EMATOLOGIA DI LABORATORIO: percorsi diagnostici e obiettivi clinici.
Milano, 11-12 Novembre 2010
Aula Magna, Fondazione IRCCS Istituto Nazionale dei Tumori

Citogenetica e genetica molecolare in oncoematologia

Francesco Cavazzini (Ferrara) 18:00-18:30

ARGOMENTI

Ruolo di: Citogenetica

Citogenetica-Molecolare (ibridazione in situ fluorescente, FISH)

diagnosi prognosi monitoraggio della malattia minima

Citogenetica e Citogenetica-Molecolare come elementi di diagnosi

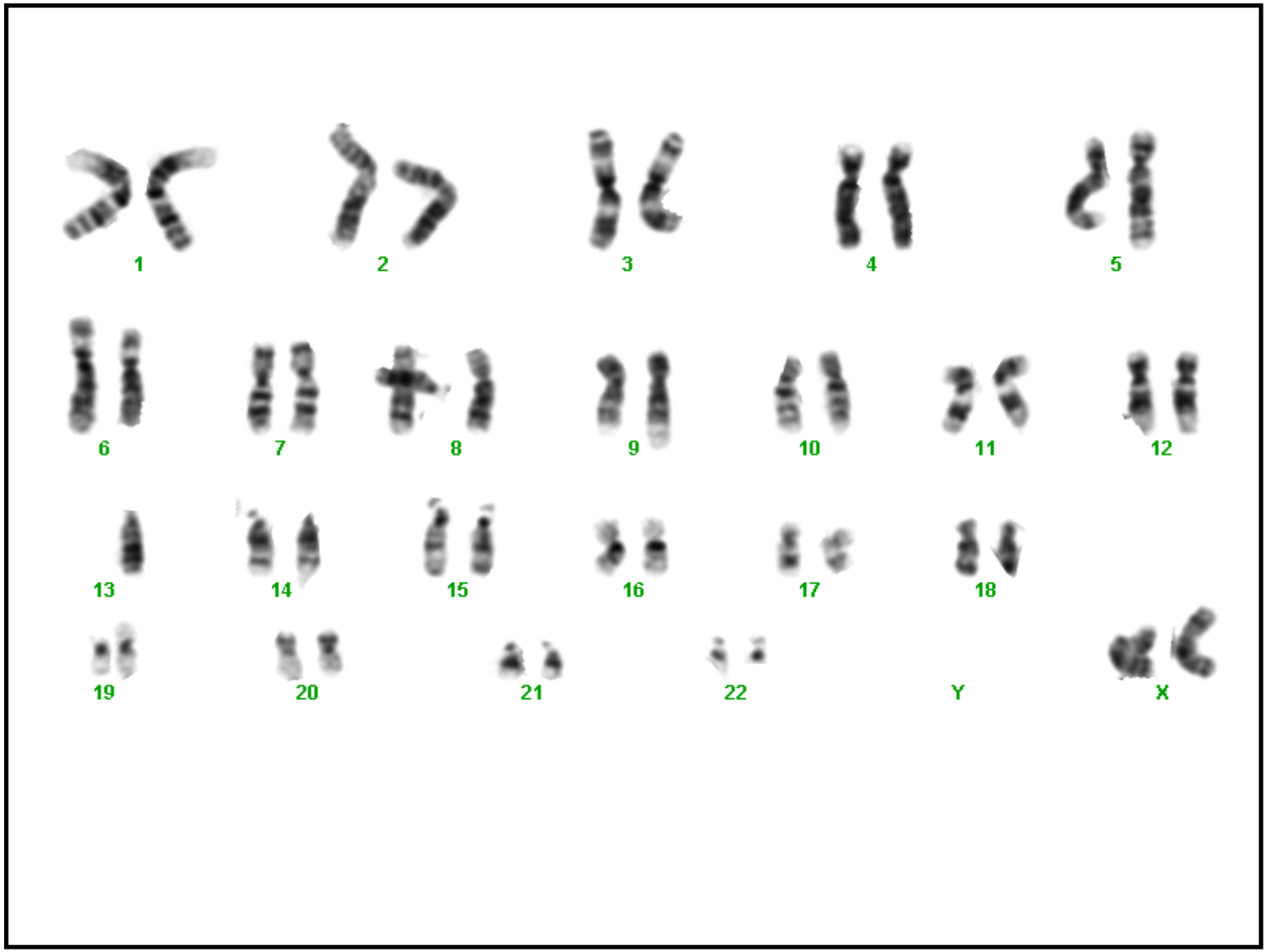
Citogenetica e Citogenetica-Molecolare come componenti integrativi di sistemi diagnostico-prognostici

Indagine citogenetica in ematologia

- Indagine di laboratorio che rileva la presenza di anomalie cromosomiche nel cariotipo delle cellule patologiche
- Riconosce le anomalie “primarie” (presenti in tutte le cellule anomale), responsabili delle fasi precoci di trasformazione
- Identifica le anomalie “ secondarie” responsabili delle fasi di evoluzione clonale
- Deve identificare le lesioni non rilevanti per la patogenesi della malattia in quanto semplice espressione della instabilità genetica

Cosa serve per l'indagine citogenetica in ematologia

- 5-20 X 10^6 cellule vive, messe in coltura sterilmente entro 24 ore dal prelievo
- Le cellule devono essere in grado di dividersi (midollo osseo, sangue periferico se contaminato da cellule immature, linfonodi, milza, biopsie tissutali patologiche)
- Passaggi tecnici relativamente semplici (blocco delle metafasi, ipotonizzazione, allestimento dei vetrini, bandeggio)
- Sistema di analisi automatizzata del cariotipo (economicamente vantaggioso per centri medio-grandi)



Ciccone M, Cavazzini F, Castoldi G.
Cytogenetics and Molecular cytogenetics in diagnosis and prognosis of hematologic malignancies. Trends in Medicine, Jan 2006, Vol 6, N 1

FISH: Fluorescence In Situ Hybridization

Metodica di citogenetica molecolare

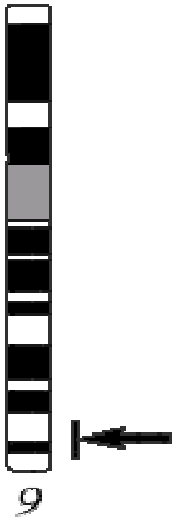
Indaga aspetti dell'assetto genomico della cellula

Utilizza sonde di acido nucleico marcate con fluorocromi diversi

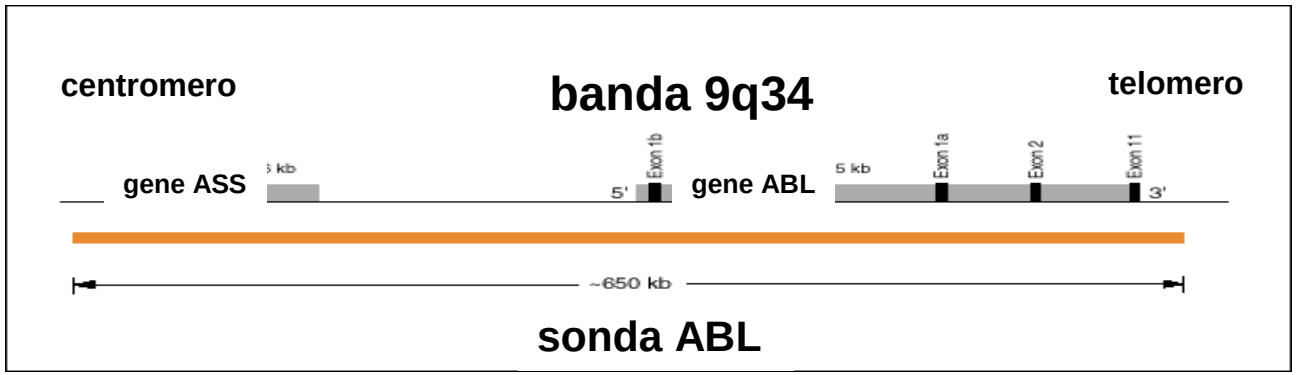
Il principio di base è l'ibridazione (hybridization, annealing) di un filamento di DNA denominato "sonda" (probe) con sequenze omologhe presenti nel genoma in studio

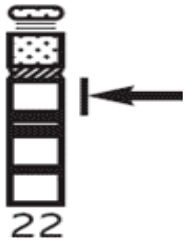
La FISH è una metodica che prevede un "target" di studio

t(9;22)(q34;q11) SISTEMA COMBINATO SEGREGAZIONE-COLOCALIZZAZIONE, Dual Color Dual Fusion

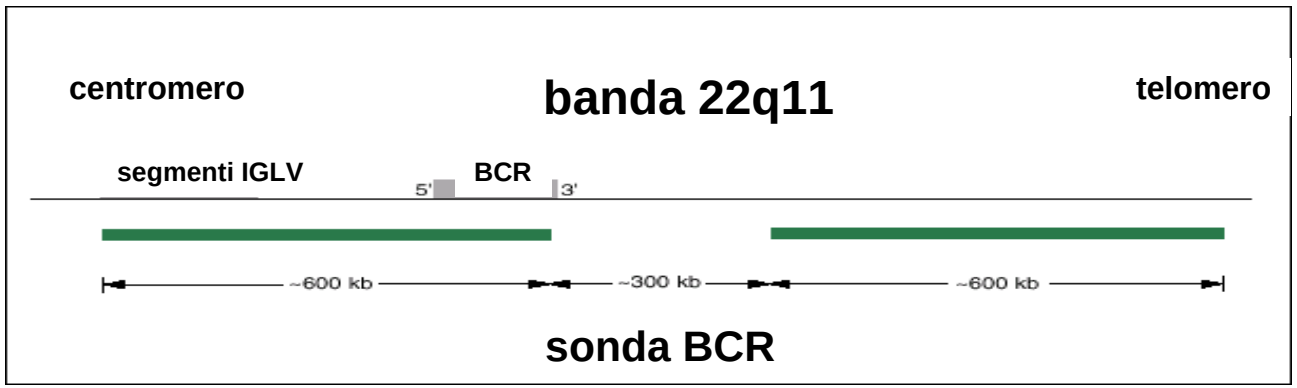


9q34 ABL

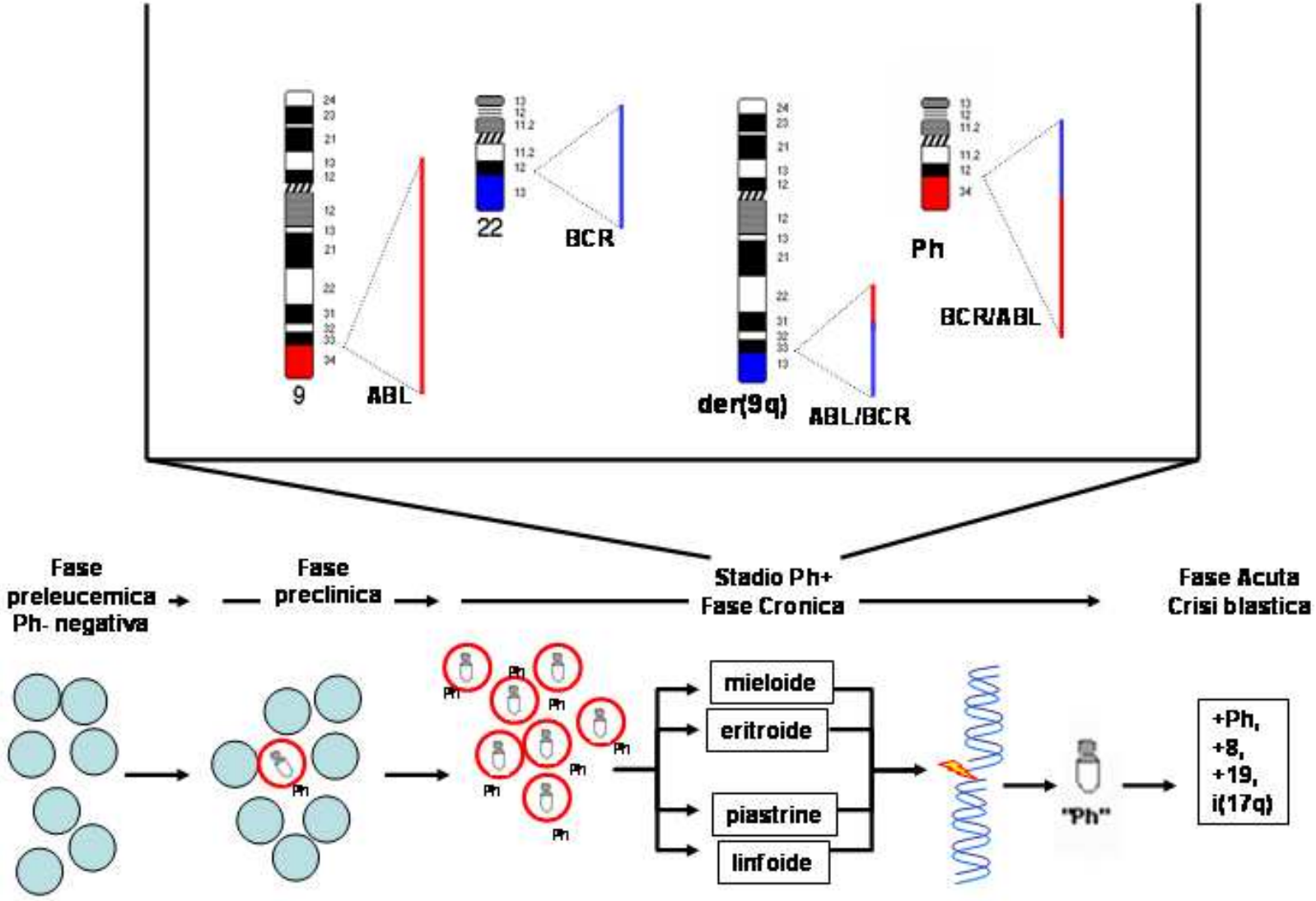




22q11 BCR

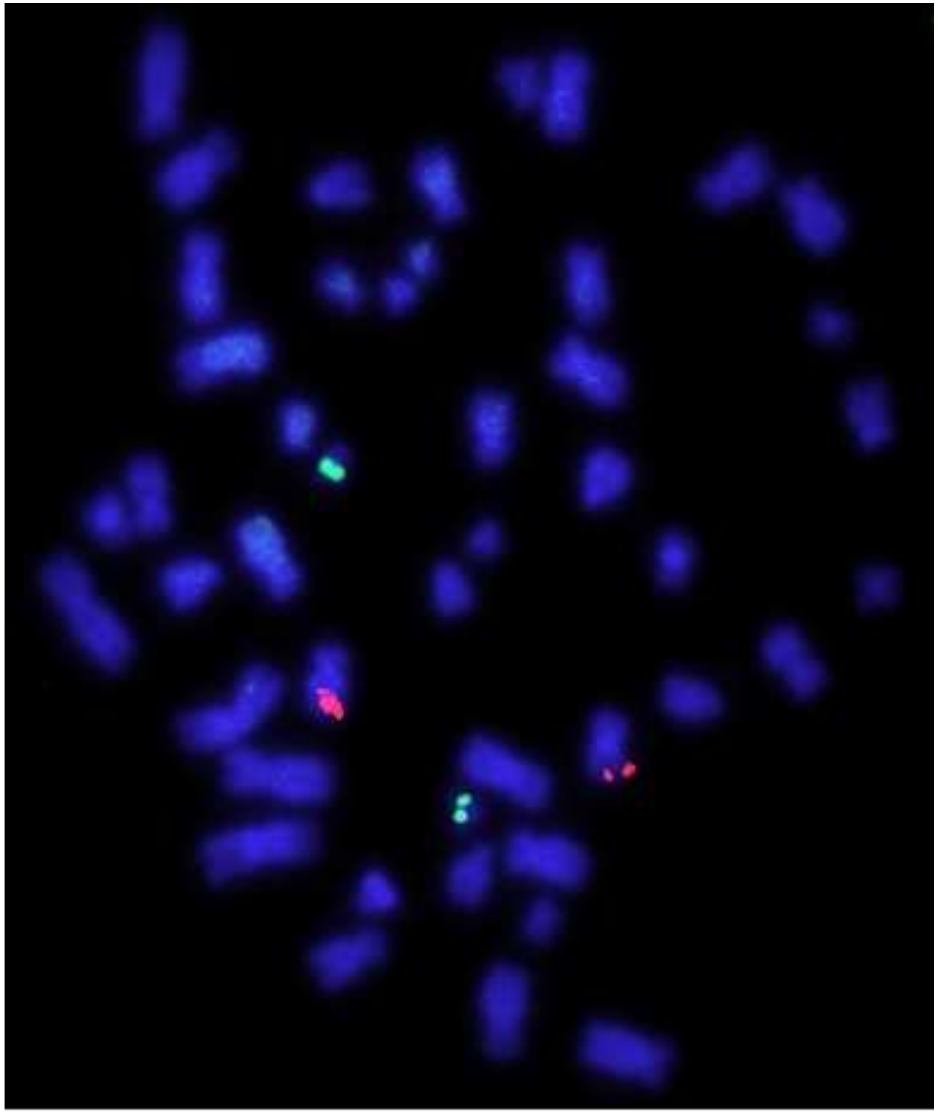


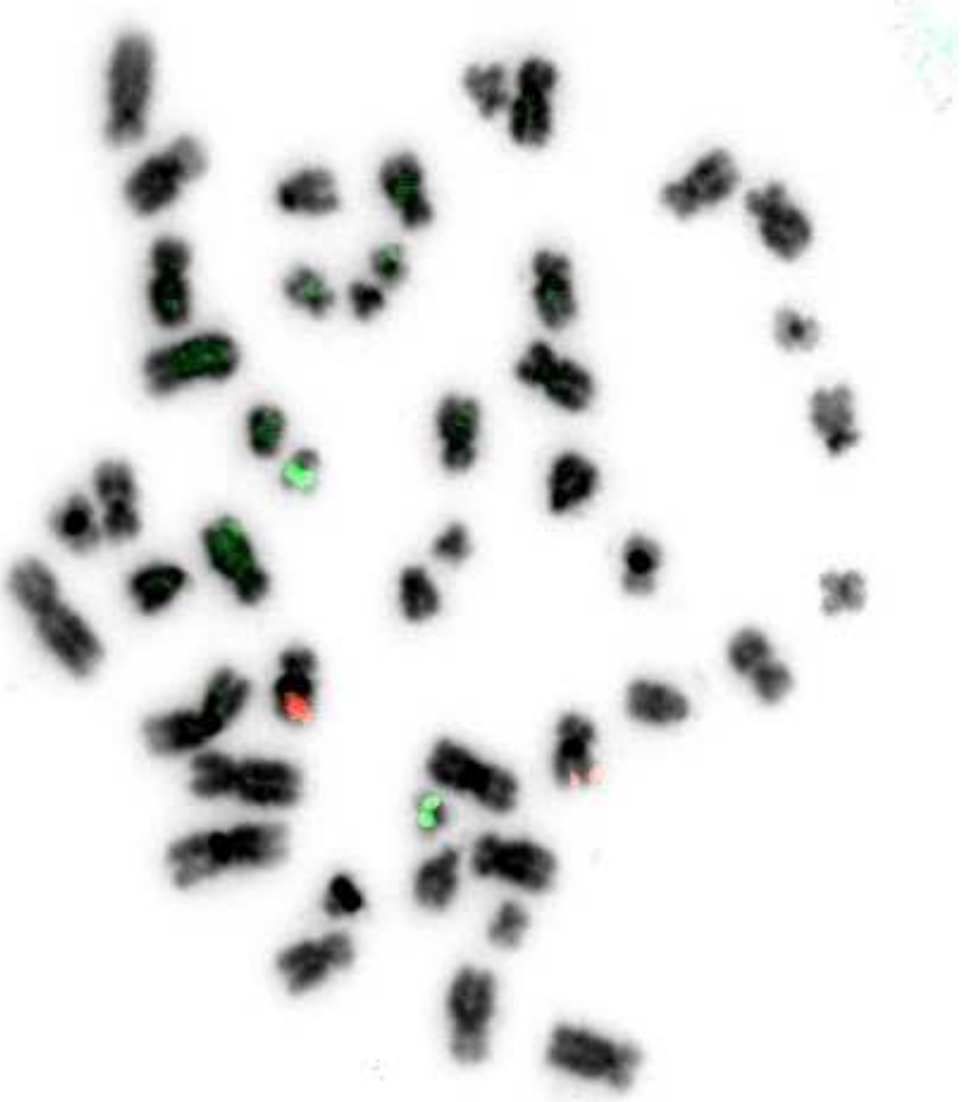
LA LEUCEMIA MIELOIDE CRONICA COME MODELLO DI LESIONE DIAGNOSTICA



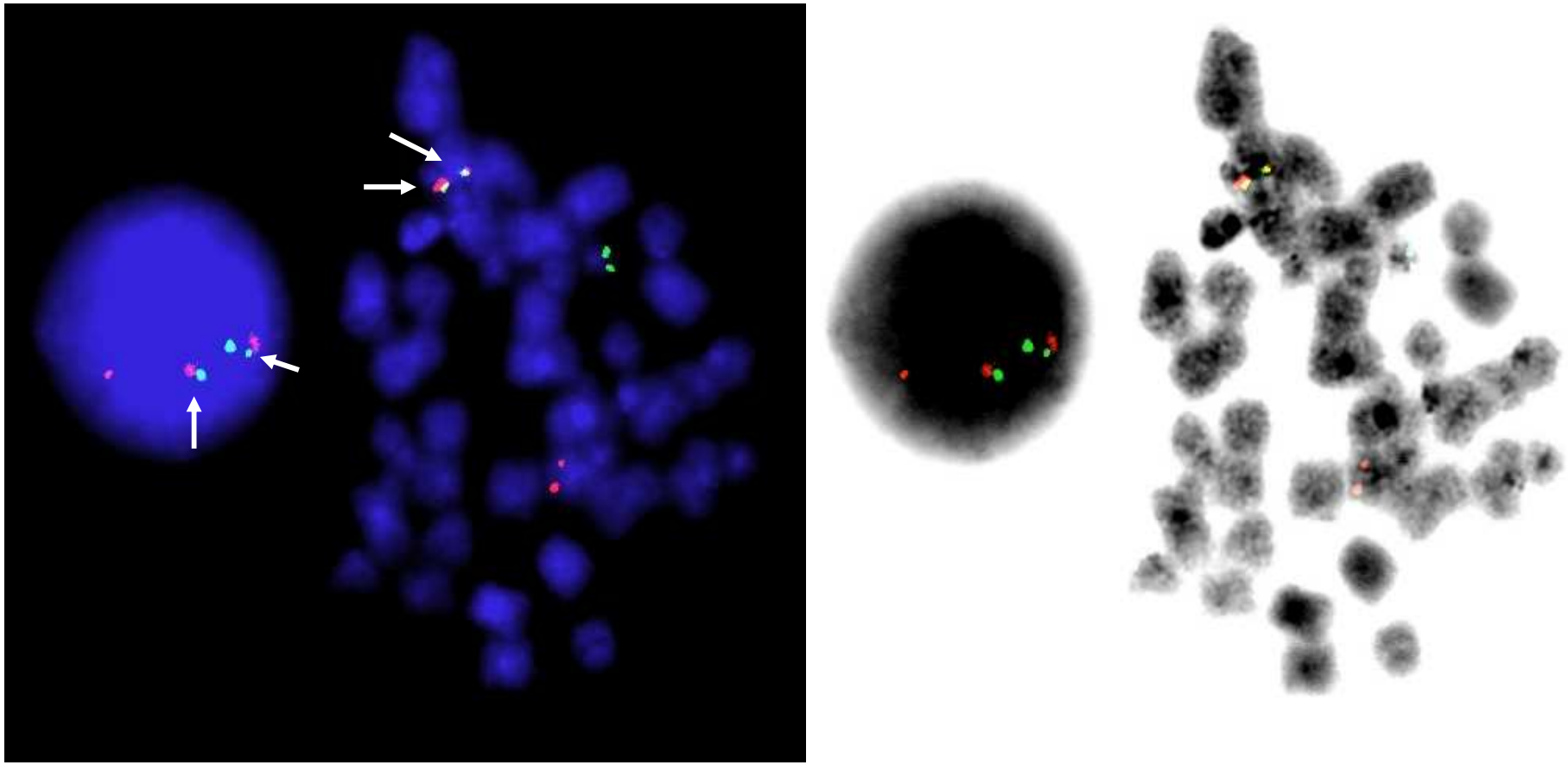
Ciccone M, Cavazzini F, Castoldi G.
Cytogenetics and Molecular cytogenetics in diagnosis and prognosis of hematologic malignancies. Trends in Medicine, Jan 2006, Vol 6, N 1

Sonda BCR/ABL DCDF – metafase normale

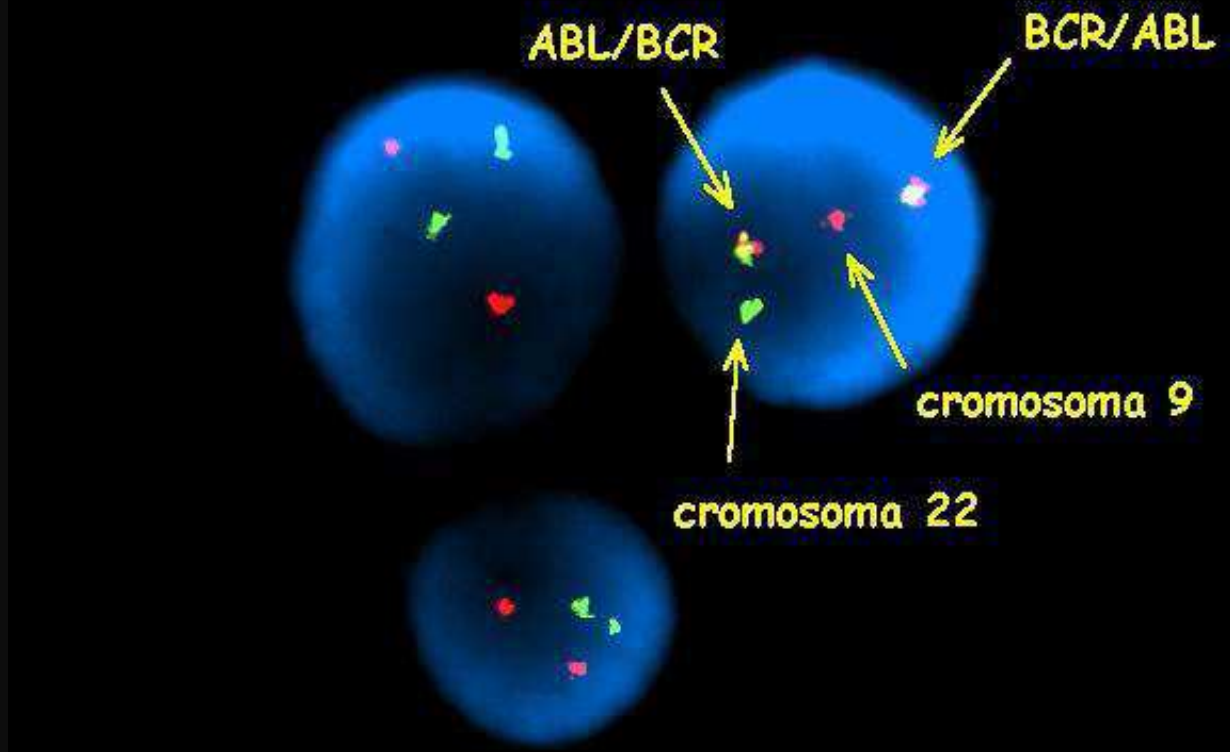




Sonda BCR/ABL DCDF – metafase Ph+

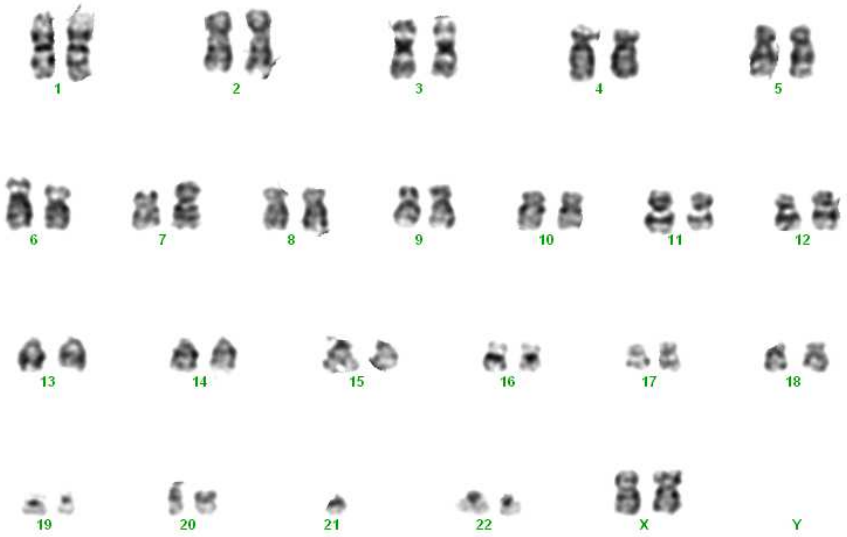


Fusione BCR/ABL nella Leucemia Mieloide Cronica



VANTAGGI INTERPRETATIVI DELLA FISH

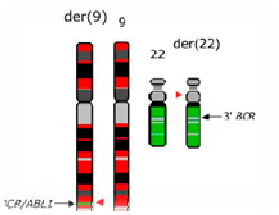
Inserzione di BCR su ABL: CASO 1

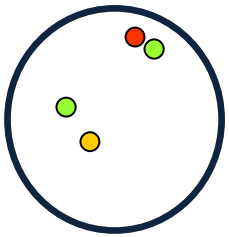


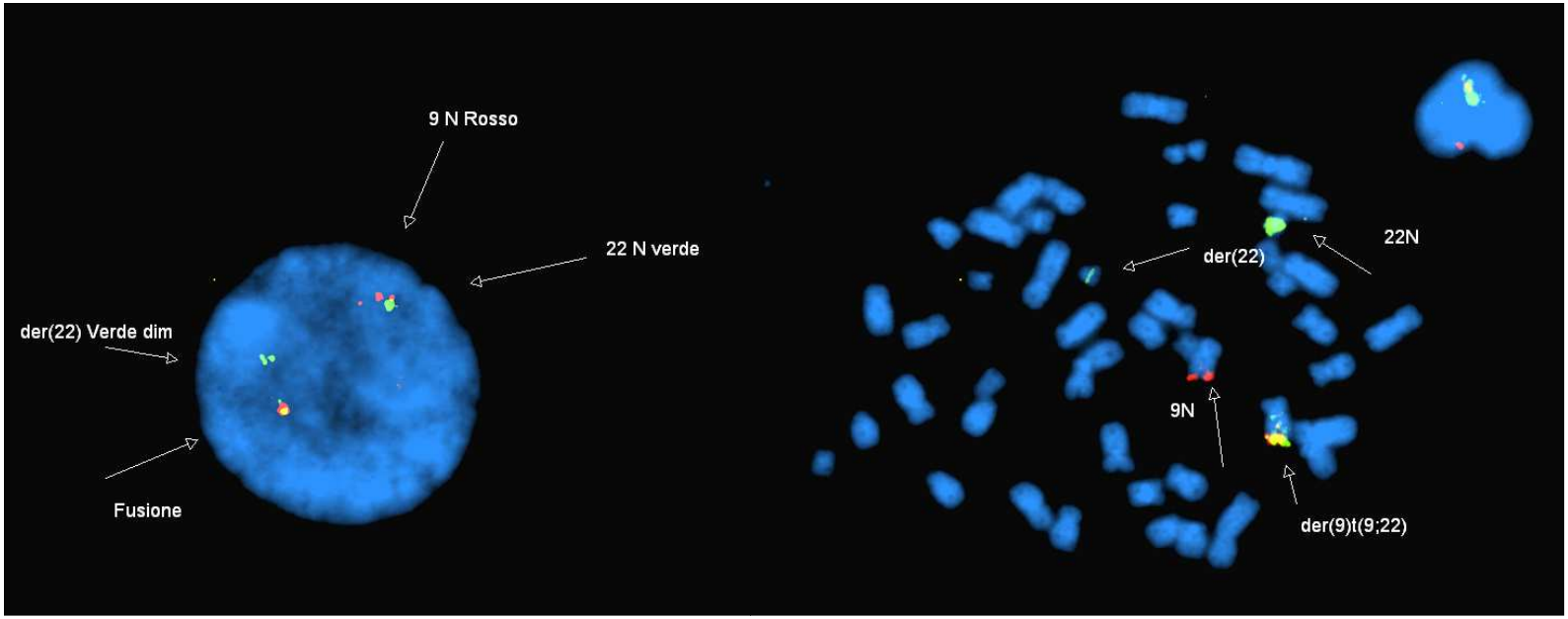
L'analisi del cariotipo non evidenzia t(9;22)

ma

All'analisi I-FISH il pattern trovando riscontro nel **76% 1F2V1R** ➔ conferma nel pattern di 15/15 metafasi che rivela **1R** sul **9N**, **1V** sul **22N** e **1V** sul **22q**. Un **segnale di fusione** sul **9q**. Non esiste evidenza di **fusione** sul **der(22)**, ma il pattern è compatibile con **l'inserzione di BCR su cromosoma 9q-.**

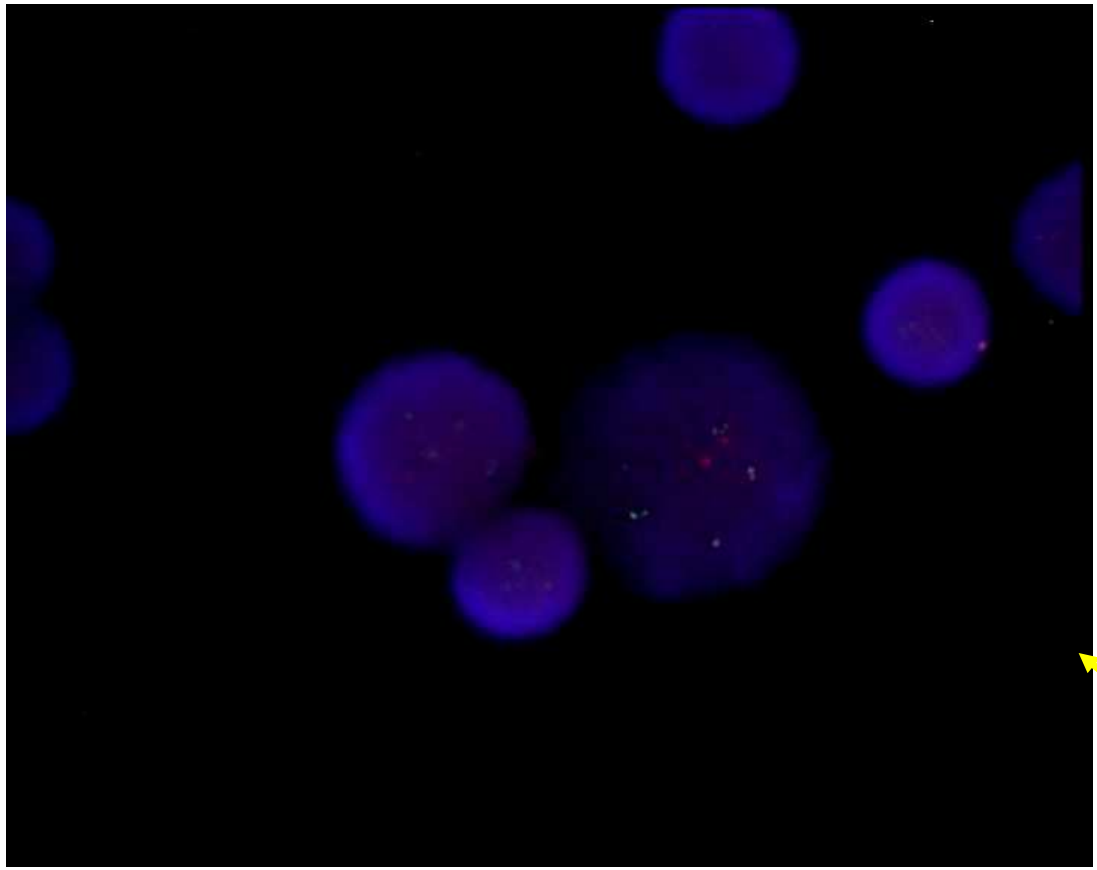




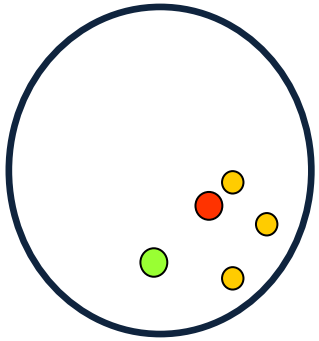


VANTAGGI INTERPRETATIVI DELLA FISH

Doppio Cromosoma Philadelphia: CASO 2

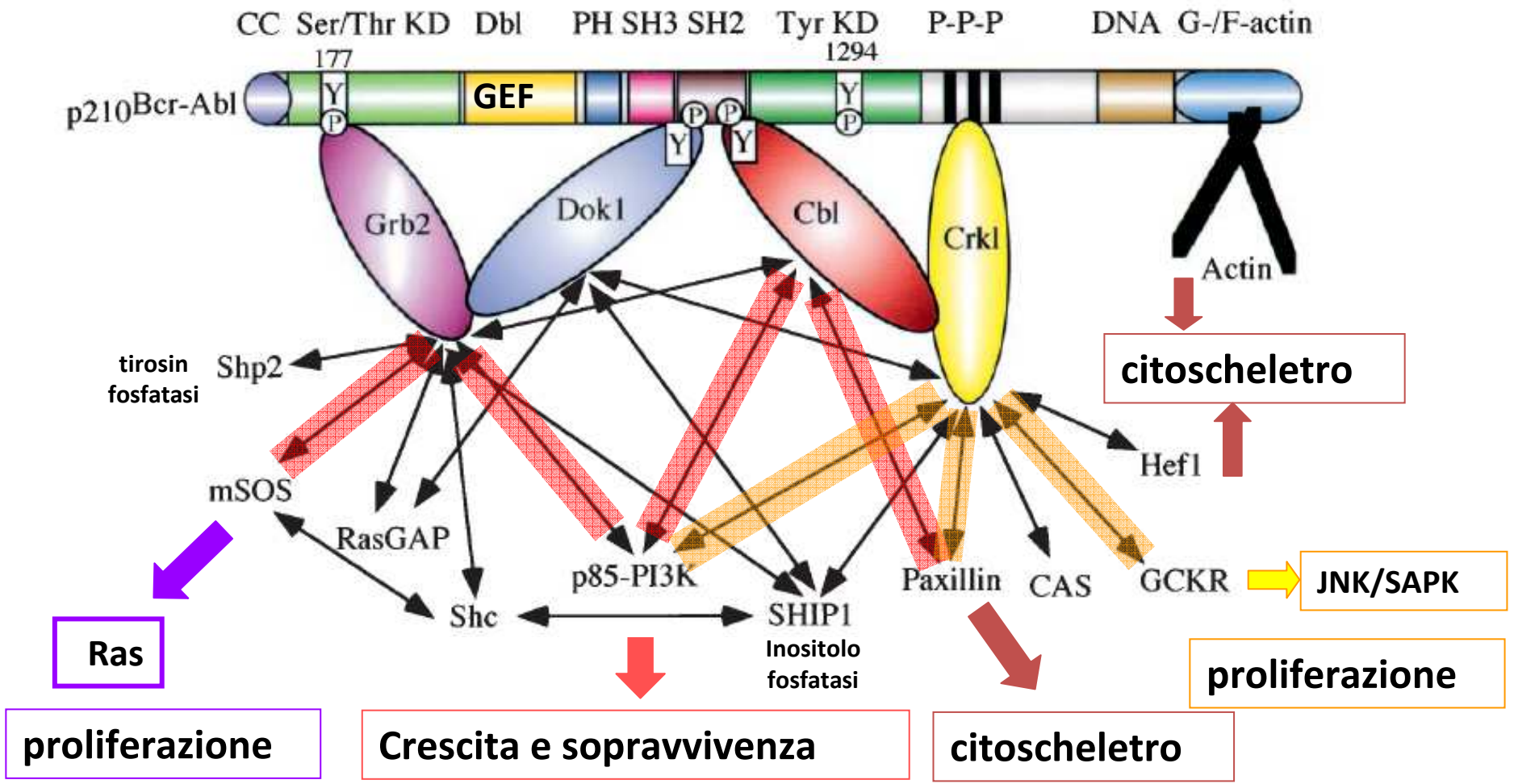


Co-esistenza del clone neoplastico convenzionale (Ph+) e di un assetto di **3 segnali gialli di fusione**, **1 segnale verde** e **1 rosso**, ad evidenza della presenza di un **clone con Ph''**



3F1V1R

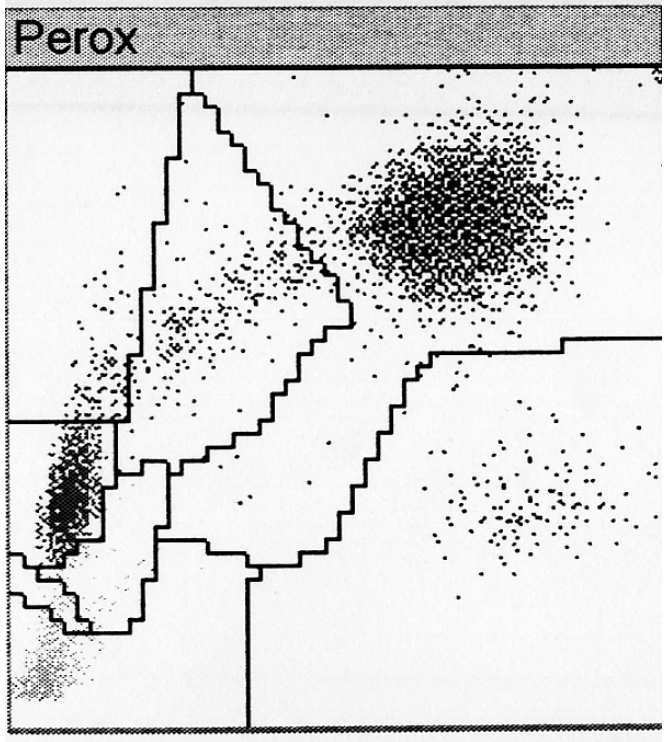
Oncogenesi *BCR/ABL*-mediata: interazioni molecolari



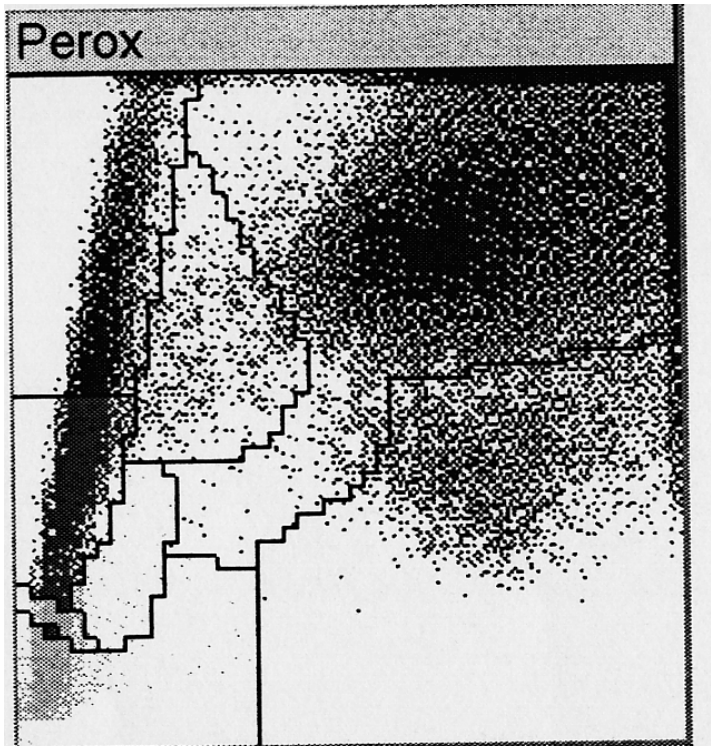
Schejien B, Oncogene 2002

Sospetto Diagnostico
Il Laboratorio

WBC				7.40		x10³/µL
Neutro		54.4		4.03		x10³/µL
Linfo		34.0		2.52		x10³/µL
Mono		4.8		0.35		x10³/µL
Eos		2.6		0.19		x10³/µL
Baso	H	2.2		0.16		x10³/µL
LUC		2.0		0.15		x10³/µL

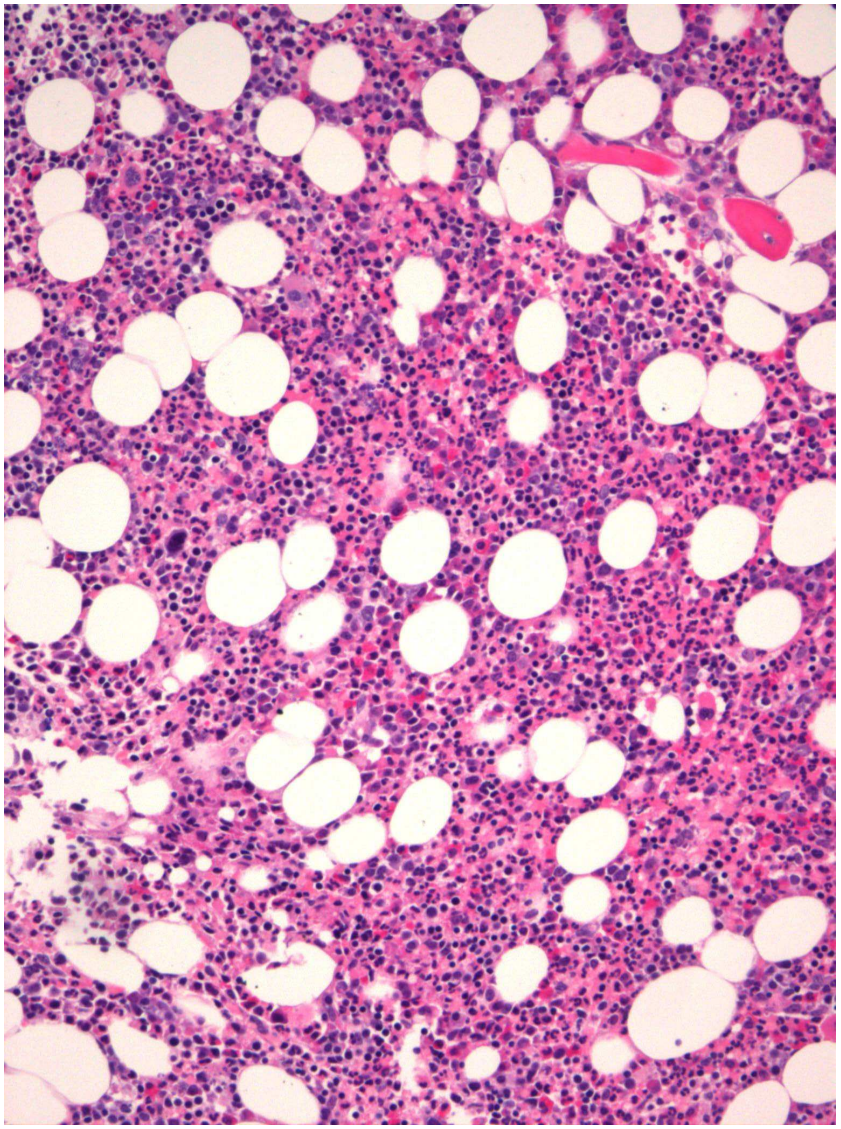


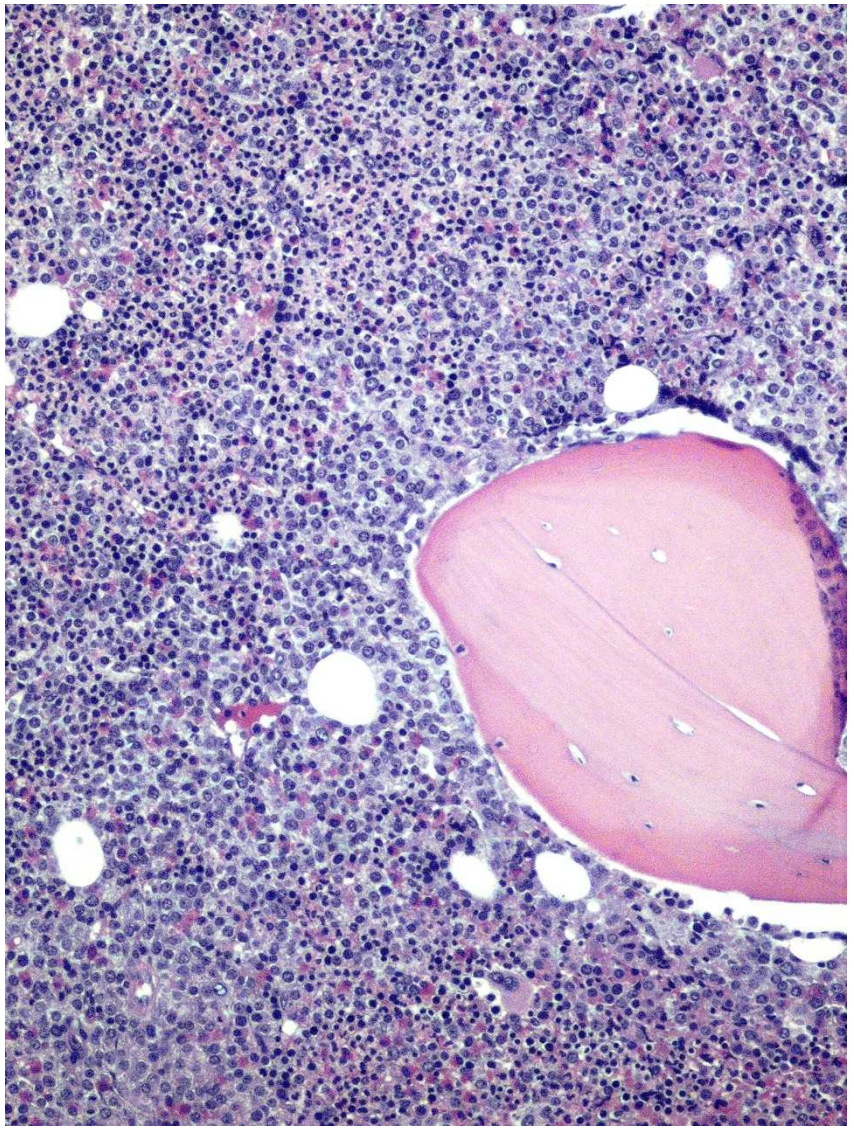
WBC				H	259.19	*	x10³/
Neutro		64.8	*	H	168.05	*	x10³/
Linfo		19.3	*	H	49.95	*	x10³/
Mono	L	1.0	*	H	2.48	*	x10³/
Eos		4.7	*	H	12.10	*	x10³/
Baso	H	13.6	*	H	35.19		x10³/
LUC	H	10.3	*	H	26.60	*	x10³/



FISIOPATOLOGIA DI ABL

La Leucemia Mieloide Cronica





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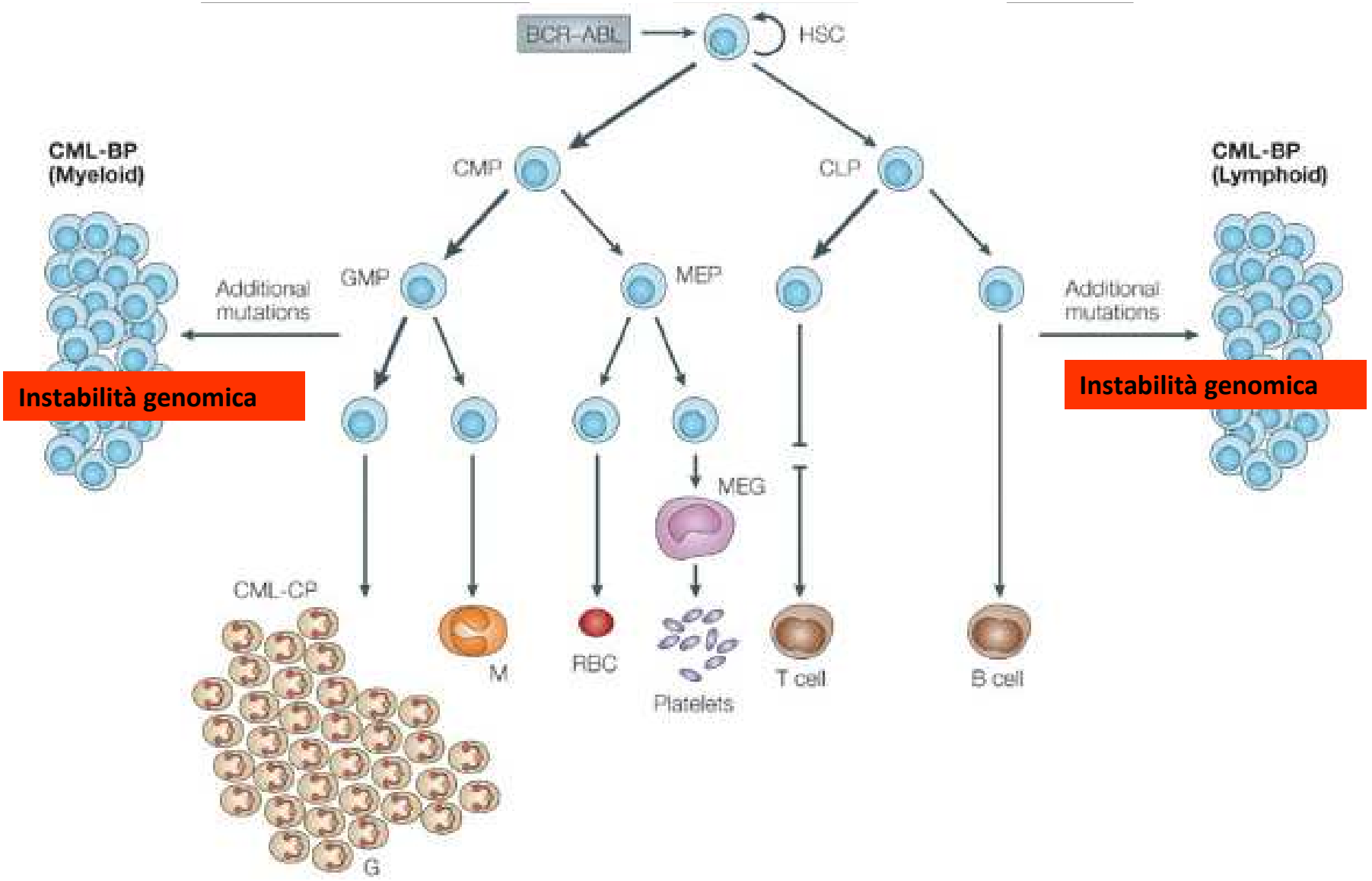
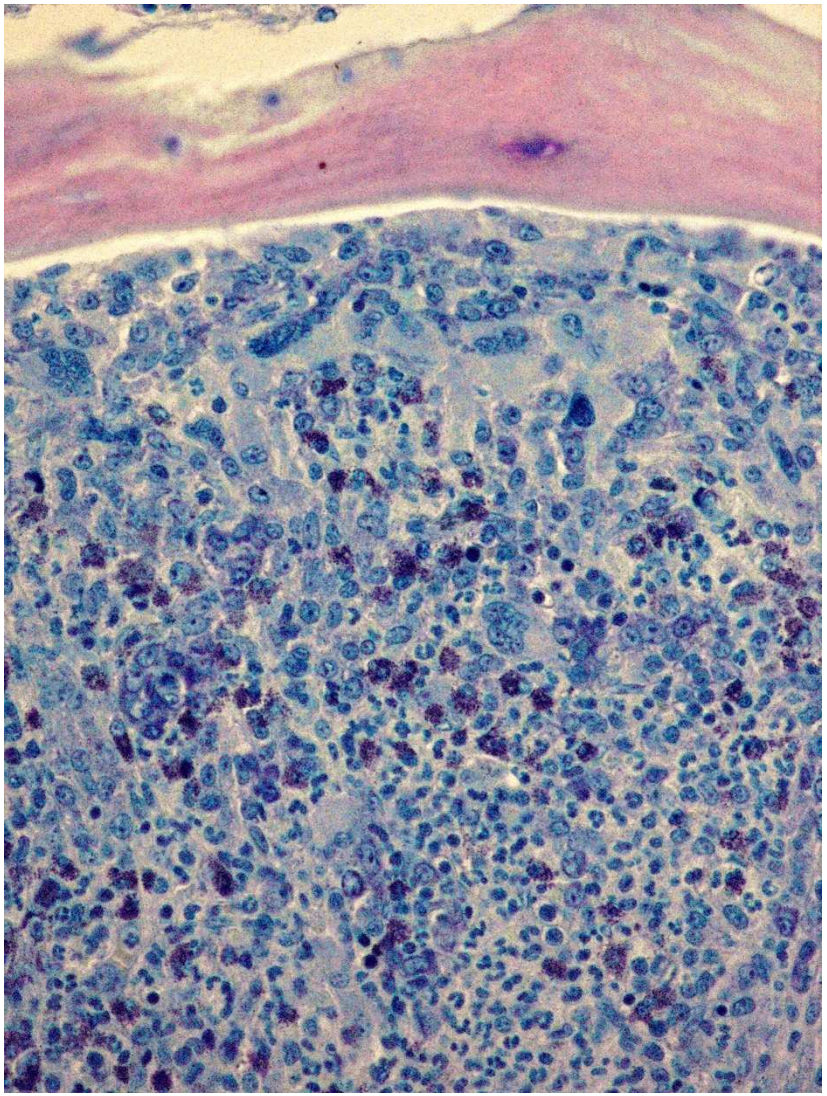
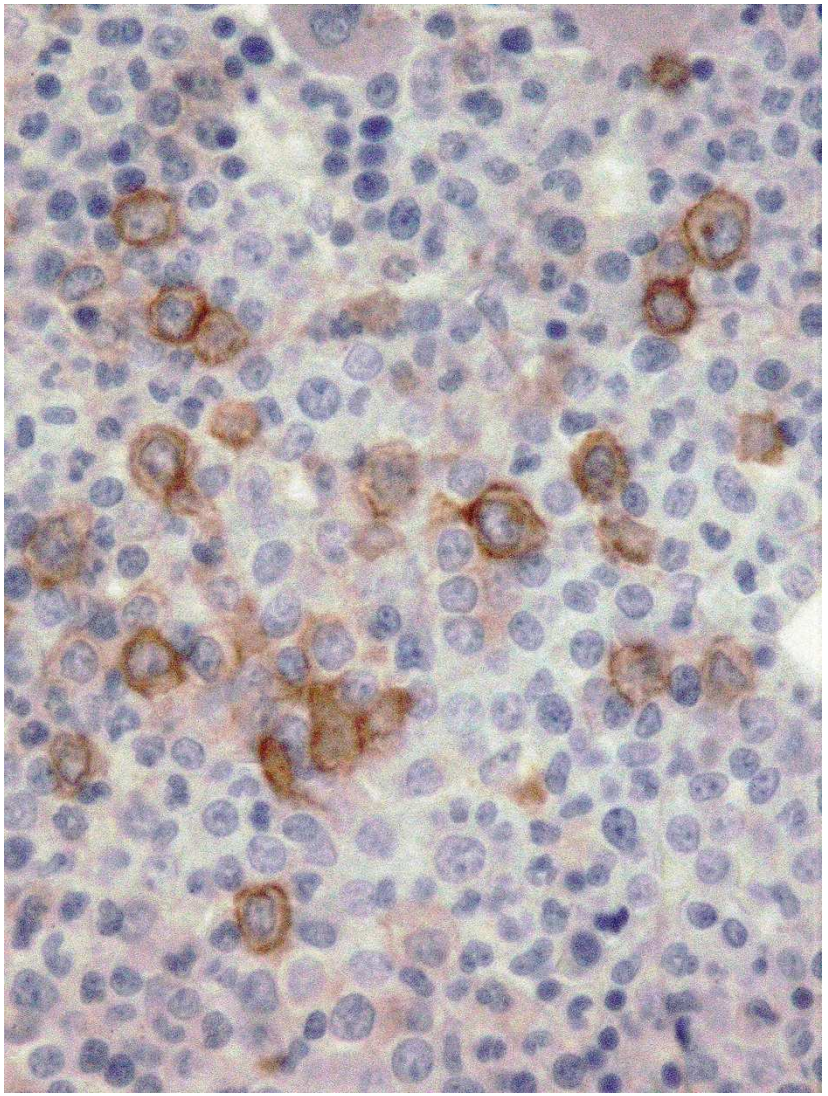
MECCANISMI DI PROGRESSIONE

STORIA NATURALE DELLA LMC



MECCANISMI DI PROGRESSIONE





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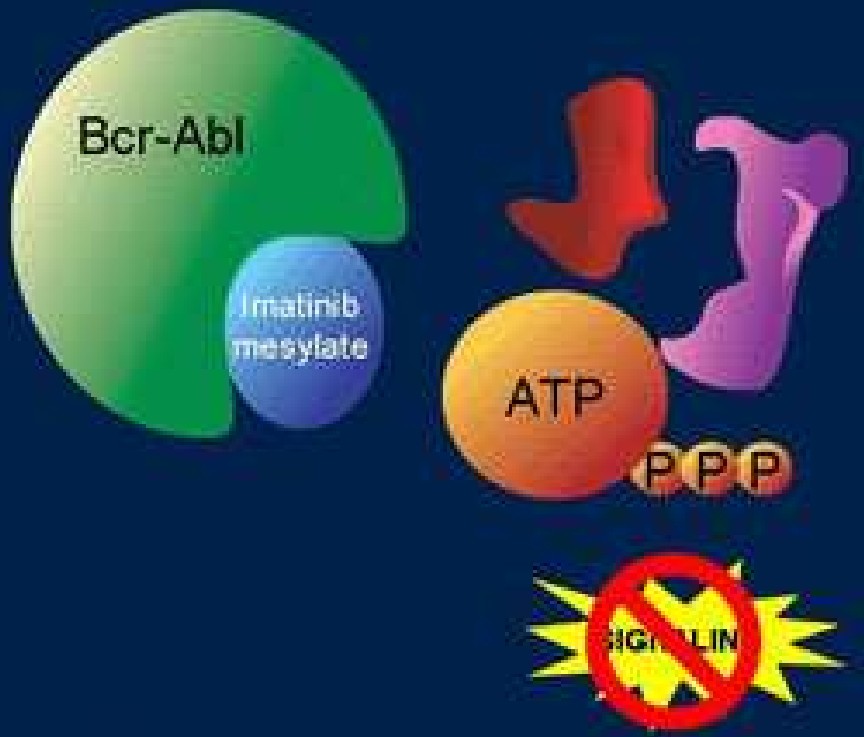
Imatinib Mesylate: Mechanism of Action

- Imatinib mesylate occupies the ATP binding pocket of the Abl kinase domain
- This prevents substrate phosphorylation and signaling
- A lack of signaling inhibits proliferation and survival



Savage DG, Antman KH. *N Engl J Med.* 2002;346:683-693.

le lesioni citogenetiche ricorrenti possono identificare entità distinte di malattia

LEUCEMIA ACUTA MIELOIDE (LAM)

Definizione:

espansione clonale di elementi immaturi della serie mieloide che hanno subito un danno genetico tale da non permettere il completamento del processo differenziativo.

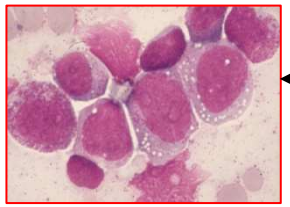
Diagnosi Clinica:

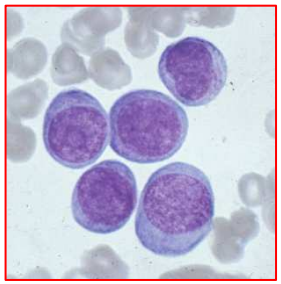
accumulo nel sangue periferico e nel midollo di precursori immaturi altamente indifferenziati:

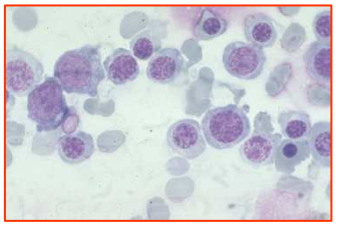
- **Leucocitosi/Leucopenia**
- **Anemia (da occupazione midollare)**
- **Piastrinopenia (idem)**

CRITERI CLASSIFICATIVI PER LE LEUCEMIE ACUTE MIELOIDI

Morfologia

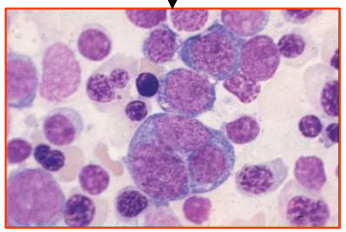






Immunofenotipo

- Linea mieloide
- Linea monocitoide
- Linea eritroide
- Linea megacariocitaria



Citogenetica

- anomalie ricorrenti
- complessità del cariotipo

normale

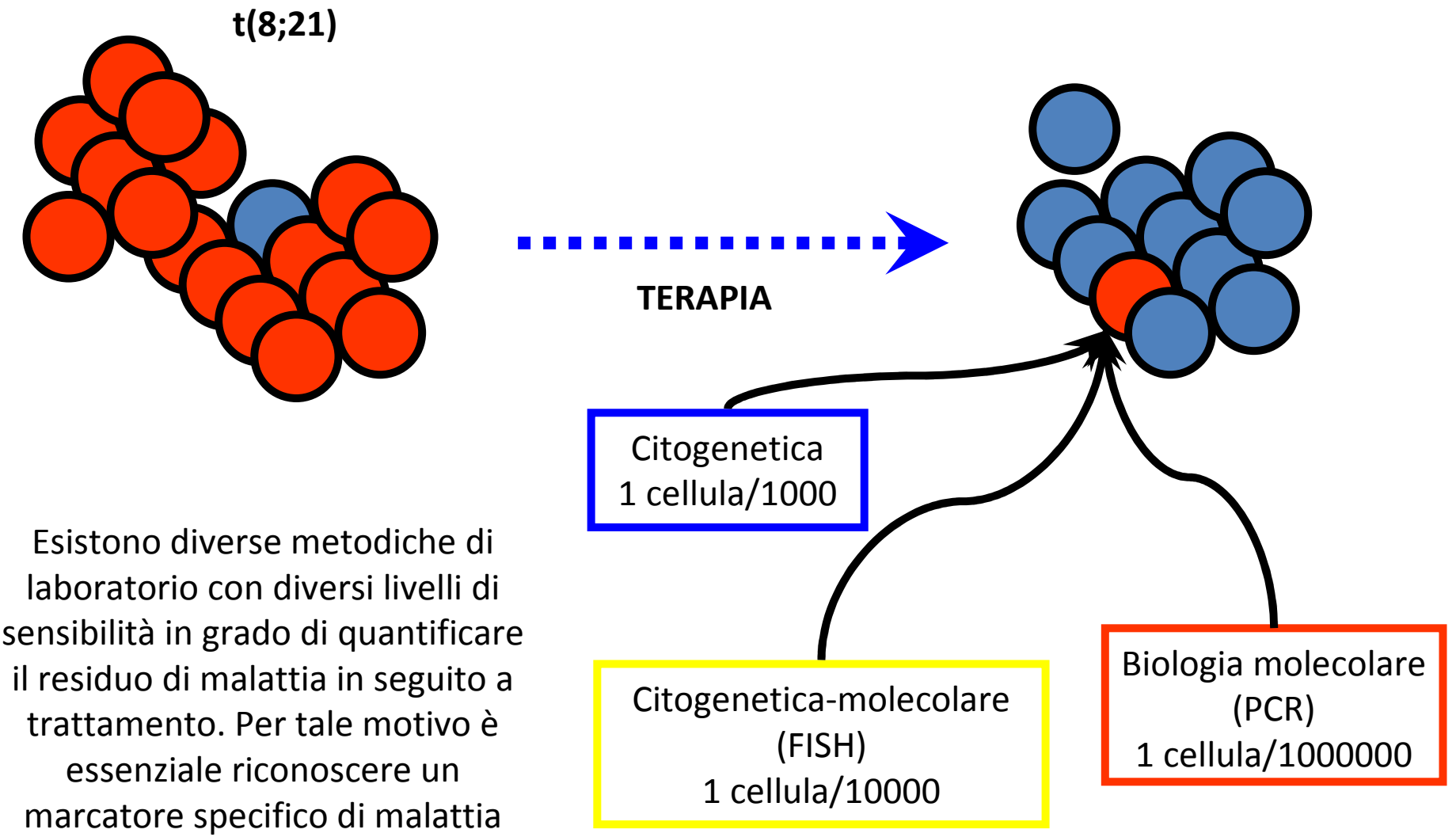
< 3 anomalie

> 3 anomalie

1) IDENTIFICAZIONE DI IDENTITA' DISTINTE DI MALATTIA
-Classificazione WHO delle Leucemie acute Mieloidi-

1. **Leucemie mieloidi acute con traslocazioni citogenetiche ricorrenti**
   - LMA con t(8;21) (q22;q22), AML1(CBFα)/ETO
   - Leucemia promielocitica acuta [LMA con t(15;17)(q22; q11-12) e varianti PML/RARα]
   - LMA con ipereosinofilia midollare [inv(16)(p13;q22) o t(16; 16)(p13;q11), CBFβ/MYH11X]
   - LMA con anomalie 11q23 (MLL)
2. **Leucemia mieloide acuta con displasia multilineare**
   - Secondaria a sindrome mielodisplastica
   - De novo
3. **Leucemia mieloide acuta e sindromi mielodisplastiche secondarie a chemioterapie**
   - Secondaria ad agenti alchilanti
   - Secondaria a epipodofillotossine
   - Altri tipi
4. **Leucemia mieloide acuta non altrimenti classificata**
   - LMA con differenziazione minima
   - LMA senza maturazione
   - LMA con maturazione
   - Leucemia mielomonocitica acuta
   - Leucemia monocitica acuta
   - Eritroleucemia acuta
   - Leucemia megacariocitica acuta
   - Leucemia basofila acuta
   - Panmielosi acuta con mielofibrosi

3) MONITORAGGIO DELLA MALATTIA RESIDUA



Esistono diverse metodiche di laboratorio con diversi livelli di sensibilità in grado di quantificare il residuo di malattia in seguito a trattamento. Per tale motivo è essenziale riconoscere un marcatore specifico di malattia

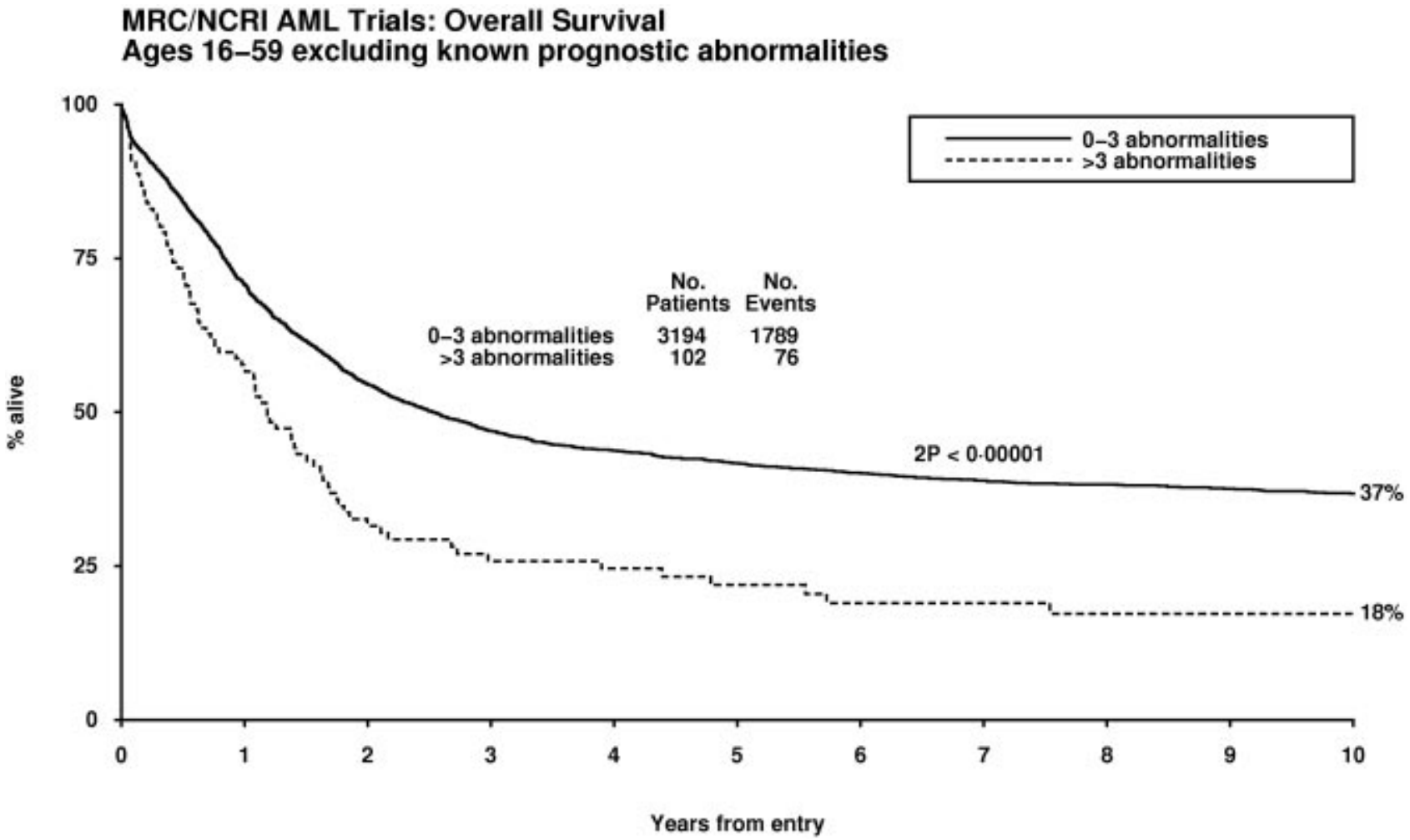


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Table 4. Revised MRC prognostic classification based on multivariable analyses

Cytogenetic abnormality	Comments
Favorable	
t(15;17)(q22;q21)	
t(8;21)(q22;q22)	Irrespective of additional cytogenetic abnormalities*
inv(16)(p13q22)/t(16;16)(p13;q22)	
Intermediate	
Entities not classified as favorable or adverse	
Adverse	
abn(3q) [excluding t(3;5)(q21~25;q31~35)],	
inv(3)(q21q26)/t(3;3)(q21;q26),	
add(5q), del(5q), −5,	
−7, add(7q)/del(7q),	Excluding cases with favorable karyotype†
t(6;11)(q27;q23),	
t(10;11)(p11~13;q23),	
t(11q23) [excluding t(9;11)(p21~22;q23) and t(11;19)(q23;p13)]	
t(9;22)(q34;q11),	
−17/abn(17p),	
Complex (≥ 4 unrelated abnormalities)	

*All favorable-risk abnormalities.
†All adverse-risk abnormalities.

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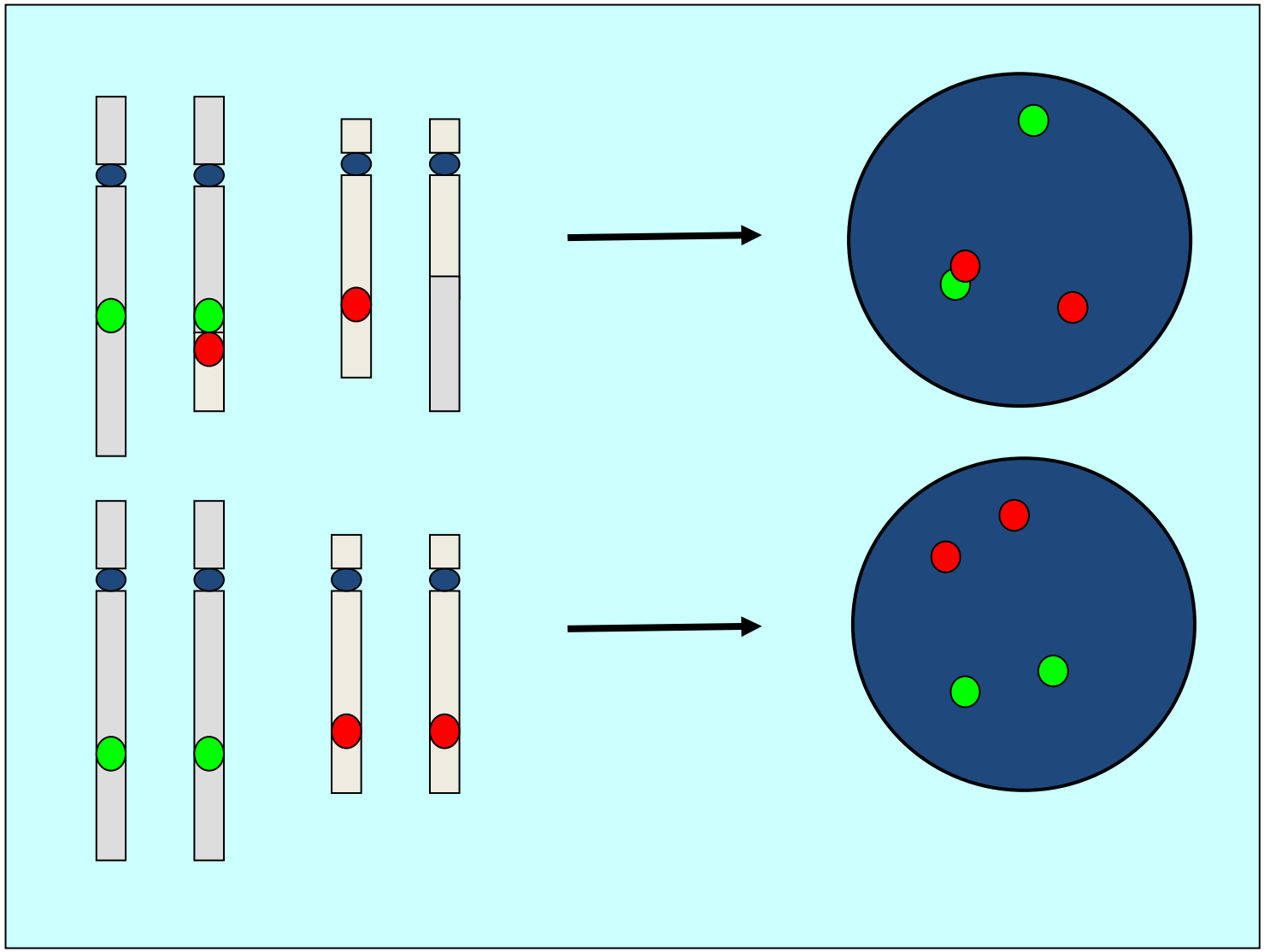
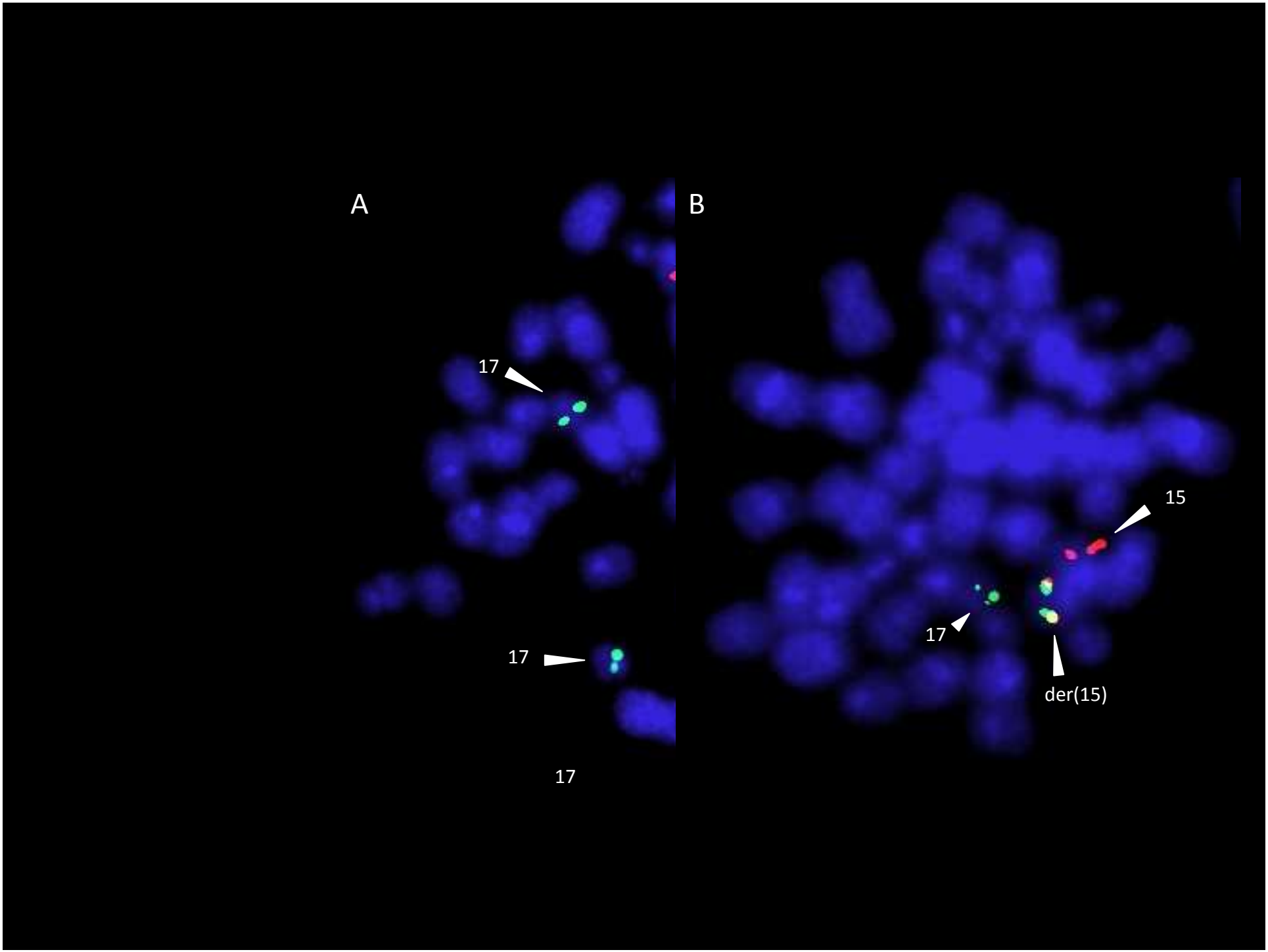
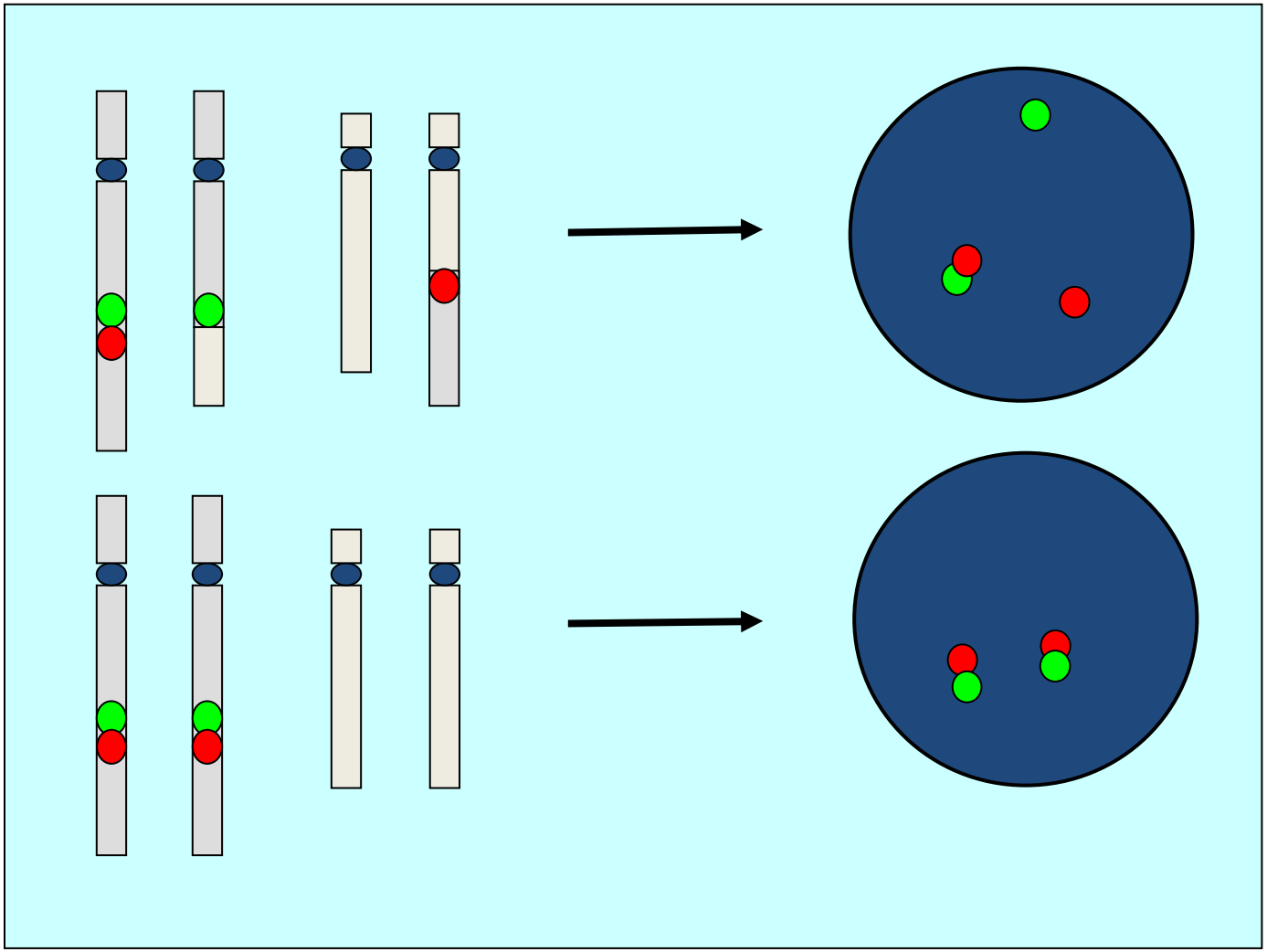
SISTEMA A COLOCALIZZAZIONE: SONDE SPECIFICHE PER GENI DIVERSI IDENTIFICANO UN SEGNALE DI FUSIONE IN PRESENZA DI TRASLOCAZIONE

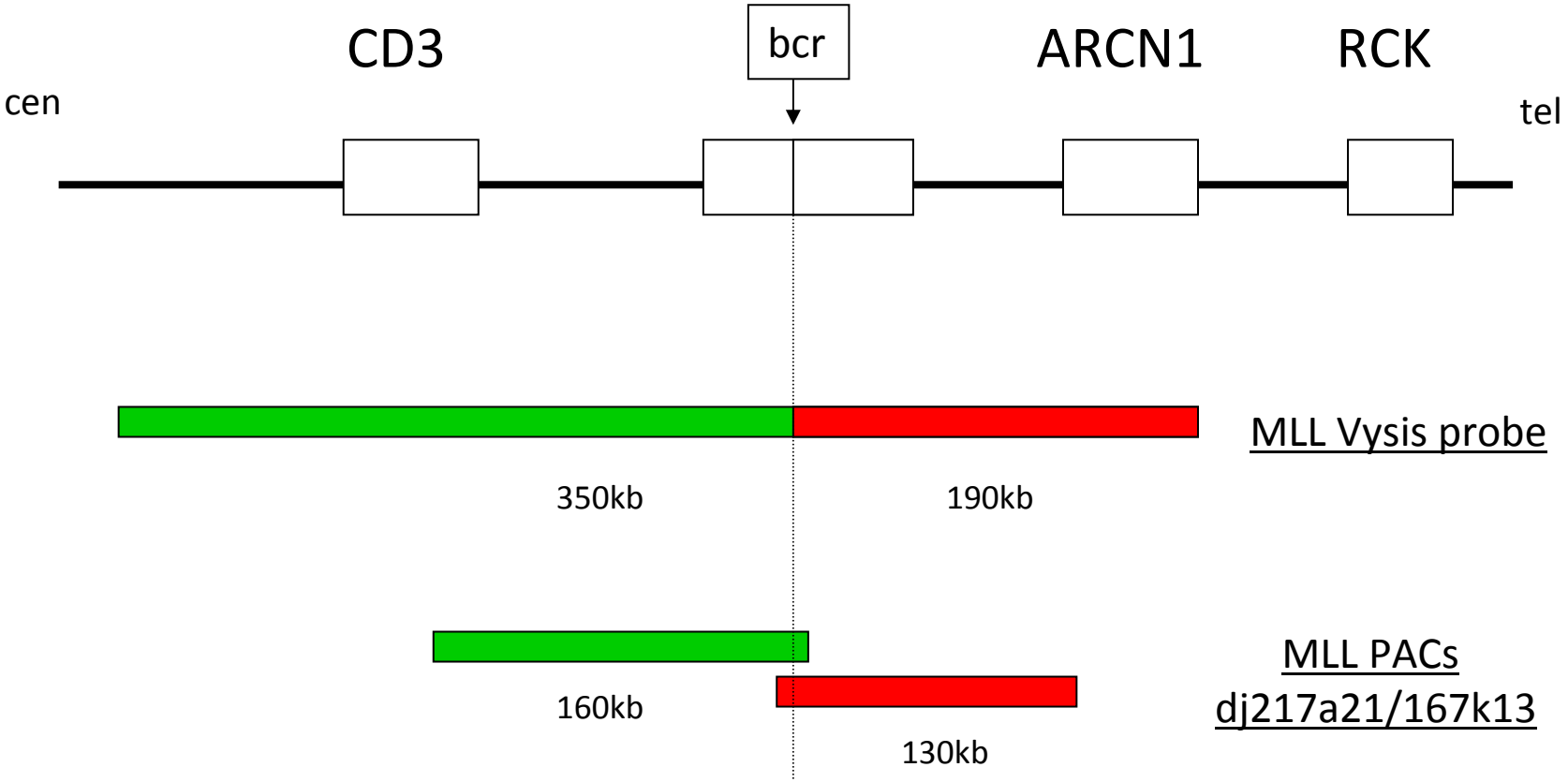


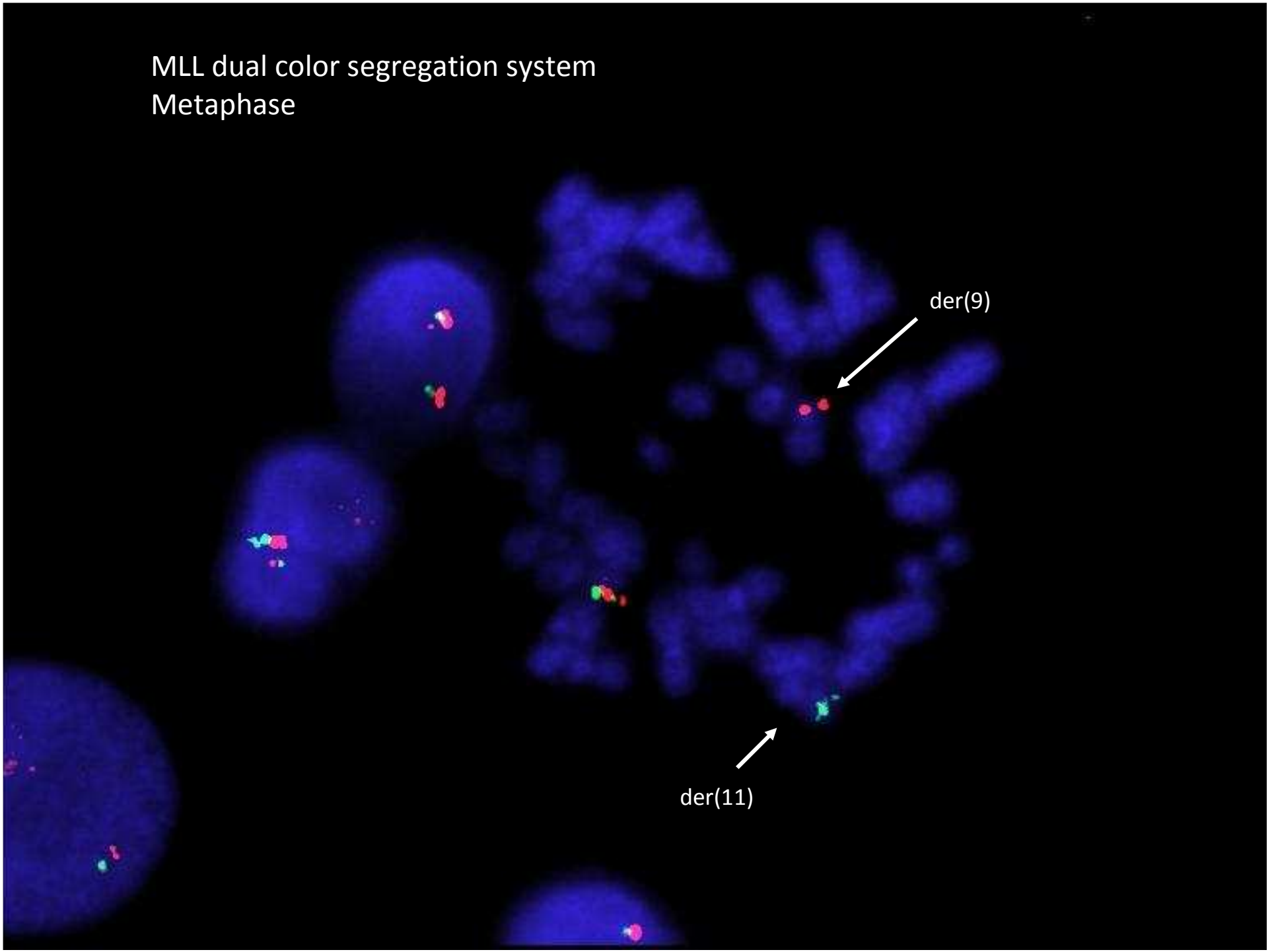

A
17
17
17
B
15
17
der(15)

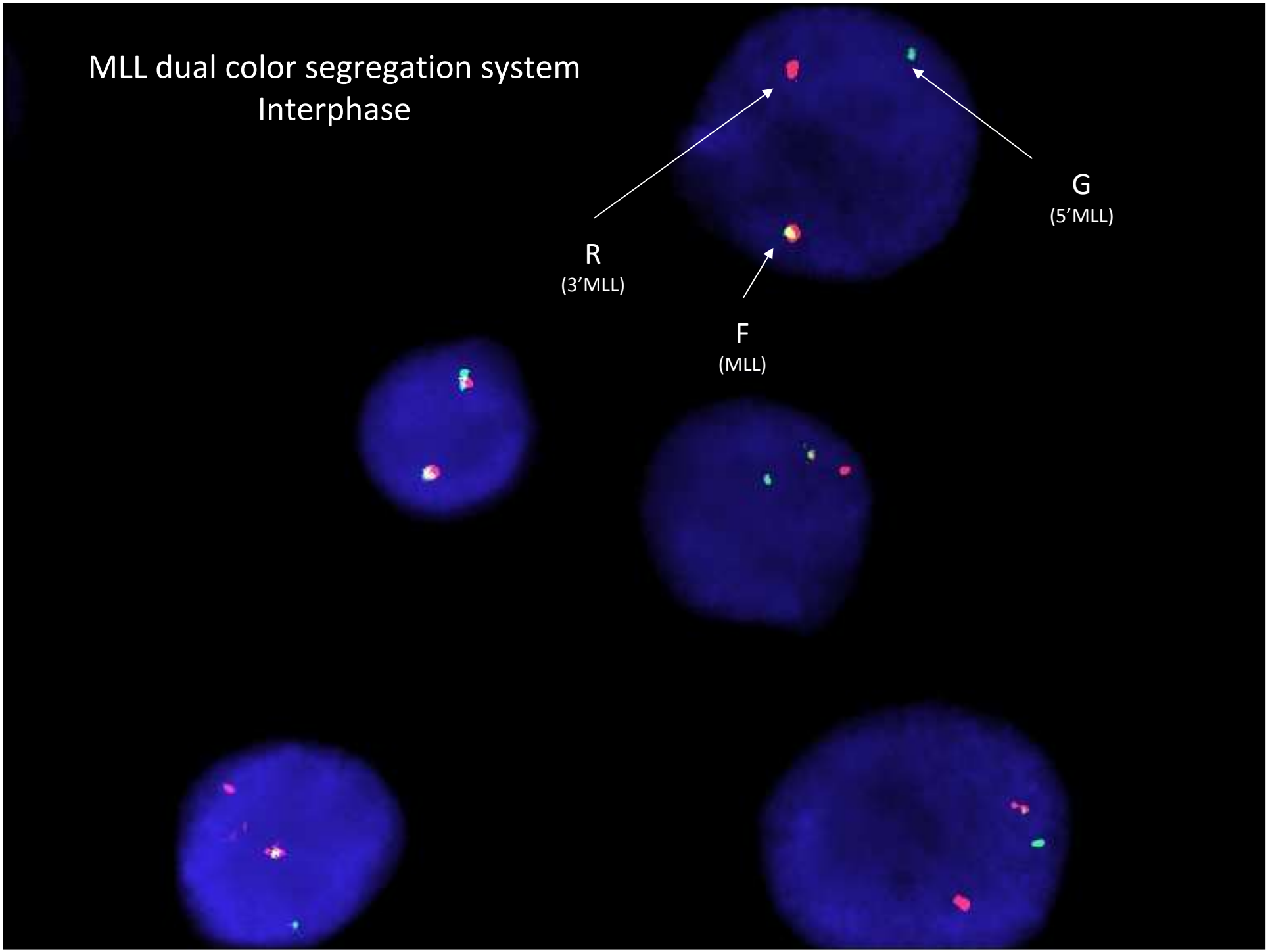
SISTEMA A SEGREGAZIONE: IL SEGNALE DI FUSIONE IDENTIFICA L'ALLELE NORMALE DEL GENE INTERESSATO NELLA TRASLOCAZIONE. QUANDO IL SEGNALE SEGREGA (LA FUSIONE SI DIVIDE IN DUE SEGNALI DI COLORE DIVERSO) è PRESENTE LA TRASLOCAZIONE

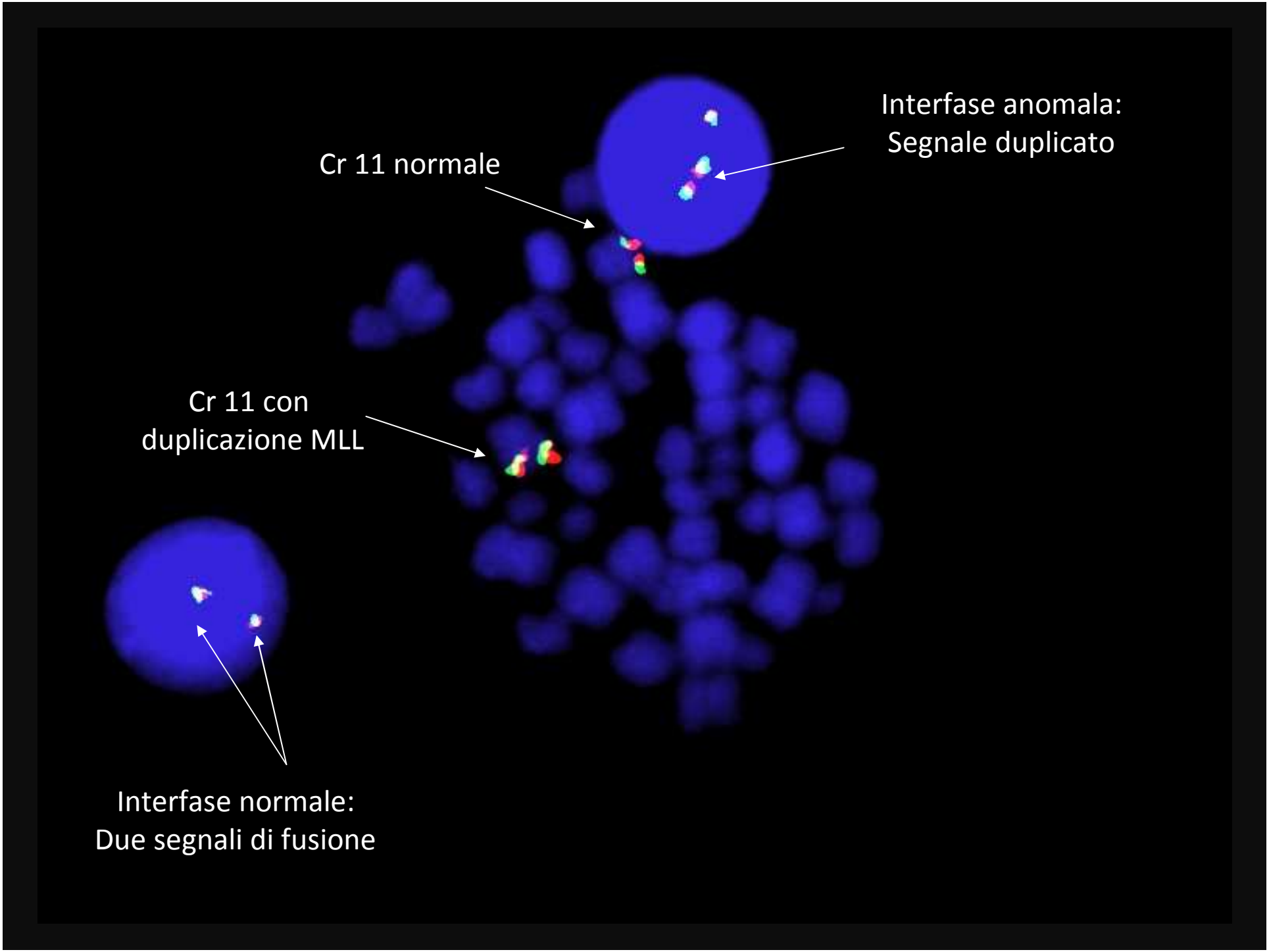


MLL probe systems

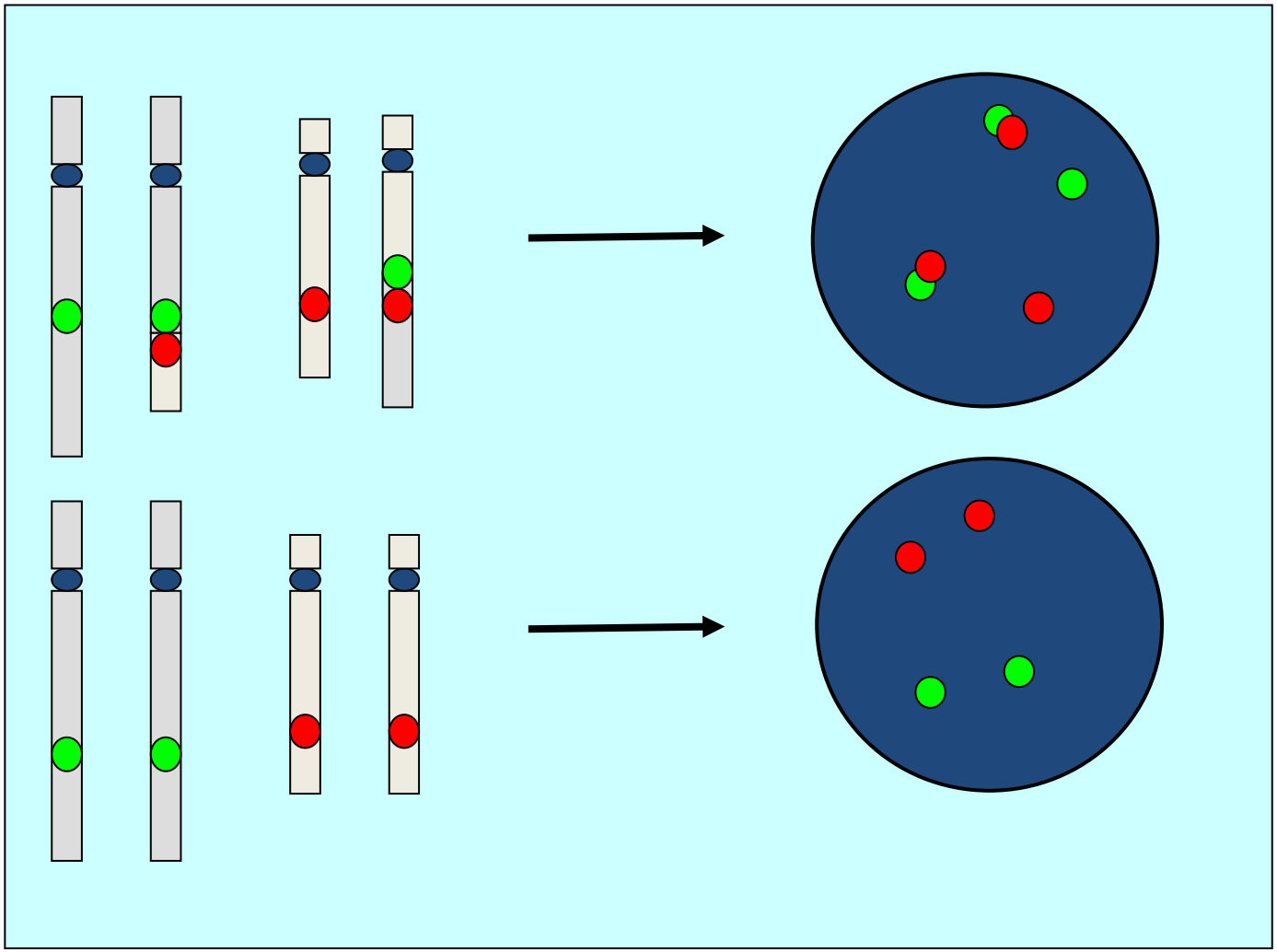



MLL dual color segregation system
Metaphase
der(9)
der(11)

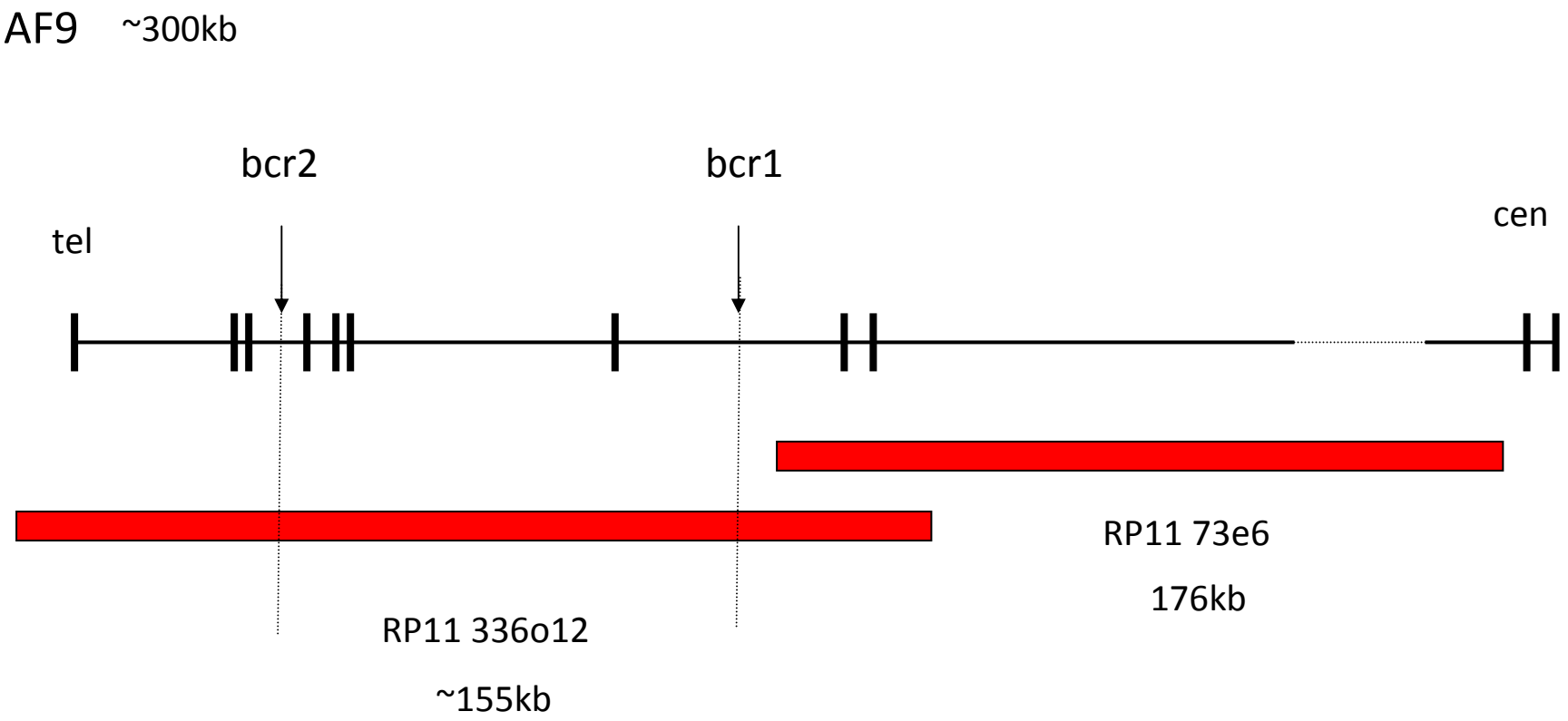

MLL dual color segregation system
Interphase
G
(5'MLL)
R
(3'MLL)
F
(MLL)

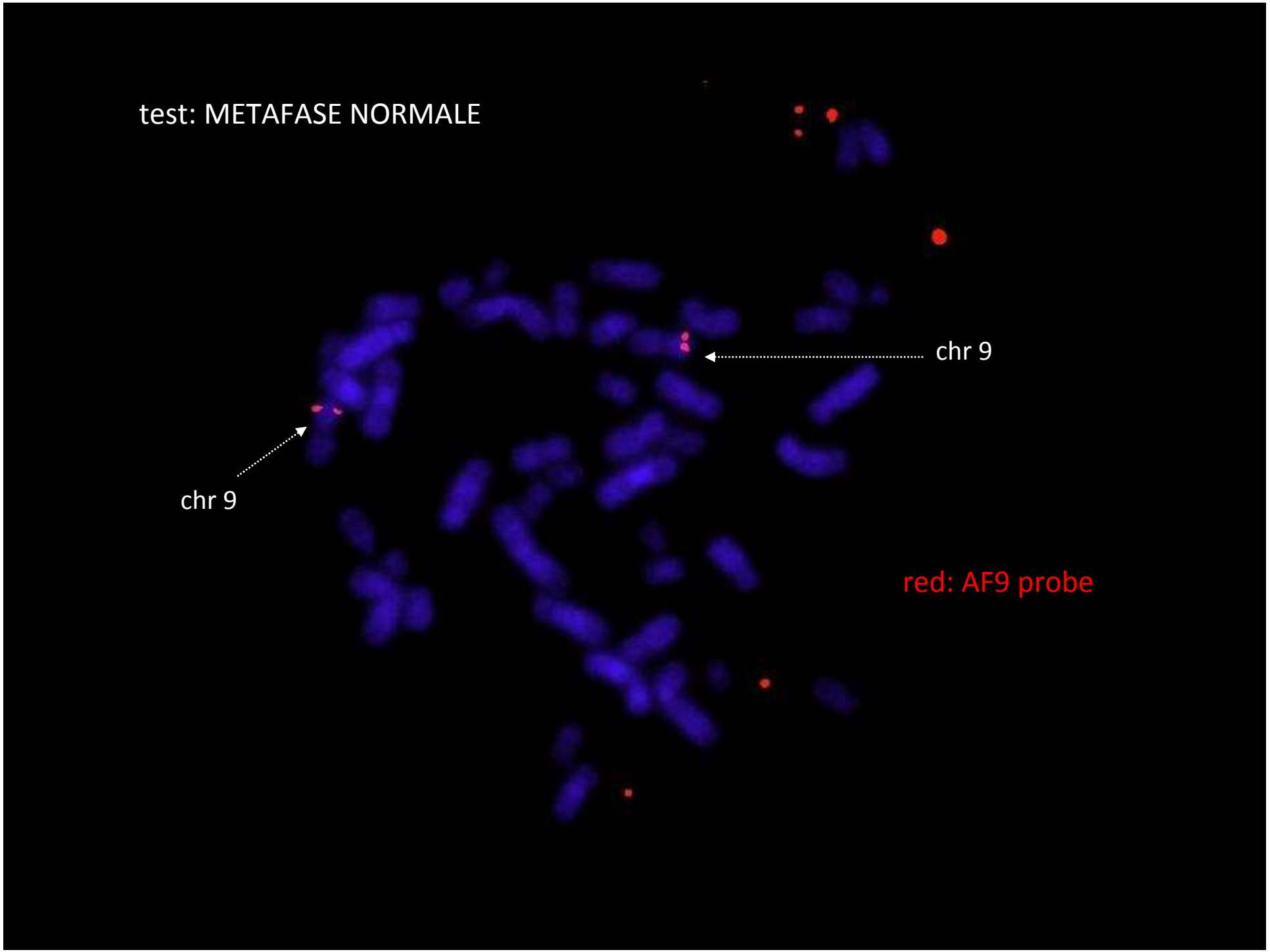

Interfase anomala:
Segnale duplicato
Cr 11 normale
Cr 11 con
duplicazione MLL
Interfase normale:
Due segnali di fusione

SISTEMA COMBINATO I: I GENI COINVOLTI NELLA TRASLOCAZIONE SONO MARCATI IN UNICO COLORE LA PRESENZA CONTEMPORANEA DI DOPPIA FUSIONE E SEGNALI A SINGOLO COLORE RAPPRESENTA UNA DISTRIBUZIONE POSSIBILE, IN UNA CELLULA 2N, SOLO IN CASO DI TRASLOCAZIONE

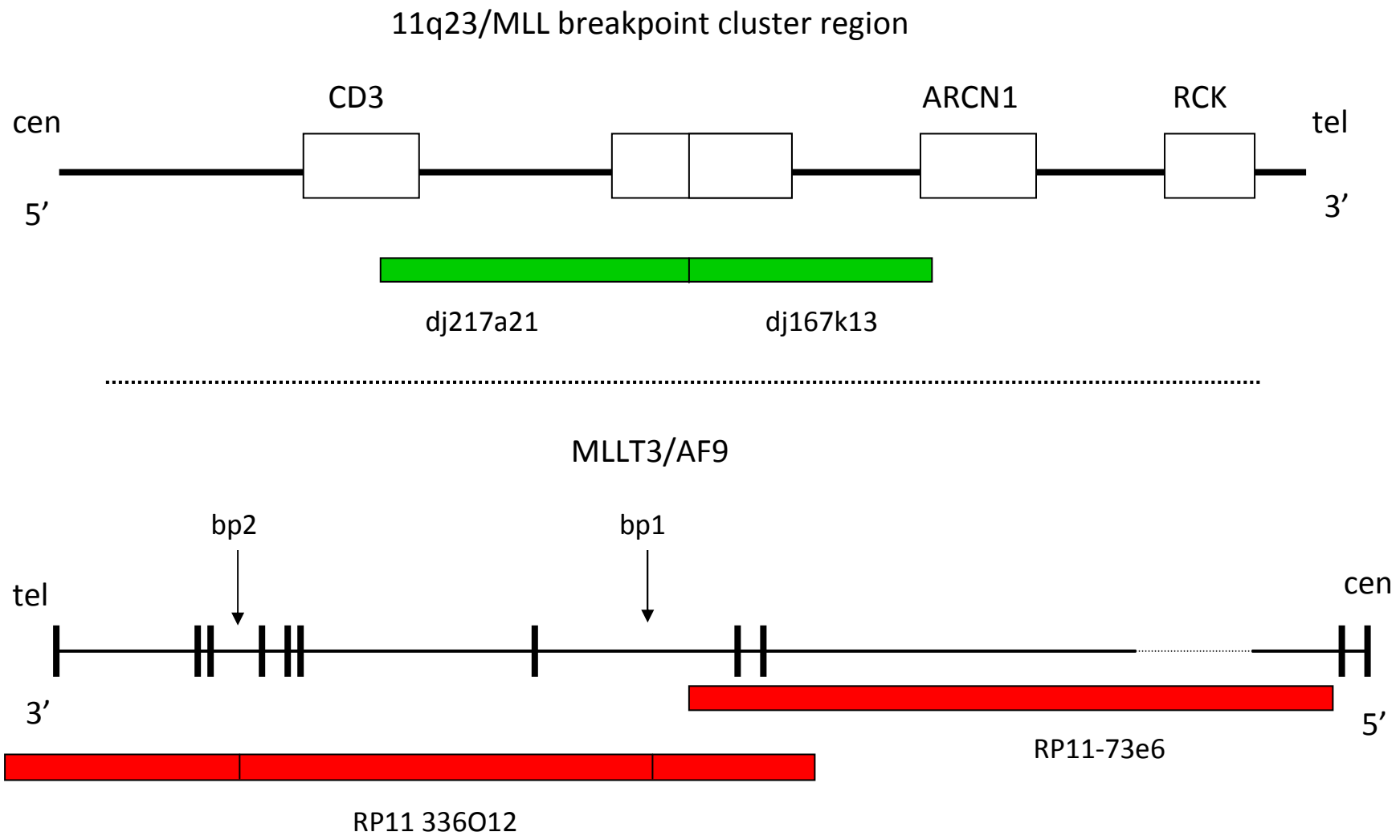


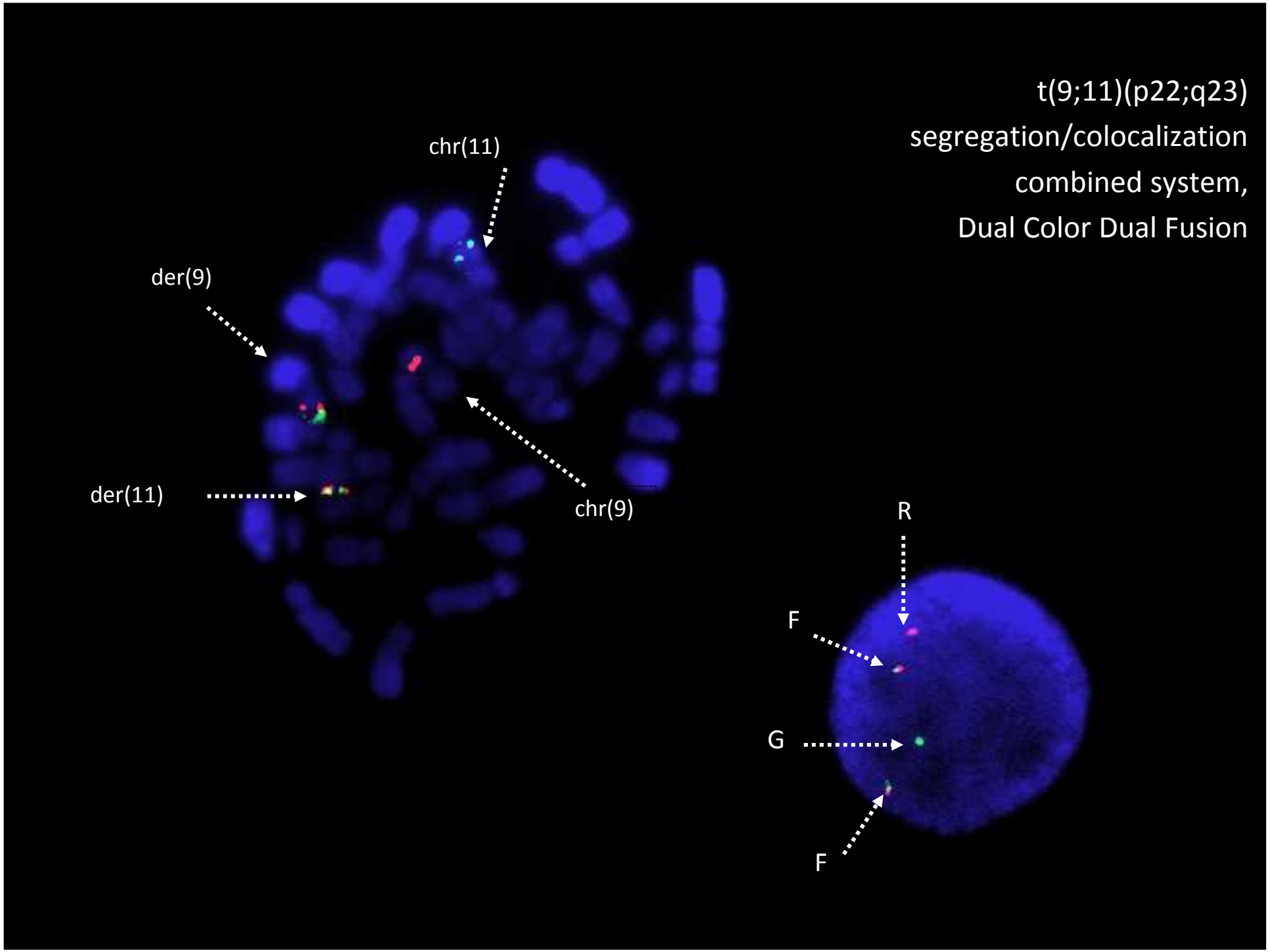
AF9 probe systems



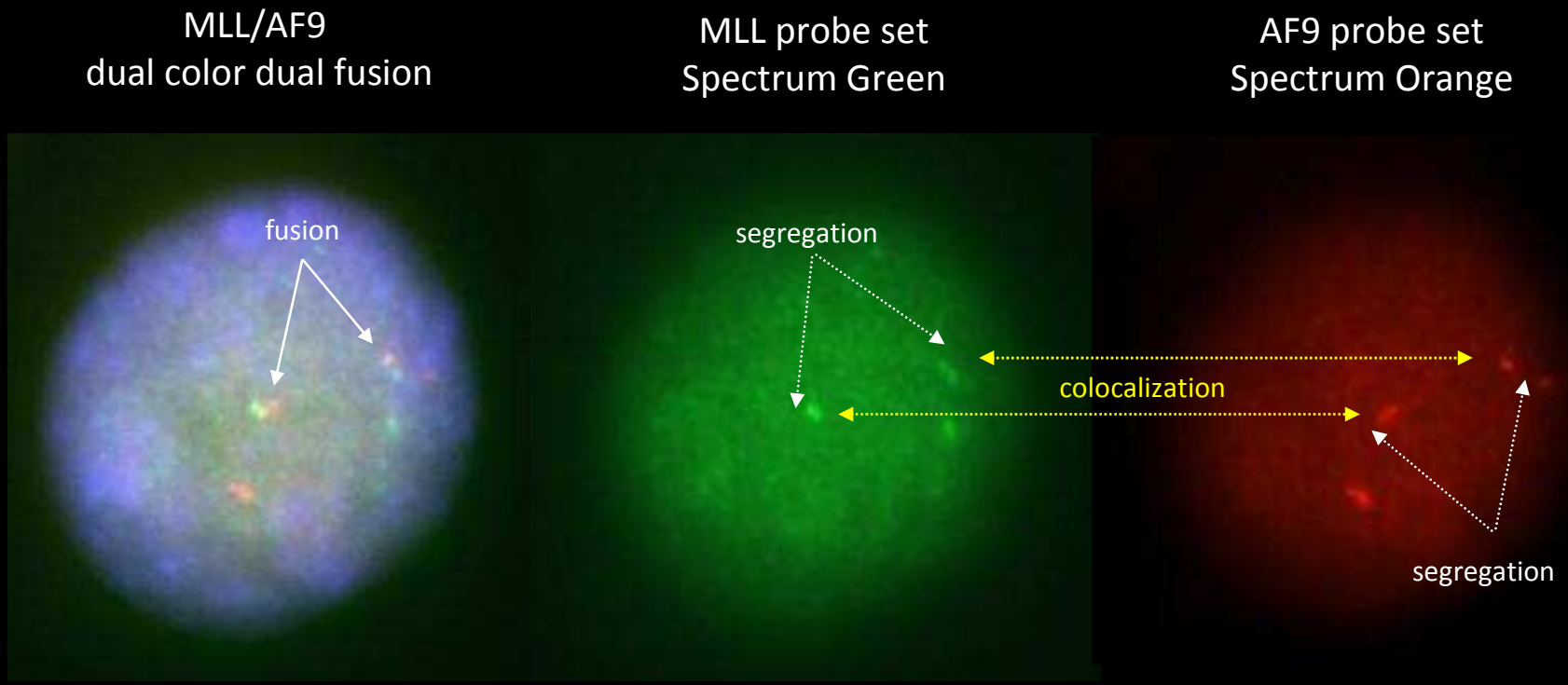

test: METAFASE NORMALE
chr 9
chr 9
red: AF9 probe

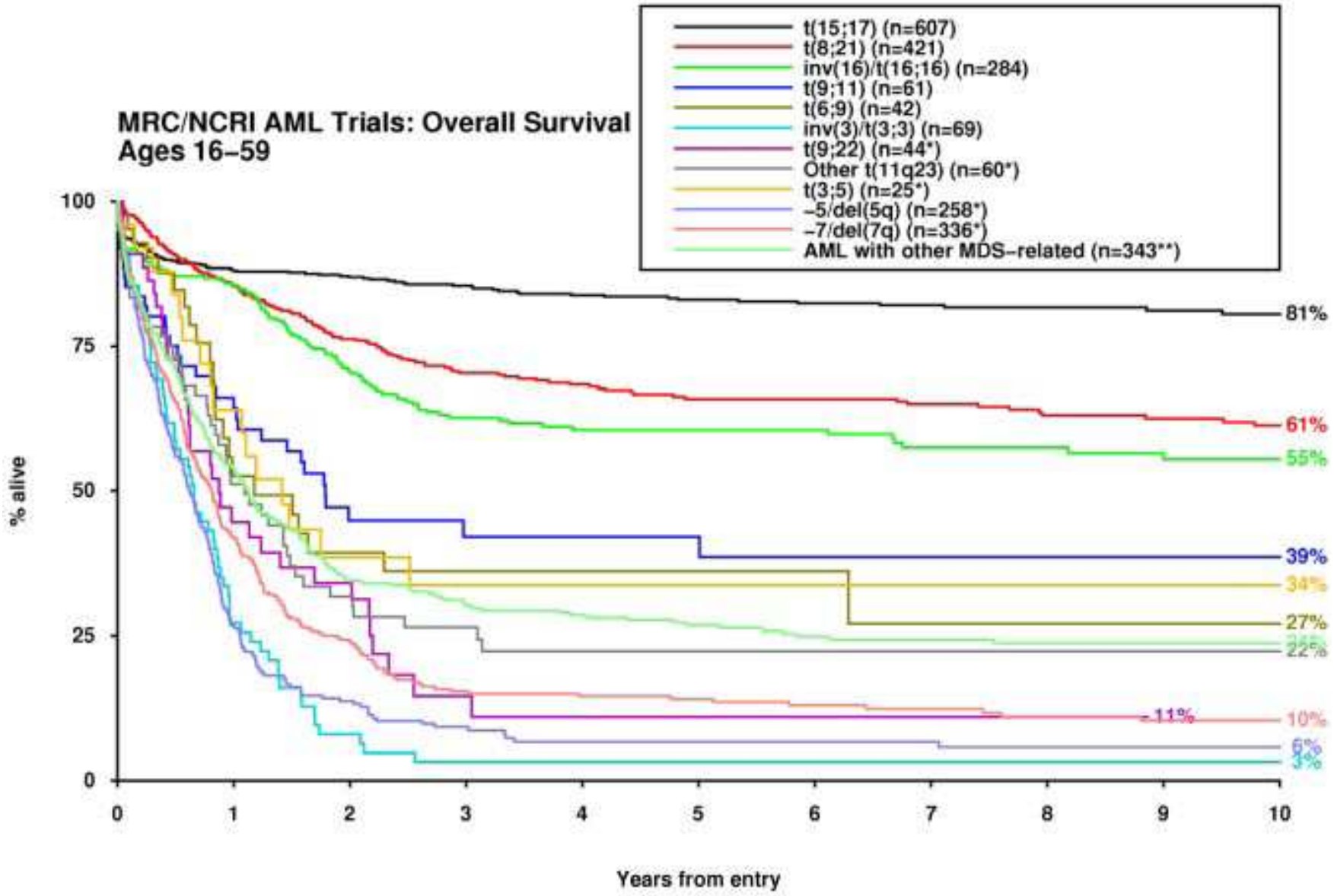
t(9;11)(p22;q23) SISTEMA COMBIANTO SEGREGAZIONE:COLOCALIZZAZIONE, Dual Color Dual Fusion




t(9;11)(p22;q23)
segregation/colocalization
combined system,
Dual Color Dual Fusion
chr(11)
der(9)
der(11)
chr(9)
R
F
G
F

MLL/AF9 dual color dual fusion system: interphase





BLOOD, 22 JULY 2010 VOLUME 116, NUMBER 3

Citogenetica e Citogenetica-Molecolare come componenti integrativi di sistemi diagnostico-prognostici

L’analisi citogenetica nelle sindromi mielodisplastiche

DIFFERENTIAL DIAGNOSIS OF Myelodysplatic Syndromes

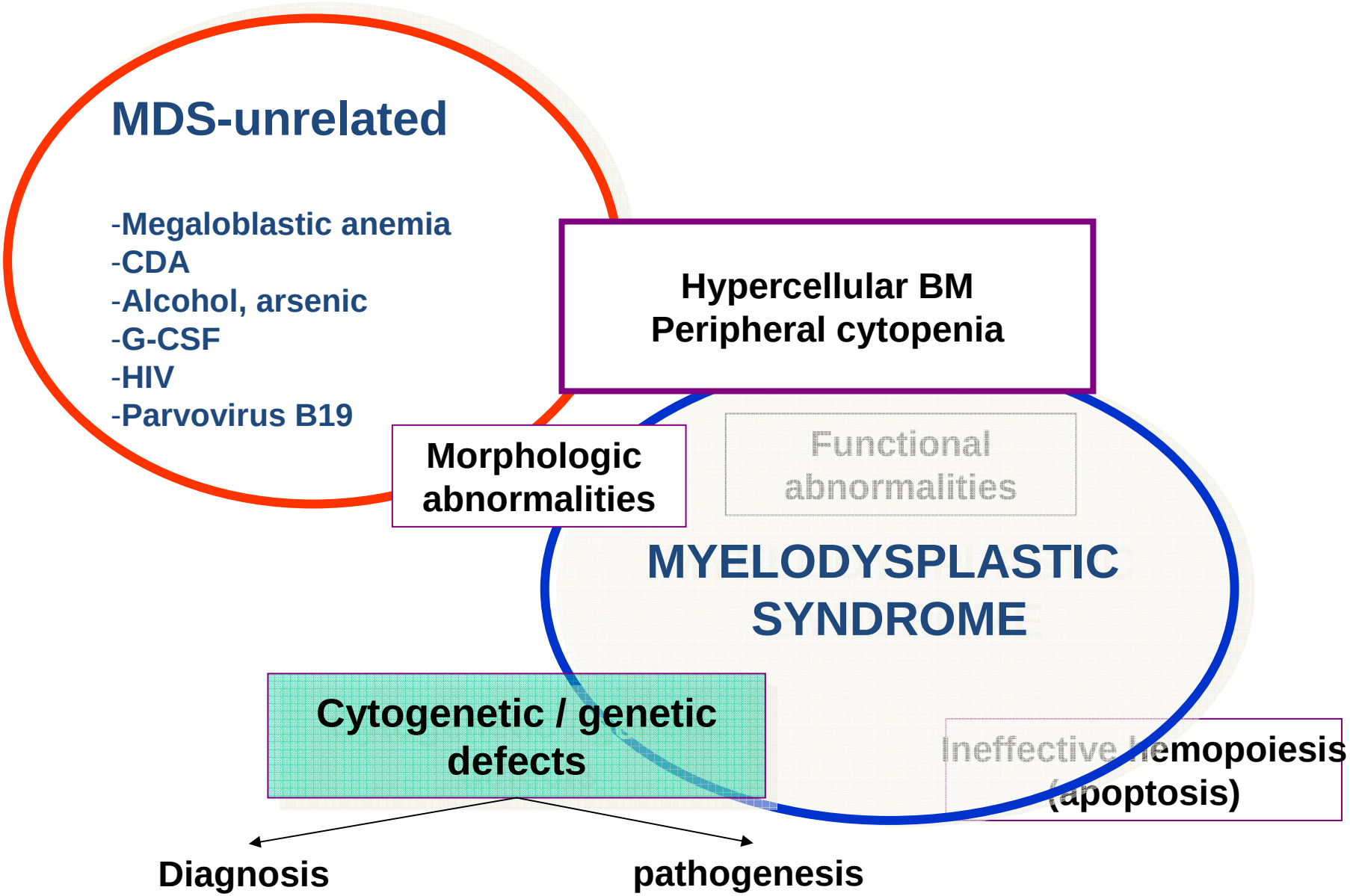


Table 2

IPSS Classification Criteria

	Score				
Prognostic variable	**0**	**0.5**	**1.0**	**1.5**	**2.0**
Bone marrow blasts	< 5%	5%–10%	—	11%–20%	21%–30%
Karyotype[a]	Good	Intermediate	Poor		
Cytopenias	0/1	2/3			

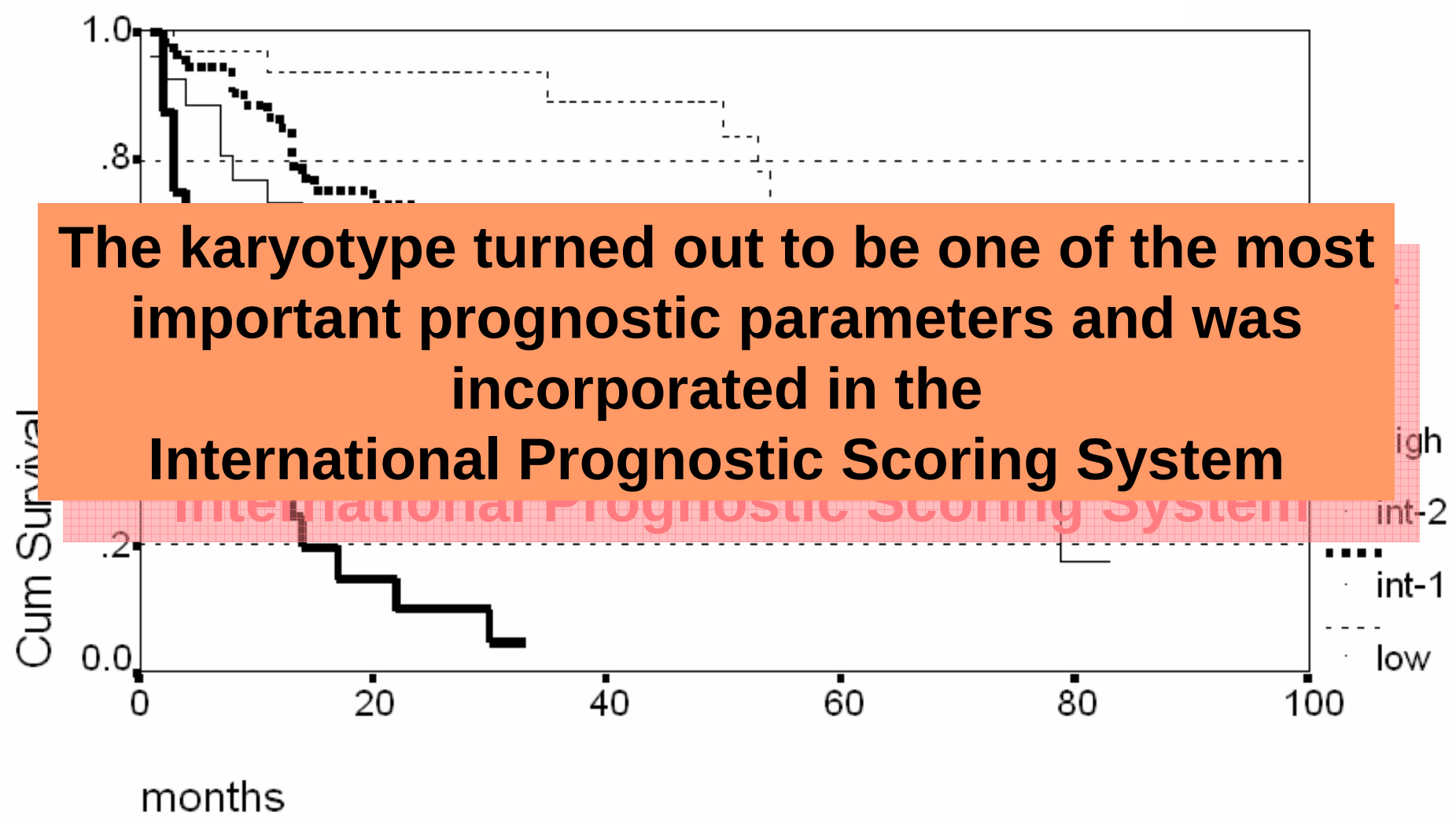
IPSS scores for risk groups are as follows:
Low = 0; Int-1 = 0.5–2.0; Int-2 = 1.5–2.0; High = 2.5

[a]Good = normal, –Y, del(5q), del(20q); poor = complex (3 abnormalities) or chromosome 7 anomalies; intermediate = other abnormalities.

Int = intermediate; IPSS = International Prognostic Scoring System.

Risk Classification according to IPSS in 134 MDS patients studied at Section of Haematology, University of Ferrara (1998-2006)

% blasts, Karyotype, cytopenia



The karyotype turned out to be one of the most important prognostic parameters and was incorporated in the International Prognostic Scoring System

CYTOGENETIC ABNORMALITIES IN MDS

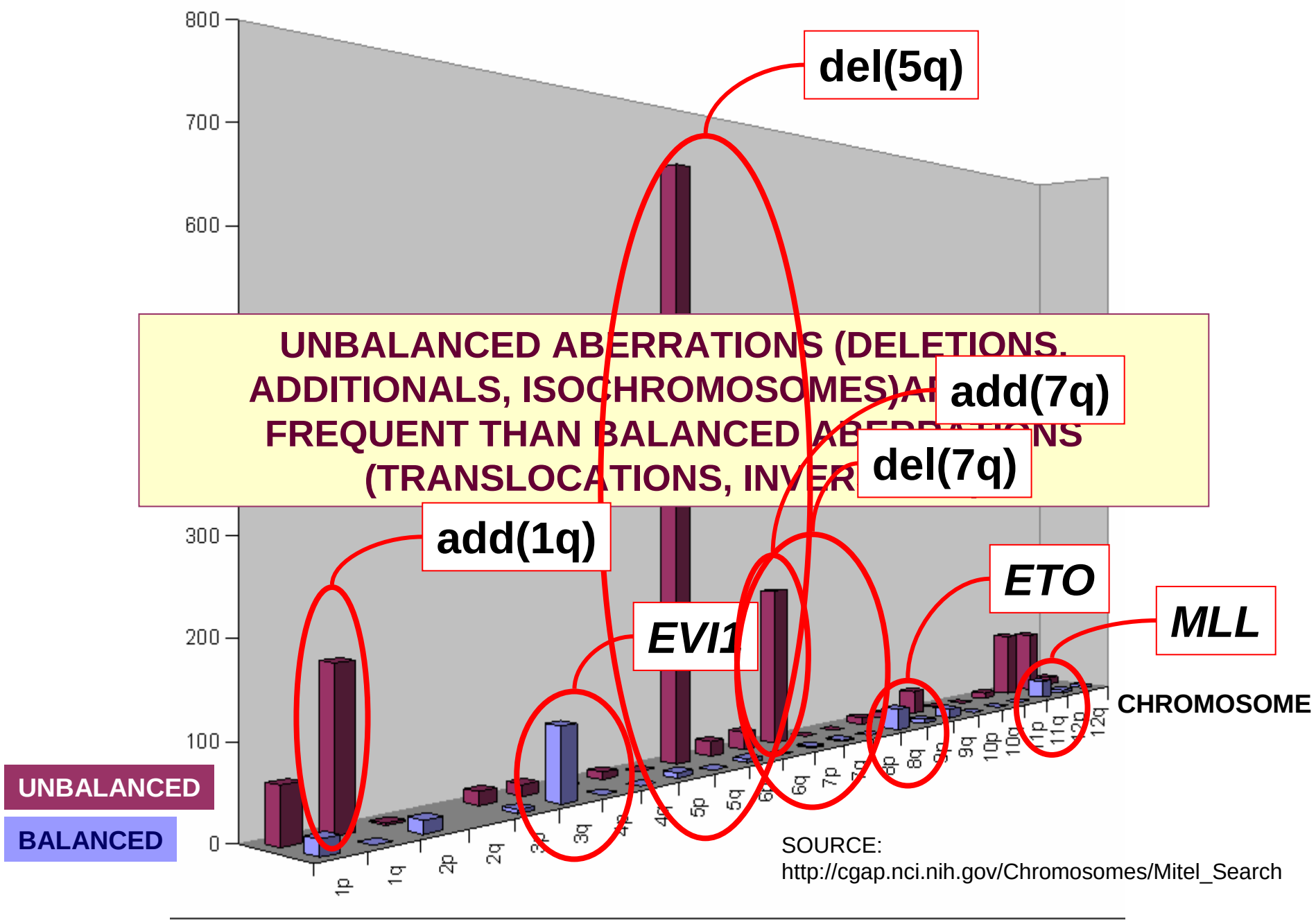
STRUCTURAL ABERRATIONS

- **BALANCED (translocations and inversions)**
- **UNBALANCED (deletions, additionals, iso)**

NUMERICAL ABERRATIONS

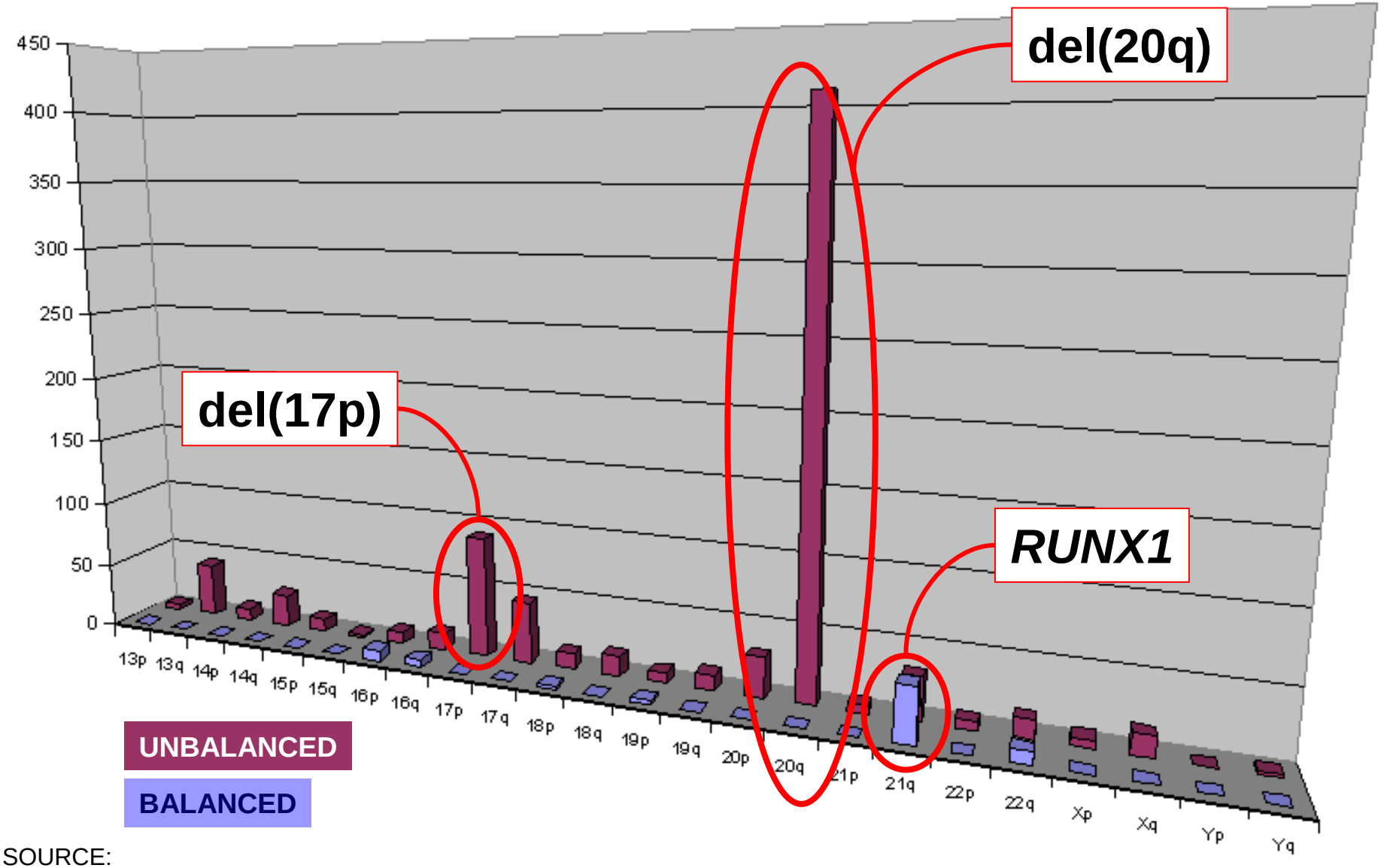
- **TRISOMIES**
- **MONOSOMIES**

STRUCTURAL ABERRATIONS (balanced and unbalanced) IN MDS



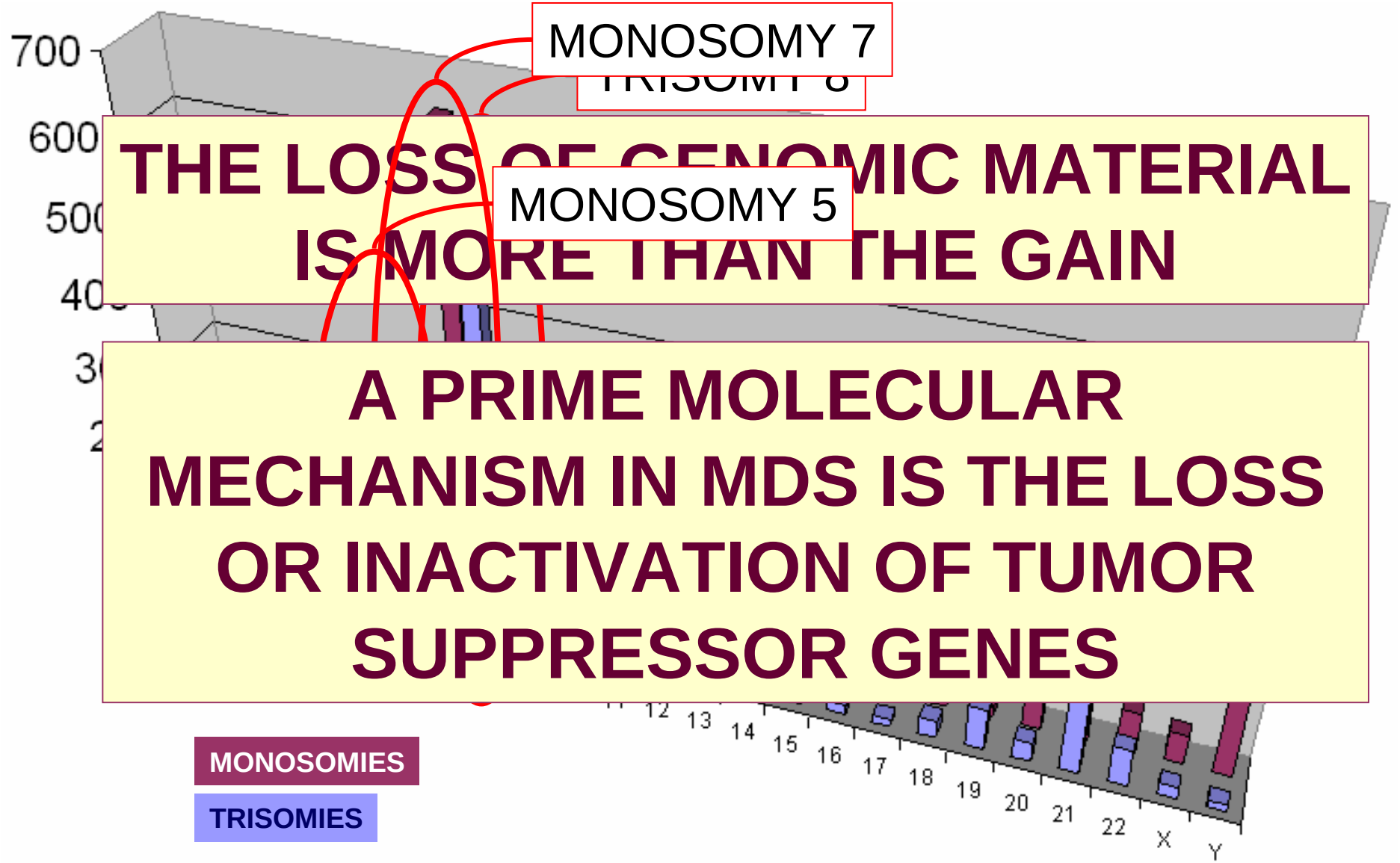
SOURCE:
http://cgap.nci.nih.gov/Chromosomes/Mitel_Search

STRUCTURAL ABERRATIONS (balanced and unbalanced) IN MDS



SOURCE:
http://cgap.nci.nih.gov/Chromosomes/Mitel_Search

NUMERICAL ABERRATIONS IN MDS



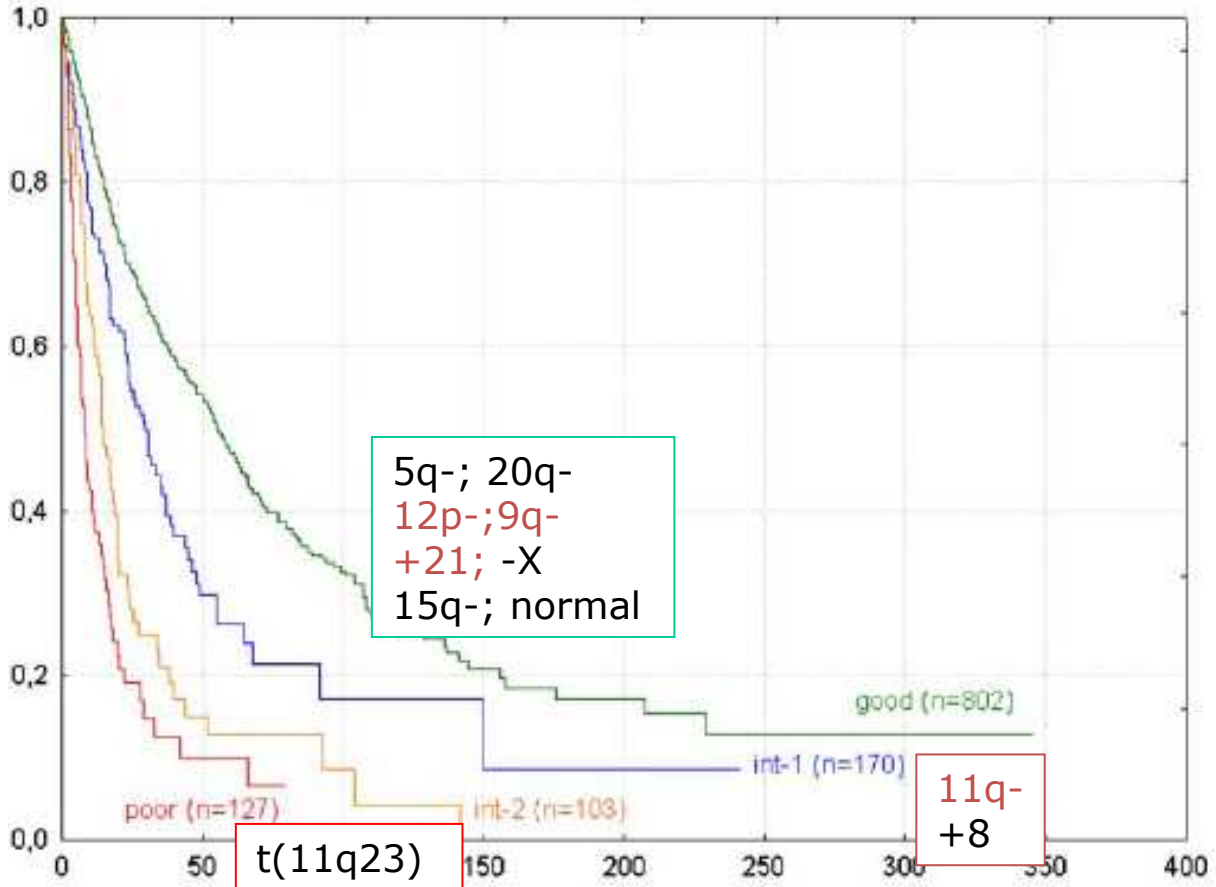
THE LOSS OF GENOMIC MATERIAL IS MORE THAN THE GAIN

A PRIME MOLECULAR MECHANISM IN MDS IS THE LOSS OR INACTIVATION OF TUMOR SUPPRESSOR GENES

SOURCE:
http://cgap.nci.nih.gov/Chromosomes/Mitel_Search

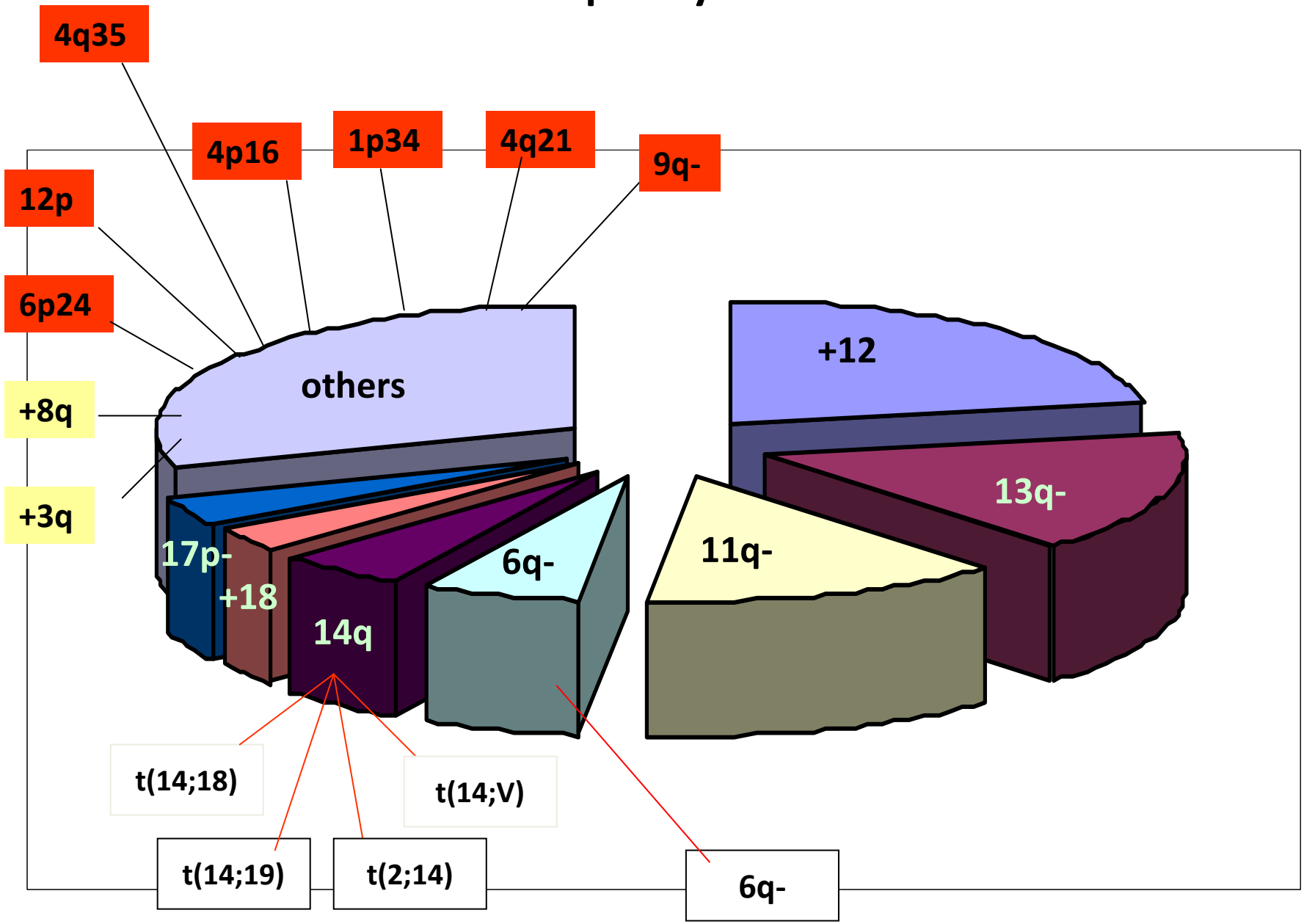
PROGNOSTIC SIGNIFICANCE OF CHROMOSOME ABERRATIONS IN MDS

Haase D. Ann Hematol, 87: 515, 2008

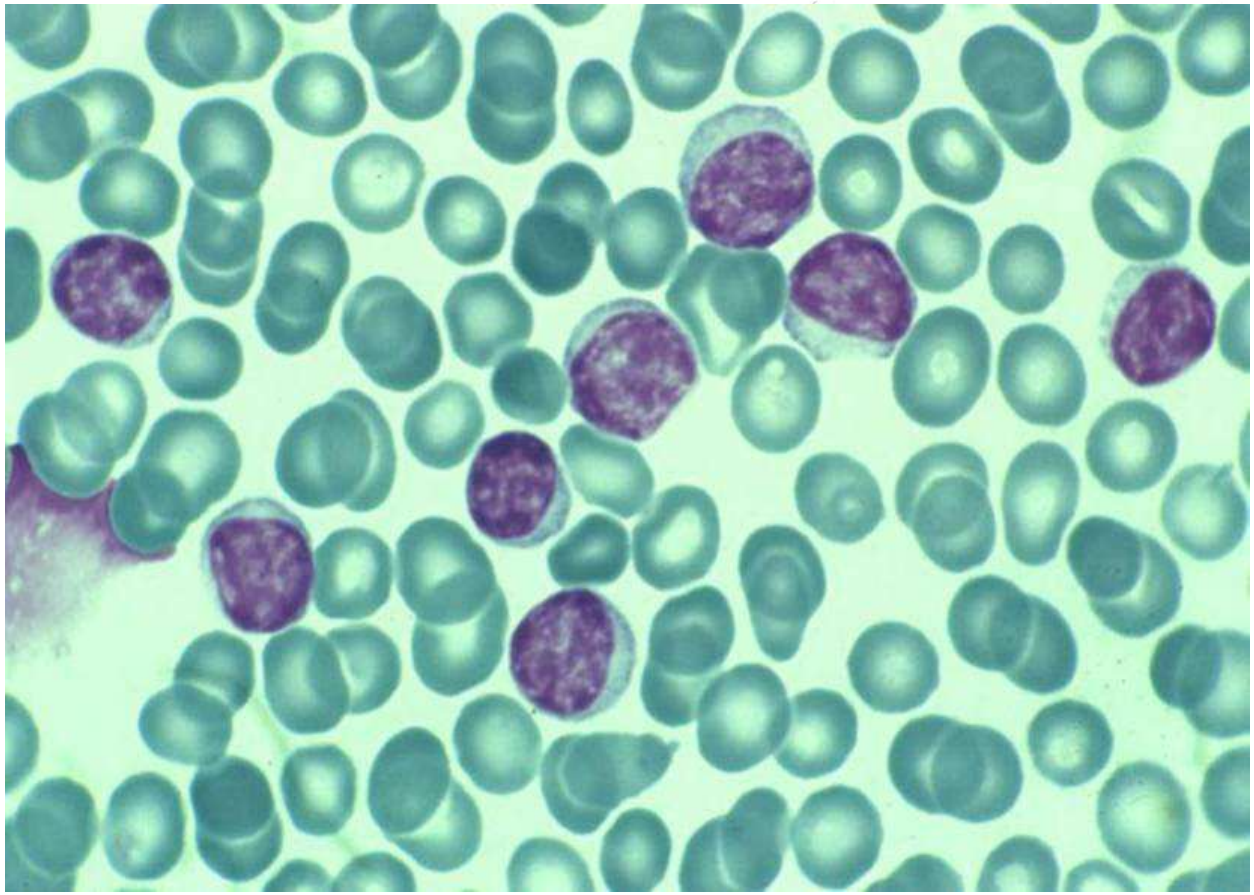


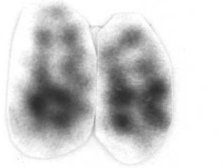
L’analisi citogenetica nella leucemia linfatica cronica

B-CLL: Frequency of chromosome aberrations



13q- and typical CLL

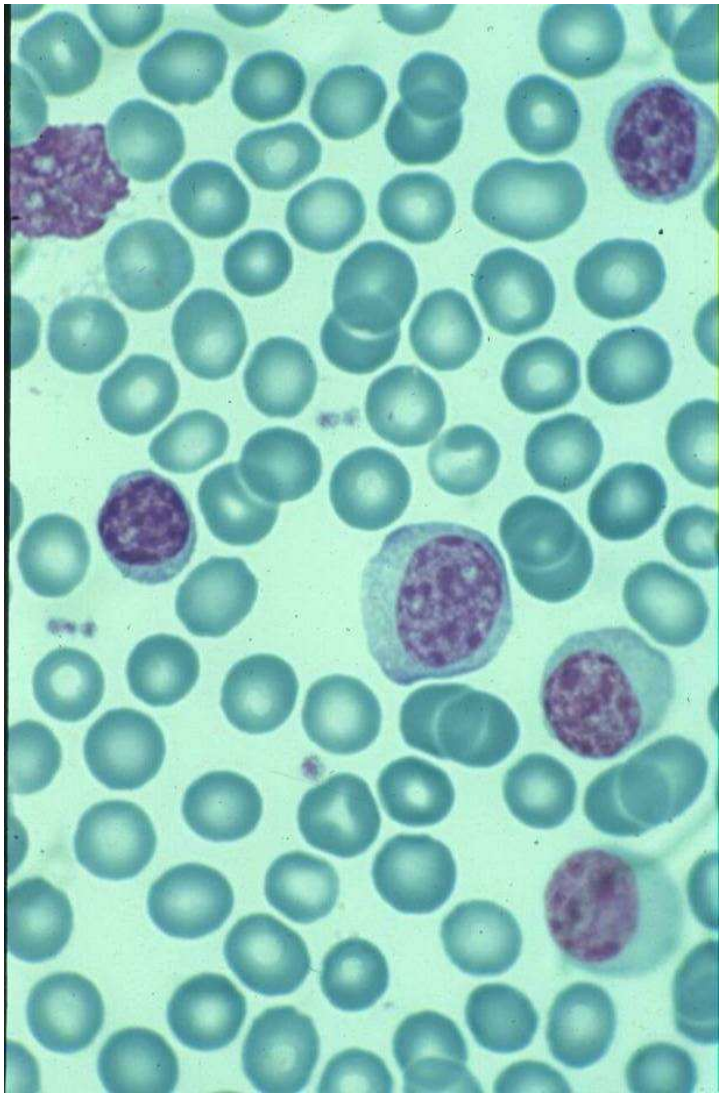


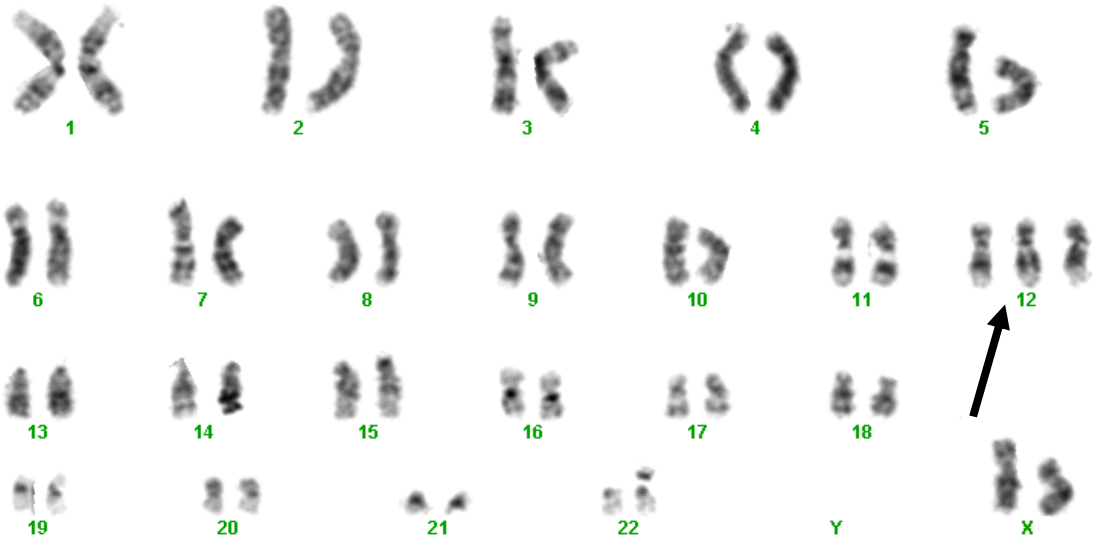


13q–

- “mutated” IgVh
- CD38- ZAP70-
- Typical CLL

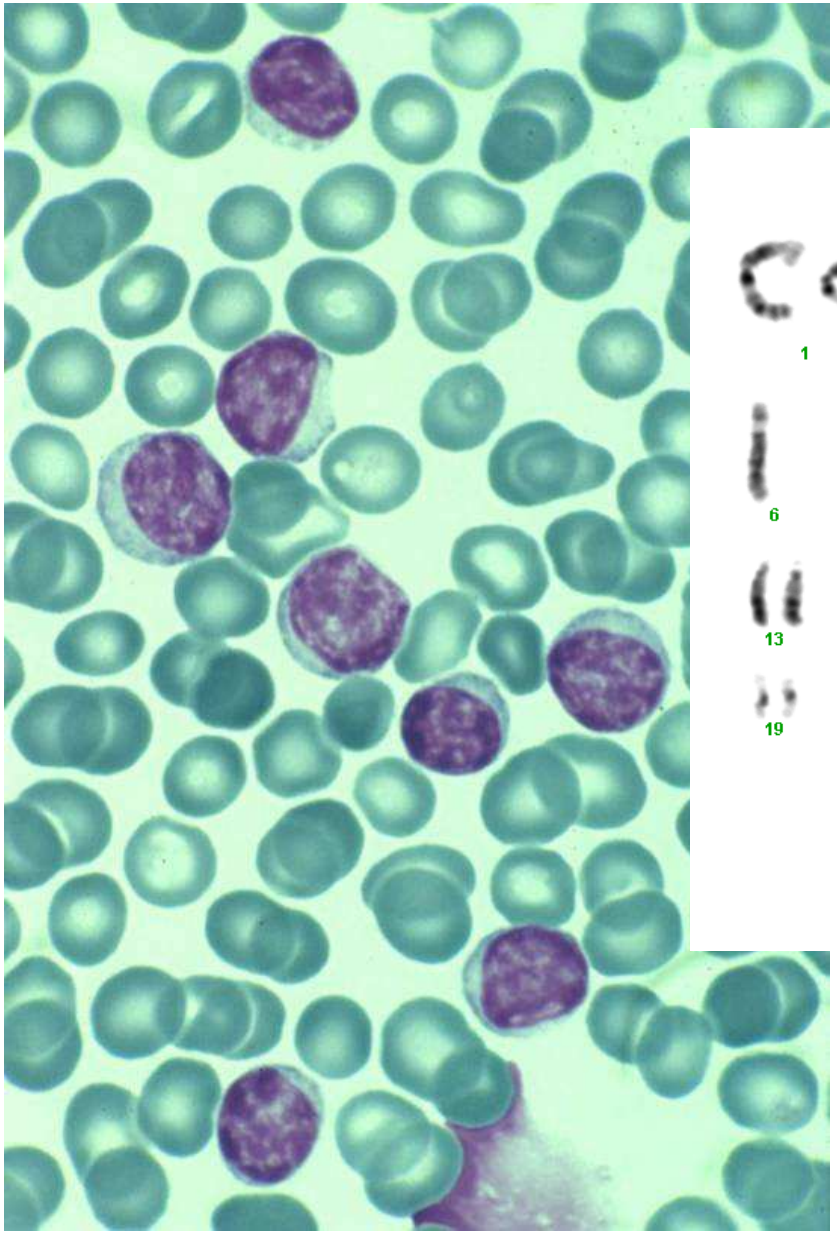
Trisomy 12 and atypical CLL

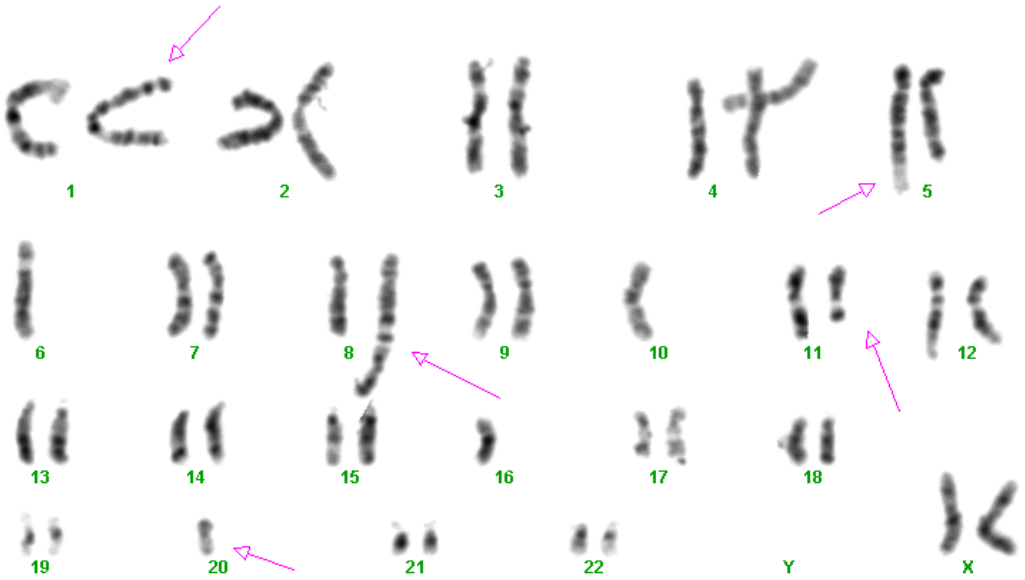




- "approx 50% mutated" IgVh
- CD38-/+ ZAP70+/-

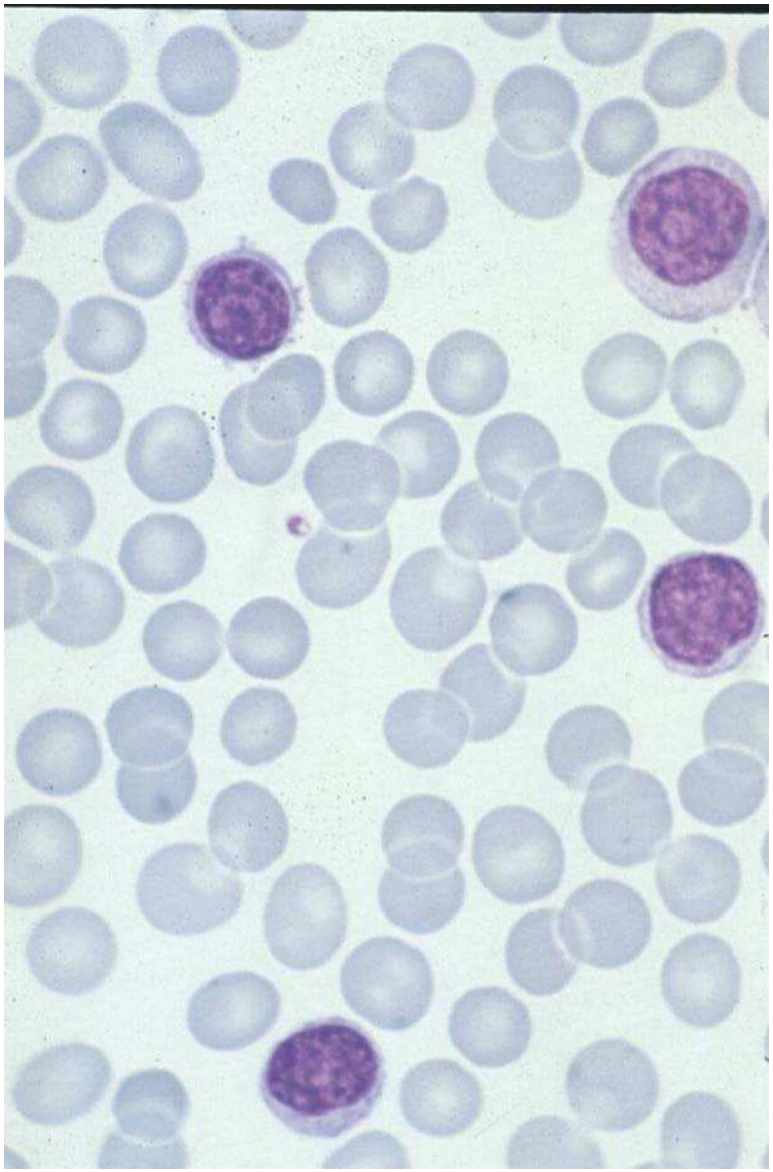
11q- and typical CLL

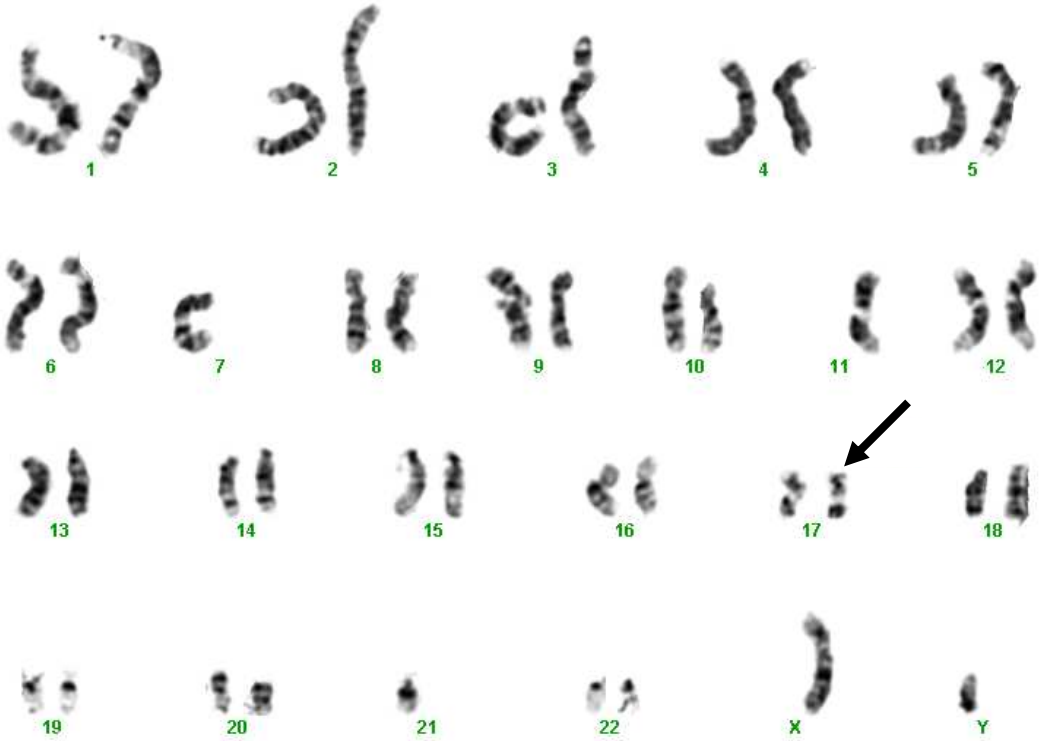




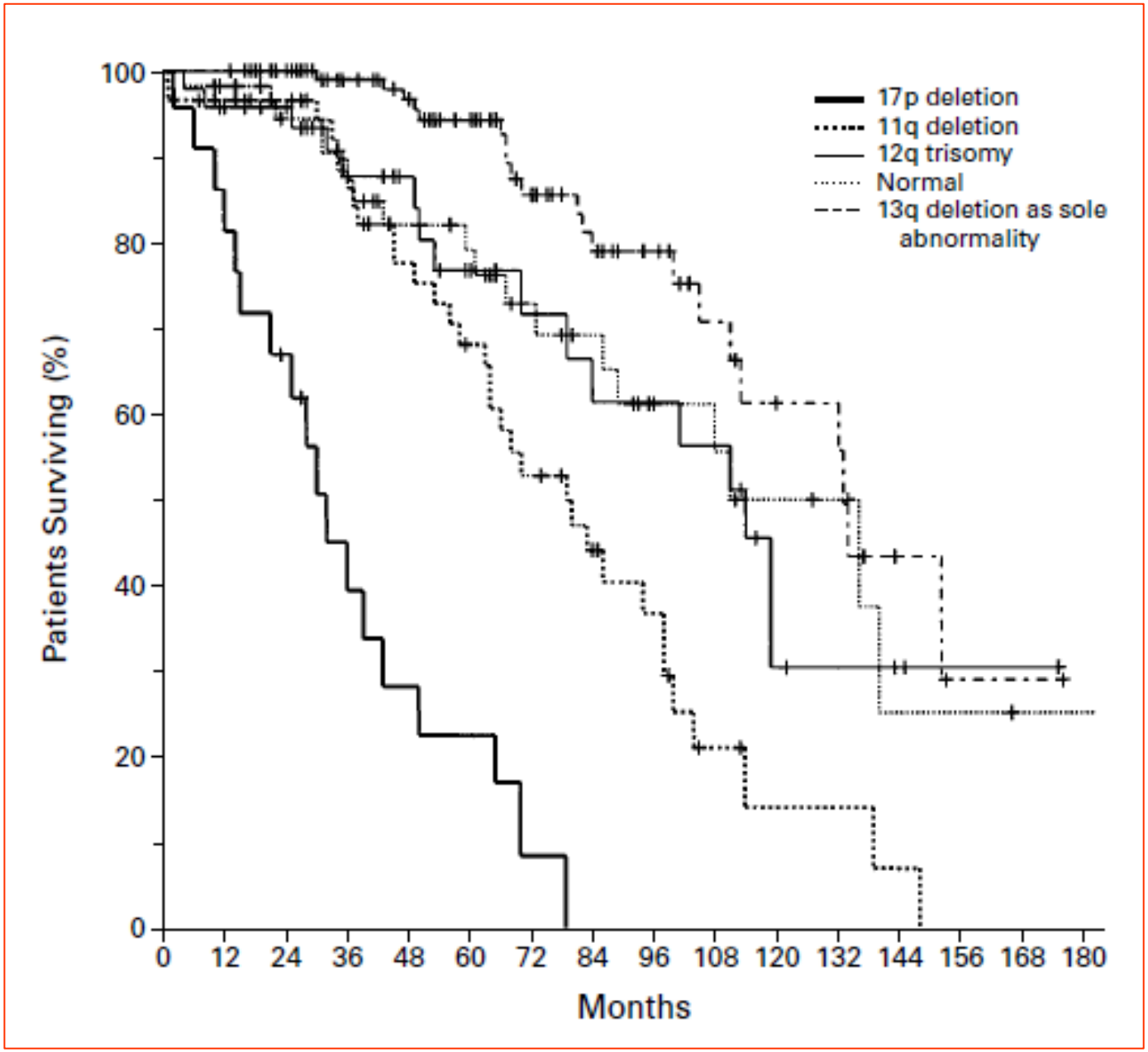
- “unmutated” IgVh
- CD38+ ZAP70+/-
- ATM deletion
- COMPLEX KARYOTYPE

17p- and CLL/PL





- "unmutated" IgVh
- CD38+/ZAP70+
- TP53 deletion
- refractory to purine analougs



NEJM, 2000. Volume 343 Number 26

Conclusioni

L'analisi citogenetica è necessaria per la diagnosi di alcune malattie oncoematologiche (LMC, APL, CEL, etc.) in cui il trattamento si basa sulla anatomia genetico-molecolare della lesione.

L'analisi citogenetica è necessaria per un completo inquadramento prognostico

L'analisi citogenetico-molecolare rappresenta un metodo rapido ed integra lo studio citogenetico convenzionale in casi a presentazione atipica

L'analisi citogenetico-molecolare offre la possibilità di un rapido screening di lesioni note alla diagnosi su nuclei in interfase