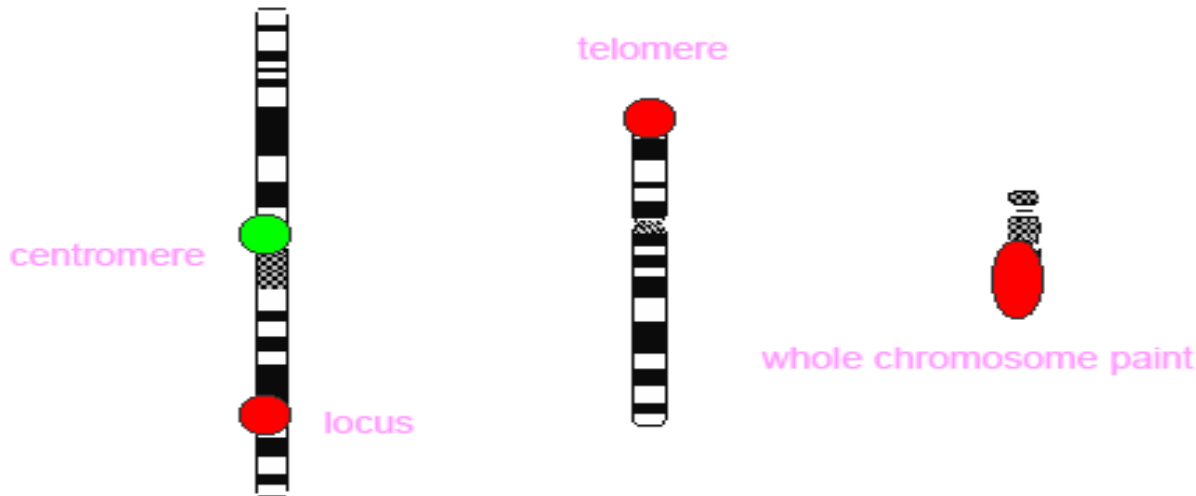




- Her prob spesifik hedefe bağlanır
- Bir işlemdeki farklı renkteki proplar farklı kromozom ve/veya farklı bölgeleri gösterir

FISH Prob Tipleri

- Sentromerik: Monozomi, trizomi, anöploidi
- Telomerik
- Tüm kromozom boyama
- Loküs spesifik: yapısal anomaliler (translokasyon, inversiyonlar, delesyonlar)

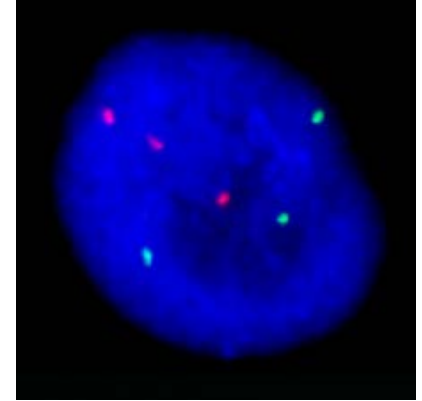
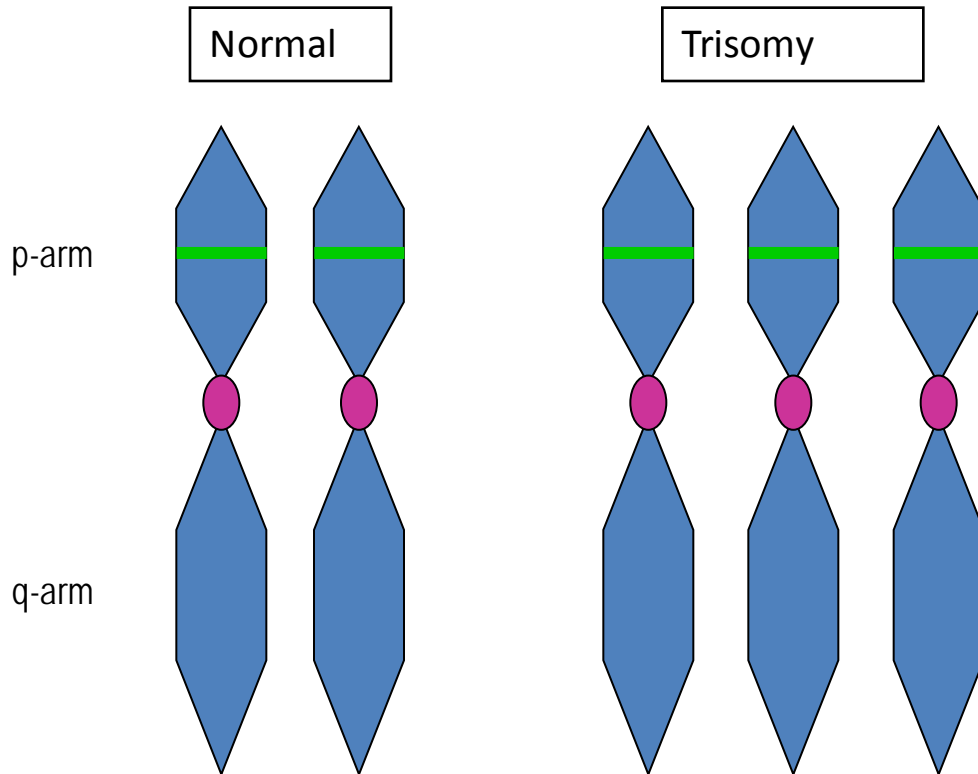


Hematolojik neoplazilerde kullanılan problemler

- **Grup 1:** sayım (enumeration) problemleri
 - CEP12 (sentromerik)
- **Grup 2:** çift renk, çift-füzyon problemleri (dual-color, dual-fusion) (loküs spesifik)
 - BCR/ABL1 [t(9;22)], PML/RARA [t(15;17)]
 - Translokasyon varyasyonları ve gizli translokasyonlar tespitini de sağlar
- **Grup 3:** tek-füzyon (single-fusion), ekstra sinyal) (loküs spesifik)
 - BCR/ABL1 ES
- **Grup 4:** Kırılma problemleri (Break-apart) (loküs spesifik)
 - MLL

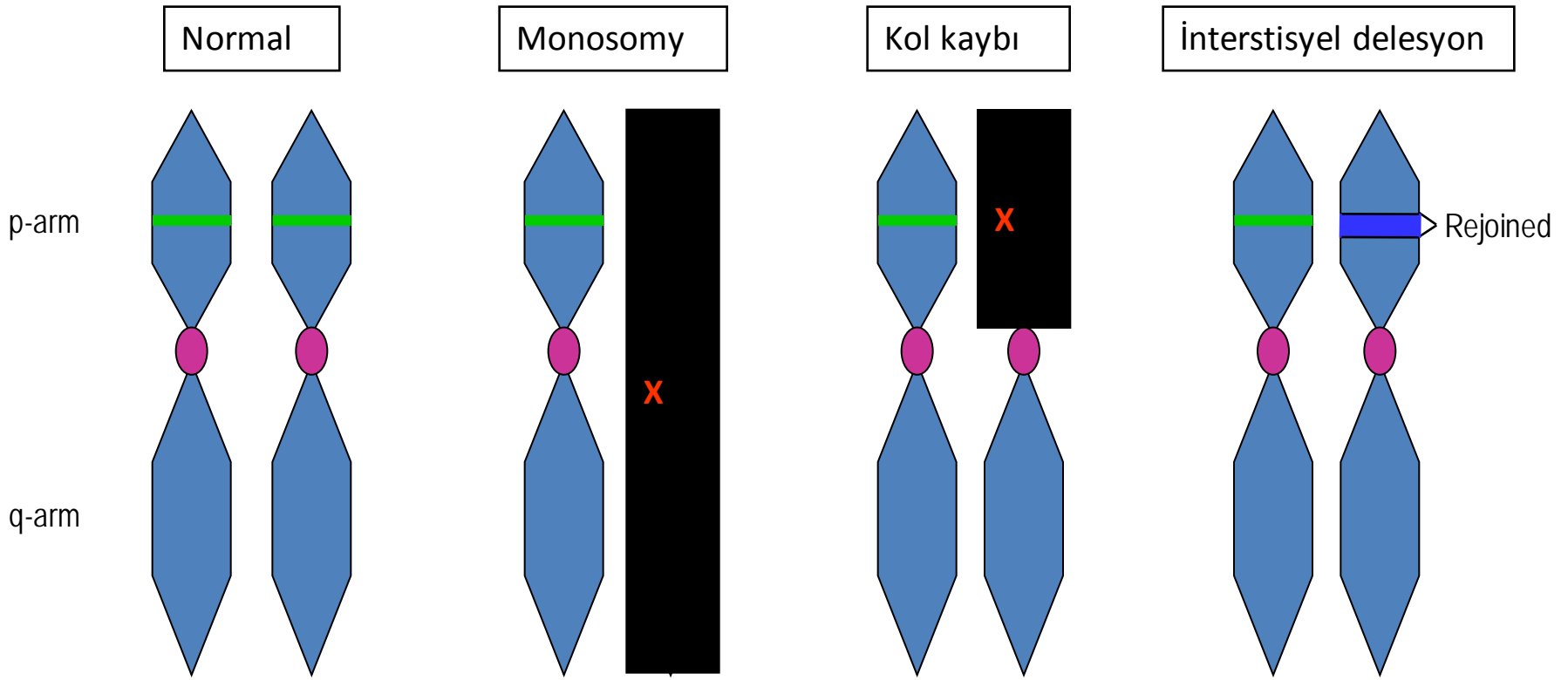
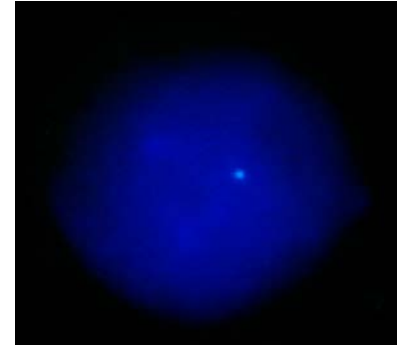
Kromozom anomalileri

Anöploidi örneği



Kromozom anomalileri

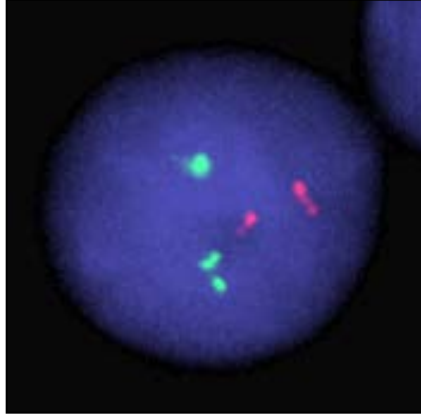
Kromozom kaybı ve delesyonlar



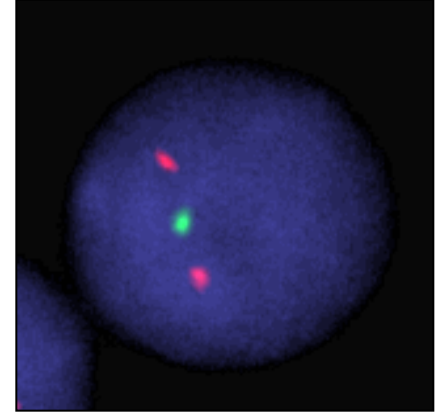
Kayıp

- KLL
 - ATM gen bölgesi kr11q22 (yeşil)
 - p53 gen bölgesi kr17p13 (kırmızı)

NORMAL
Her probtan
iki sinyal var



ANORMAL
iki p53 probu
fakat
ATM prob
kaybı

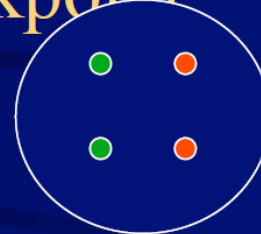


Translokasyonları ve inversiyonlarının tespiti için FISH prob dizaynı

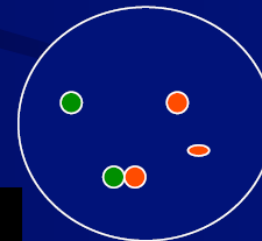
- Dual color, single füzyon (çift renkli tek füzyon) ve ekstra sinyal (ES)
- Dual color, Break-apart (çift renkli-ayrılma)
- Dual color, dual füzyon (çift renkli çift füzyon)

ES dizayn: probun biri kırılma noktasını kapsar;
diğeri yan taraftan bağlanır

ES Design: One Probe Flanks, Other Probe Spans Breakpoint

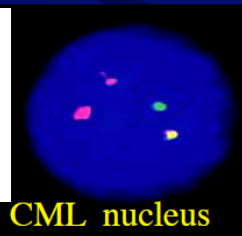


Normal 2O2G



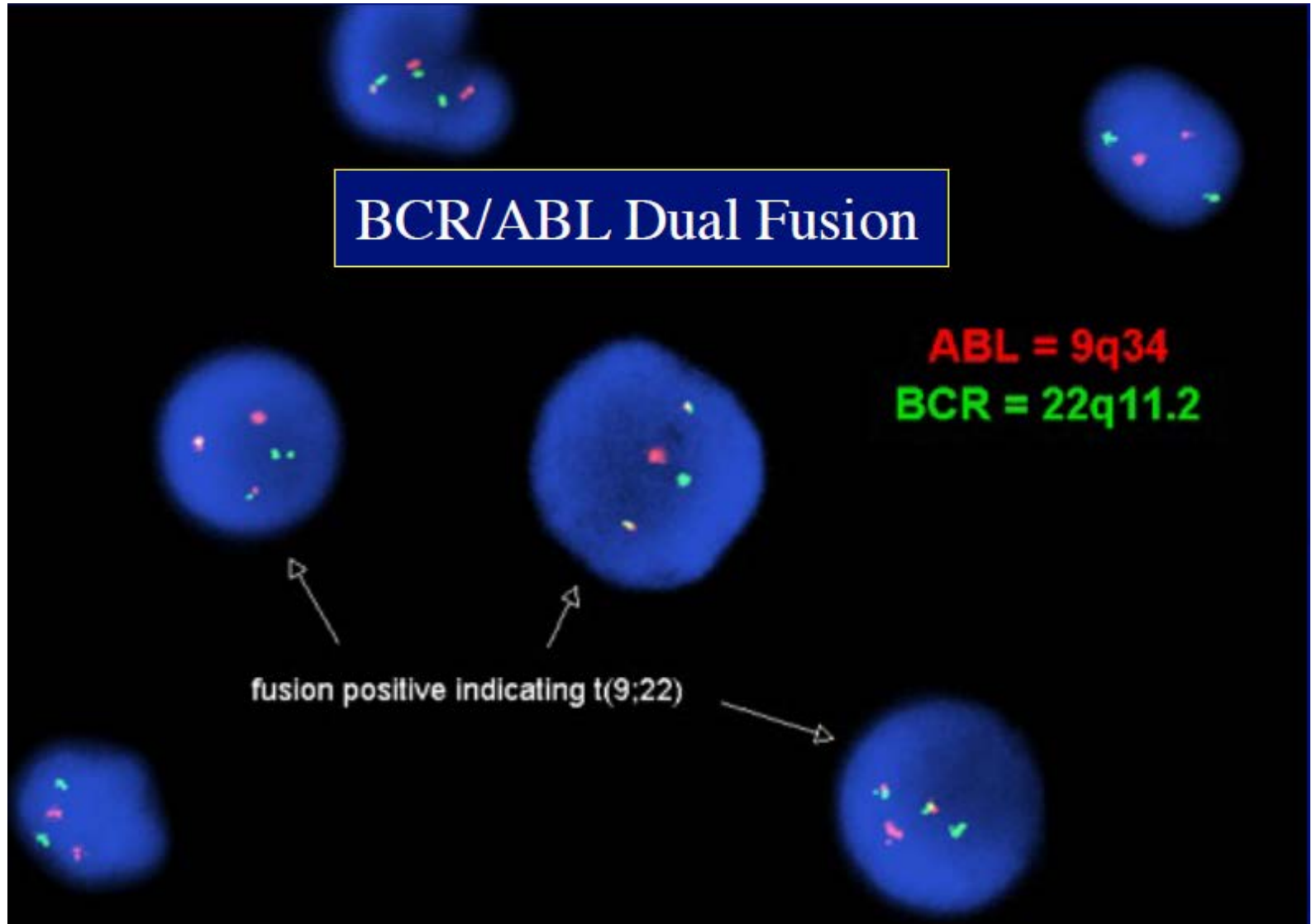
Abnormal
2O1G1F

•Cut-off %3

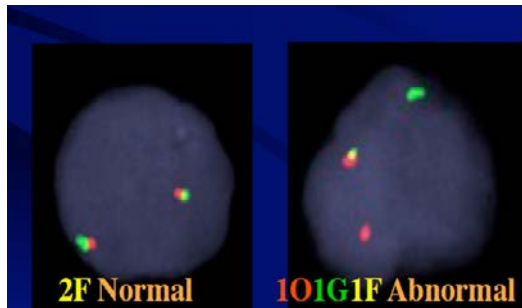


CML nucleus

BCR/ABL Dual color, Dual Fusion

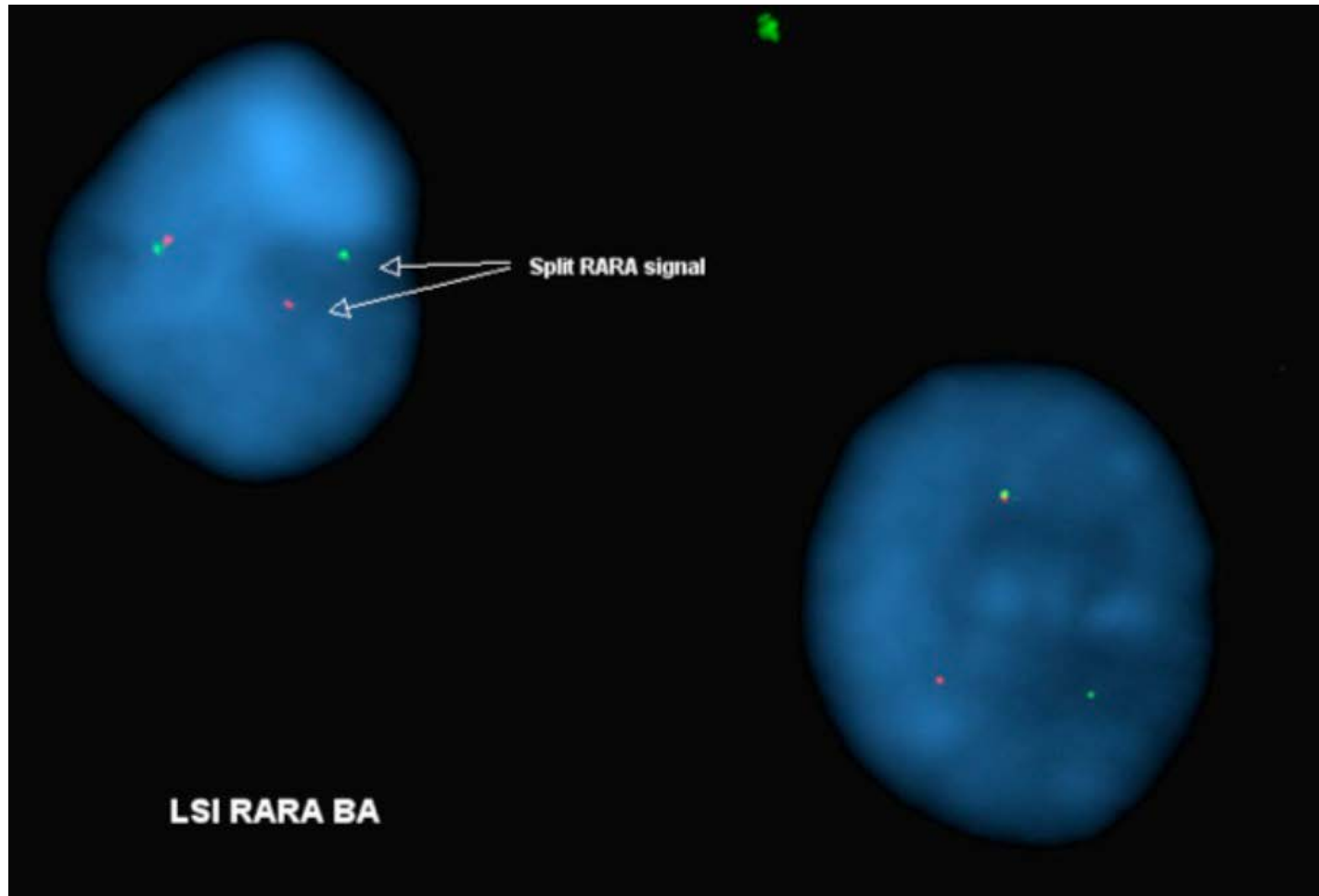


Dual color, Break-apart: her iki prob bir kırılma noktasını gösterir

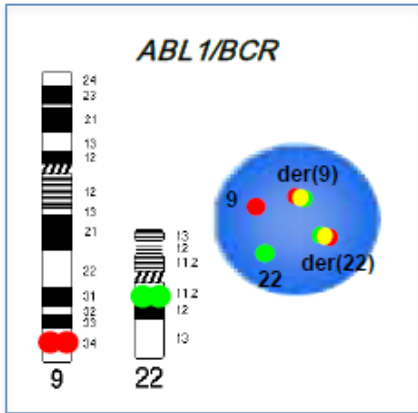


- Özellikleri:
 - Cutoff. %1-3
 - Multiple translokasyonlar için faydalıdır: IGH, MLL, BCL-6, ALK, MYC, BCR/ABL

Akut promyelositik lösemi PML/RARA t(15;17)

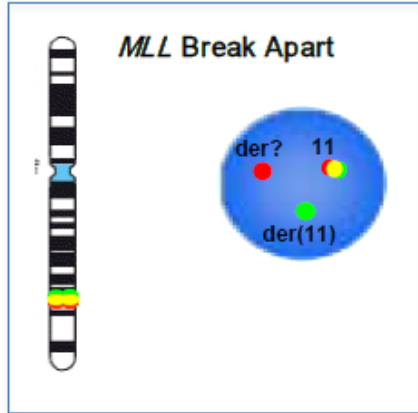


Translokasyon Problemleri



Füzyon probu:

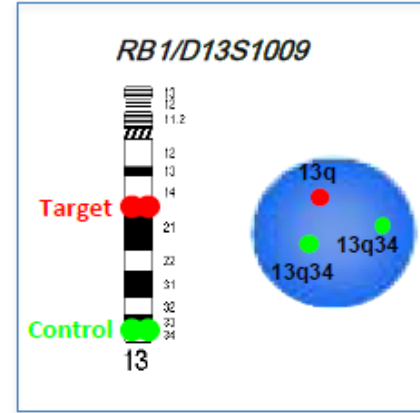
1 kırmızı, 1 yeşil ve 2 füzyon



Break apart probu:

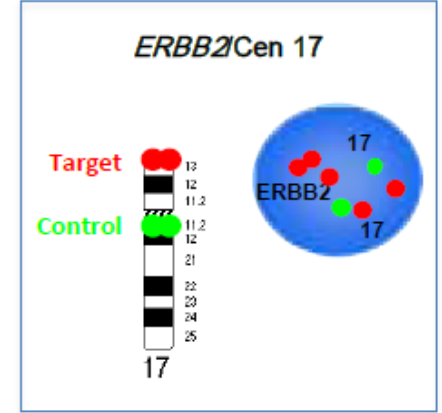
1 kırmızı, 1 yeşil ve 2 füzyon

Kopya sayı Problemleri



Delesyon

1 kırmızı, 2 yeşil



Amplifikasyon

2'den fazla kırmızı, 2 yeşil

Translokasyonlar için kullanılan FISH Problar

Prob Dizaynı	Normal için üst sınır
Basit single füzyon	3-5%
ES füzyon	3%
Break apart	1-3%
Dual füzyon	<1%

Cutoff oranını tespit etmenin bir yolu örneğin 20 sağlıklı BCR/ABL negatif bireyin herbirinden dual color dual füzyon prob ile 500'er hücre sayarak istatistiksel olarak tespit etmektir

Minimal rezidüel hastalık tespiti

metod	sensitivite
Işık mikroskopi	10^{-1} - 10^{-2}
Multiparametre flowsitometri	10^{-2} - 10^{-5}
Klasik sitogenetik	10^{-1} - 10^{-2}
FISH	10^{-1} - 10^{-4}

FISH sesitivitesini etkileyenler

- Spesmen tipi
- Sayılan nükleus sayısı
- Prob dizaynı

FISH'in avantajları ve sınırları

Avantajlar

- Dokuların çeşitliliği
- Metafaz&interfaz
- Spesmen yapıları intakt kalır
- Hücre hücre analiz
- Sitogenetik olarak gizli olan anomalileri tespit eder
- Bir önceki testten kontaminasyon diye bir şey olmaz

Dezavantajlar

- Çok küçük genetik lezyonları saptayamaz
- Real-time PCR kadar hızlı değildir
- Bazı moleküler tetkikler gibi henüz otomatize değildir



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