Introduction to Clinical Cytogenetics: Lecture 3

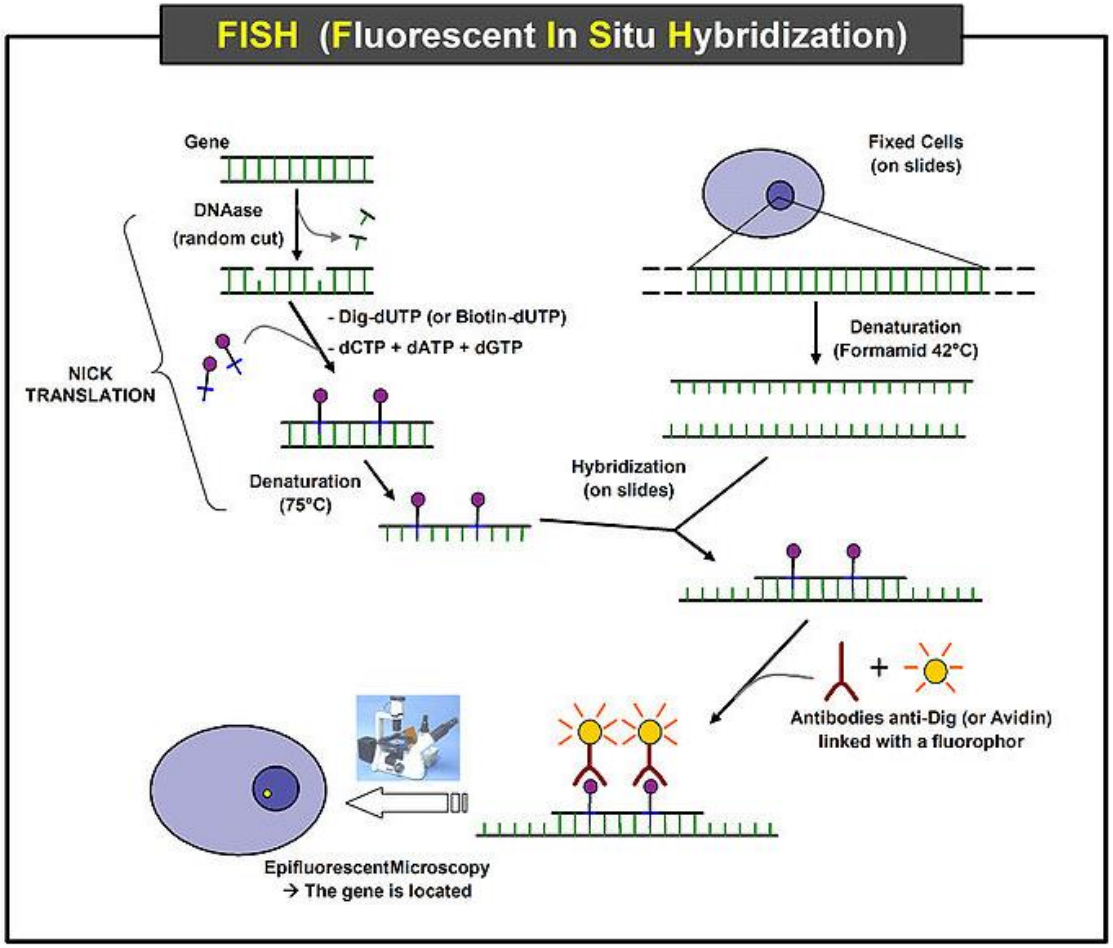
- Cinthya J Zepeda Mendoza, PhD
- Medical Director, Cytogenetics and Genomic Microarray, ARUP Laboratories
- Assistant Professor, Department of Pathology, University of Utah
- Email: Cinthya.Zepeda@aruplab.com

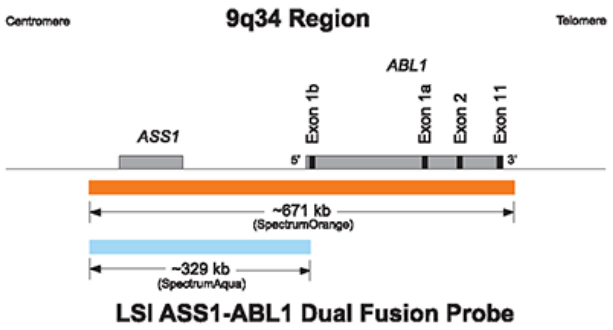
Introduction to Cytogenetics III

- FISH technique overview
- FISH advantages & disadvantages
- Cancer FISH applications
- Genomic Microarray
- Cancer CMA applications
- Cytogenetics technique summary

FISH Procedure

- A probe consisting of a specific DNA sequence is designed to target a locus
- A fluorescent tag is attached to the probe to allow for microscopic visualization
- Probe types: Enumeration (counting), dual fusion, break-apart



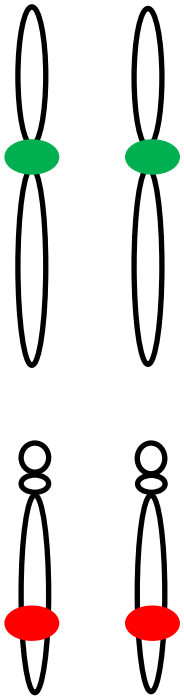


200 cells are analyzed for interphase FISH

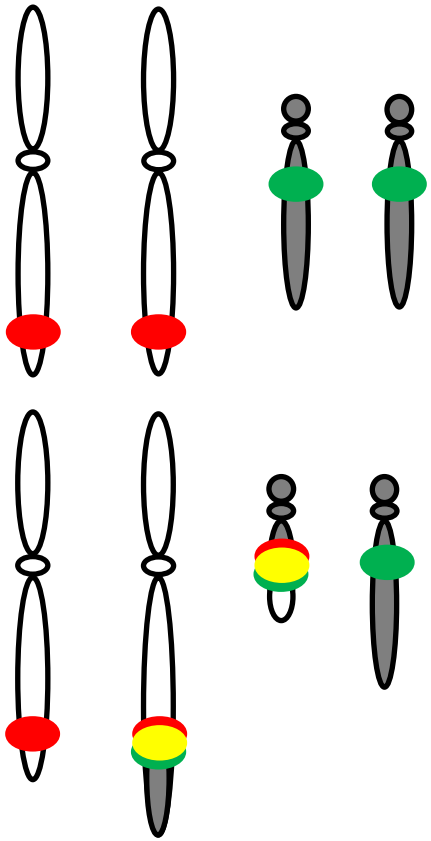
Lower limit of detection is probe-specific, generally between 1-5 %

FISH probe strategies

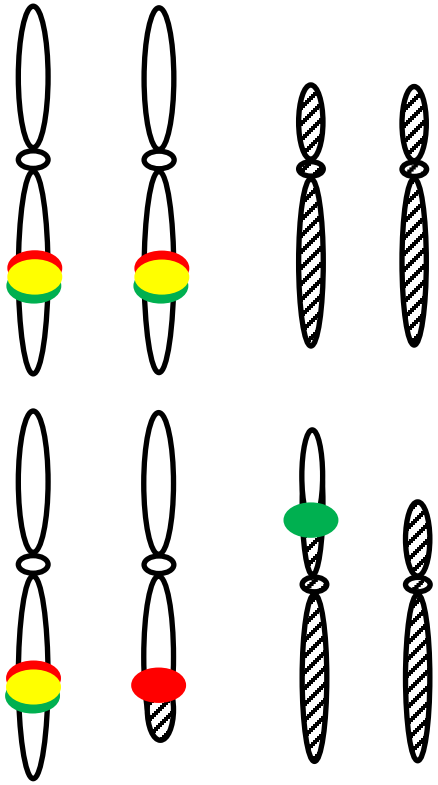
Enumeration (counting)



Dual fusion

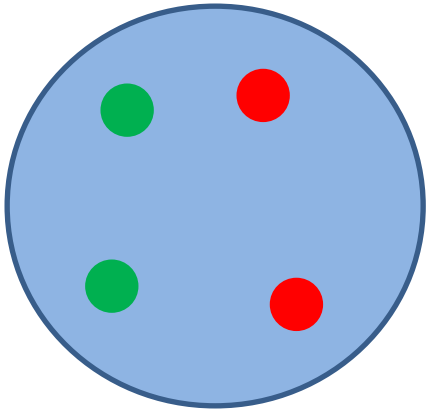


Break-apart

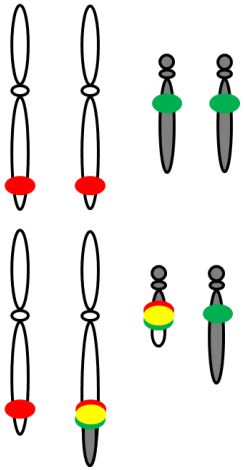


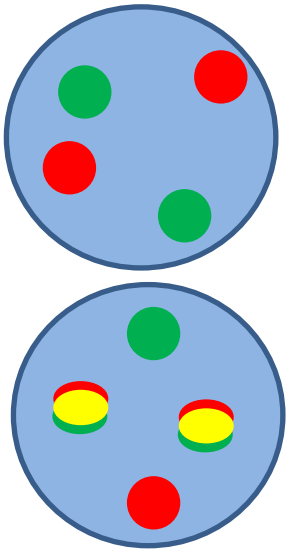
FISH probe strategies

Enumeration (counting)

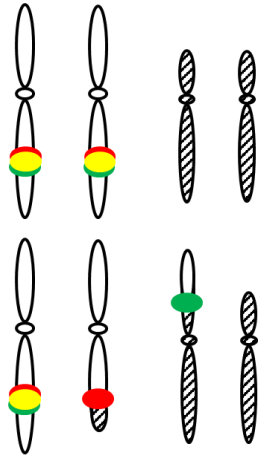


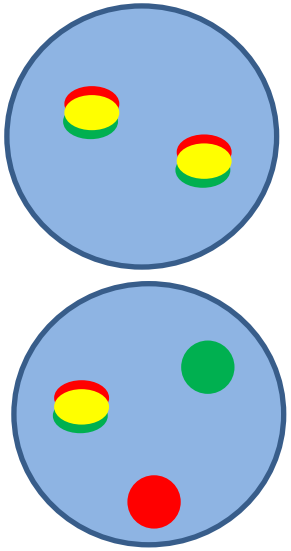
Dual fusion





Break-apart

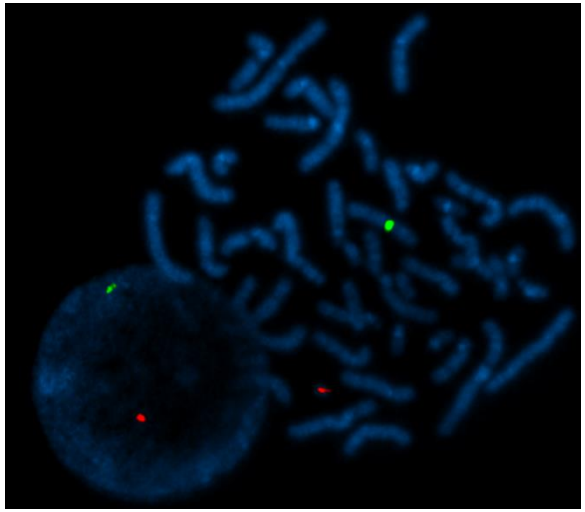




Fluorescence *in situ* hybridization (FISH)

- A fluorescently labeled DNA fragment is used to detect a chromosome, region or gene *in situ*
- Advantages:
  - Much higher resolution compared to G-banding for identifying deletions, duplications, insertions, and translocation breakpoints (down to the 100's of kb range)
  - Can use cells in any state of the cell cycle (interphase or metaphase), as well as archived tissue
  - Does not require culturing = shorter TAT
  - Greater sensitivity for low level mosaicism detection compared to chromosomes
- Limitation:
  - Targeted approach: only analyzing the region of the genome that is complementary to your probe

FISH for X and Y centromeres on an interphase and metaphase cell

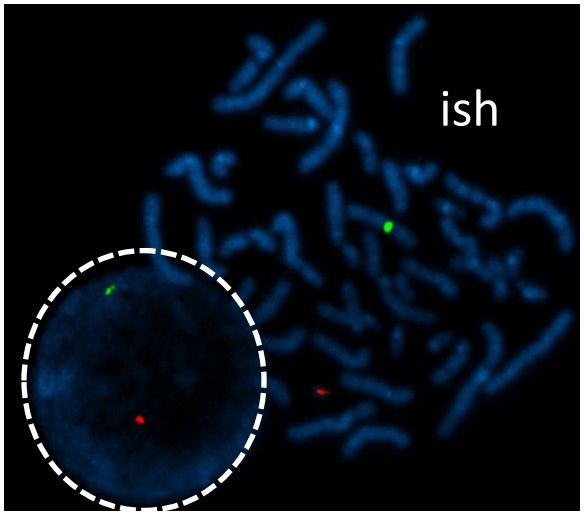


When to FISH?

- Detecting small (submicroscopic) changes
  - Deletions, duplications, translocations, insertions, inversions
  - For undiagnosed patients, GMA is recommended
- Detecting abnormalities in non-dividing (interphase) cells
- Detecting mosaicism below the limit of detection of chromosome analysis and genomic microarray

FISH Nomenclature

Two types of strategies:



Normal female:
46,XX.ish X(DXZ1x2,SRYx0)

Normal 22q11.2 region:
46,XX.ish 22q11.2(D22S75x2)

Deletion of probe at 22q11.2:
46,XX.ish del(22)(q11.2q11.2)(D22S75-)

Two copies of ERBB2:
nuc ish (D17Z1,ERBB2)x2[200]

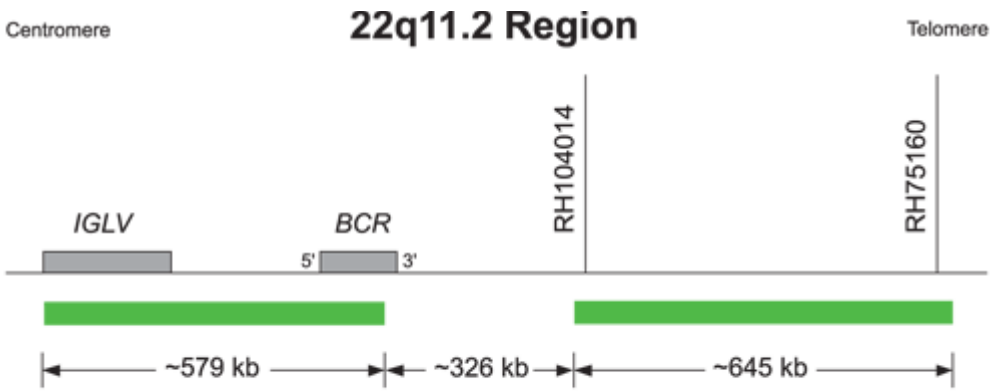
Homozygous D13S319 deletion:
nuc ish (D13S319)x0[50/200]

ERBB2 amplification:
nuc ish (ERBB2 amp)[200]

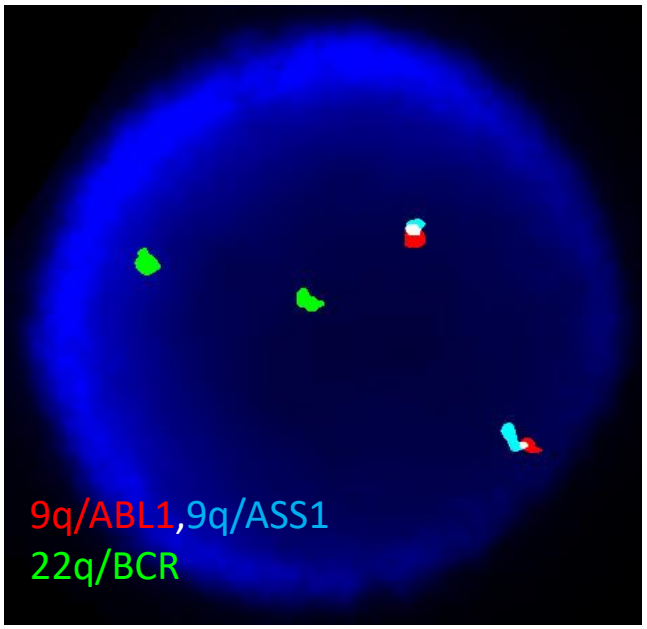
Two copies of BCR and ABL1:
nuc ish (ABL1,BCR)x2[200]

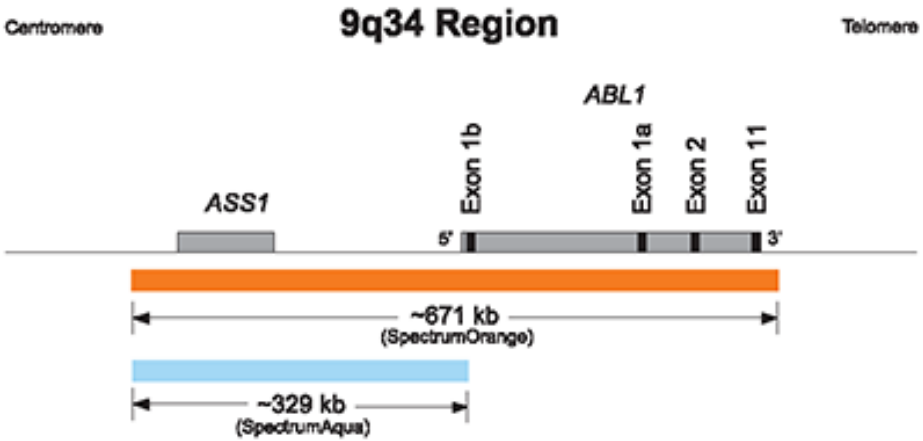
Typical BCR/ABL1 translocation:
nuc ish (ABL1,BCR)x3(ABL1 con BCRx2)[50/200]

nuc ish (ABL1,BCR)x2[200]

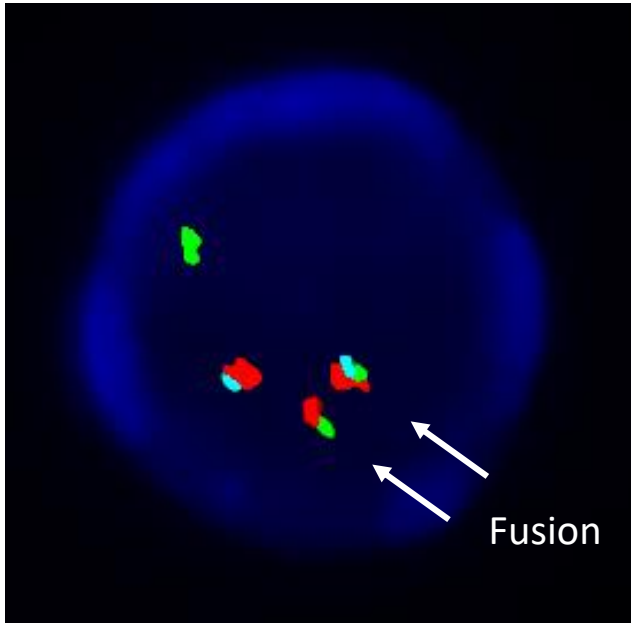


LSI BCR SpectrumGreen Dual Fusion Probe





LSI ASS1-ABL1 Dual Fusion Probe

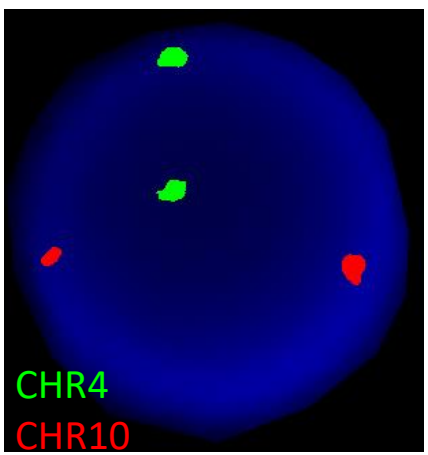


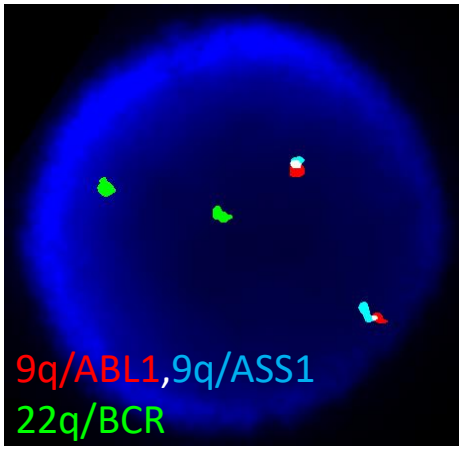
nuc ish (ABL1,BCR)x3(ABL1 con BCRx2)[50/200]

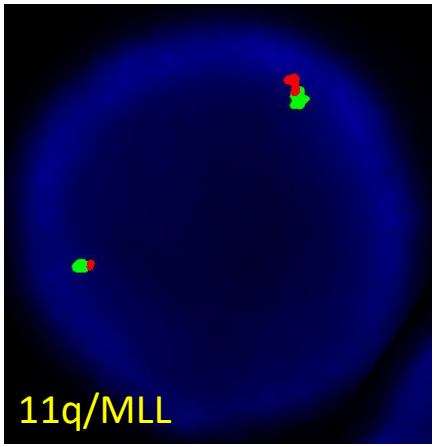
FISH Applications in Oncology Studies

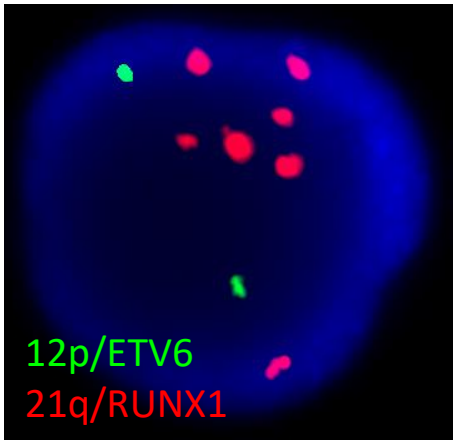
- Diagnosis: often using panels targeting recurrent and/or prognostic/therapeutic alterations, some cytogenetically cryptic
- Monitoring: using a FISH probe(s) specific to the abnormal primary clone or using a panel to simultaneously monitor for residual disease and markers of disease progression

Pediatric ALL Panel

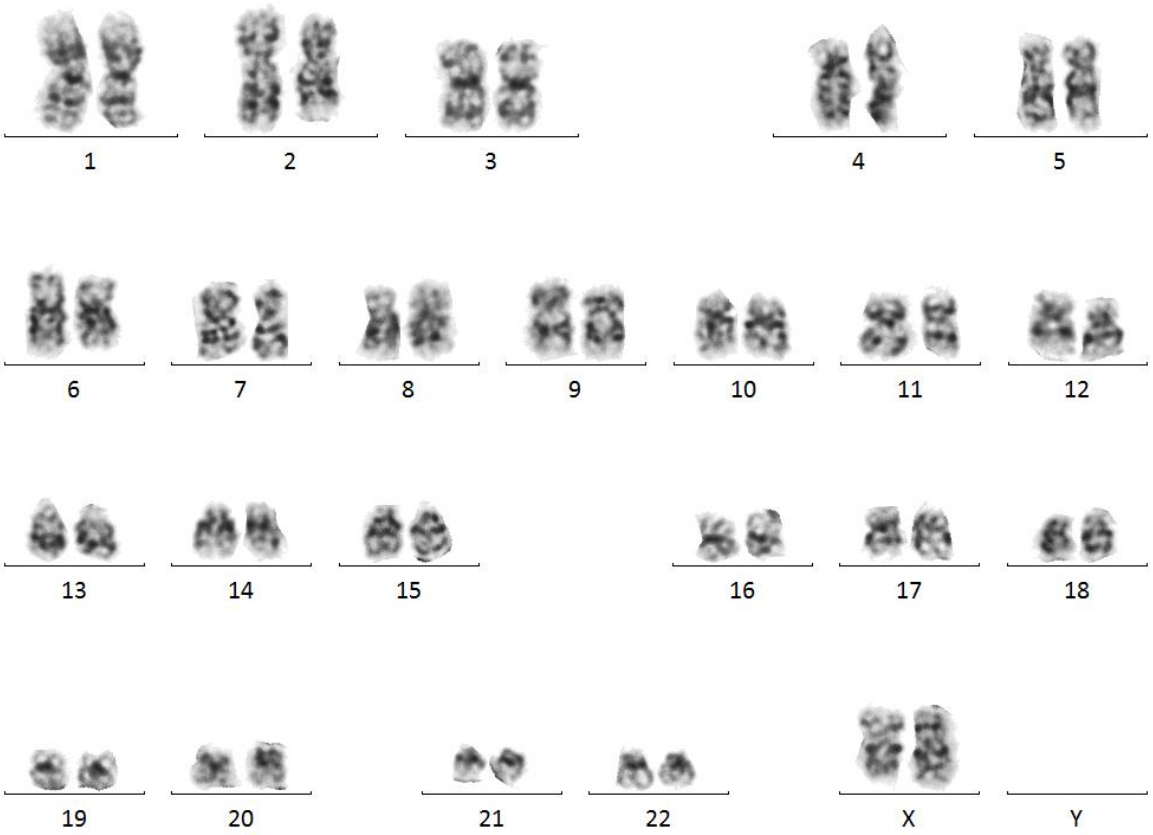






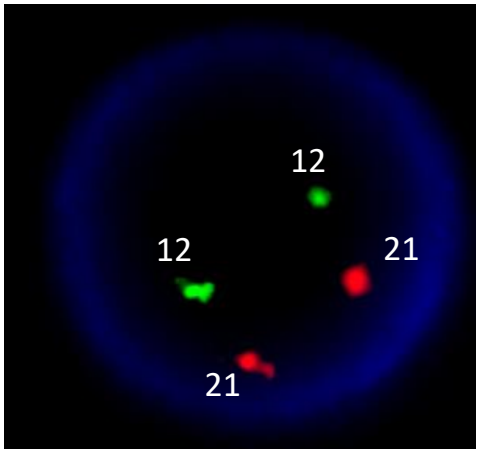


Utility of FISH in B-ALL

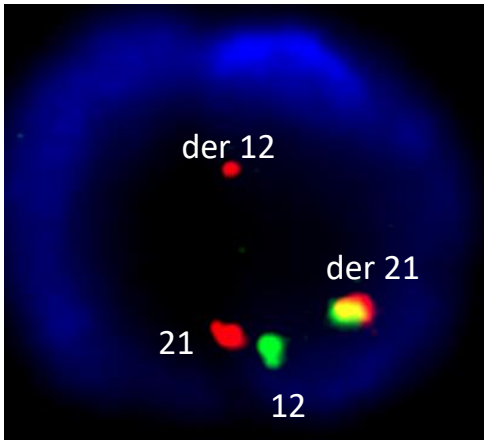


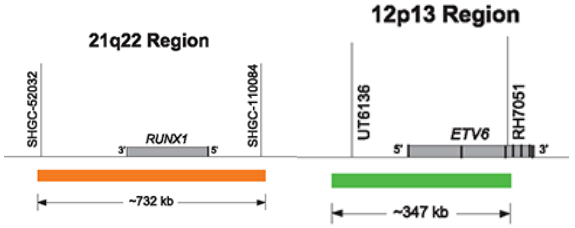
46,XX[20]

Normal signal pattern

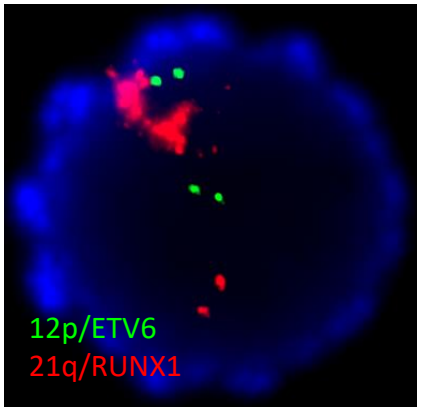


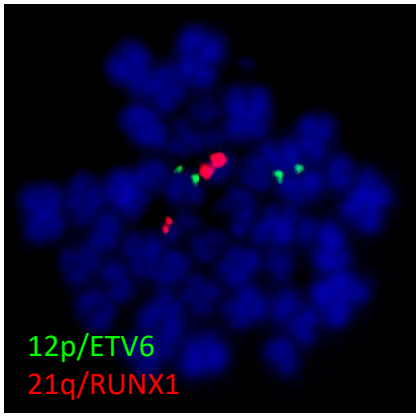
***ETV6/RUNX1* fusion pattern**

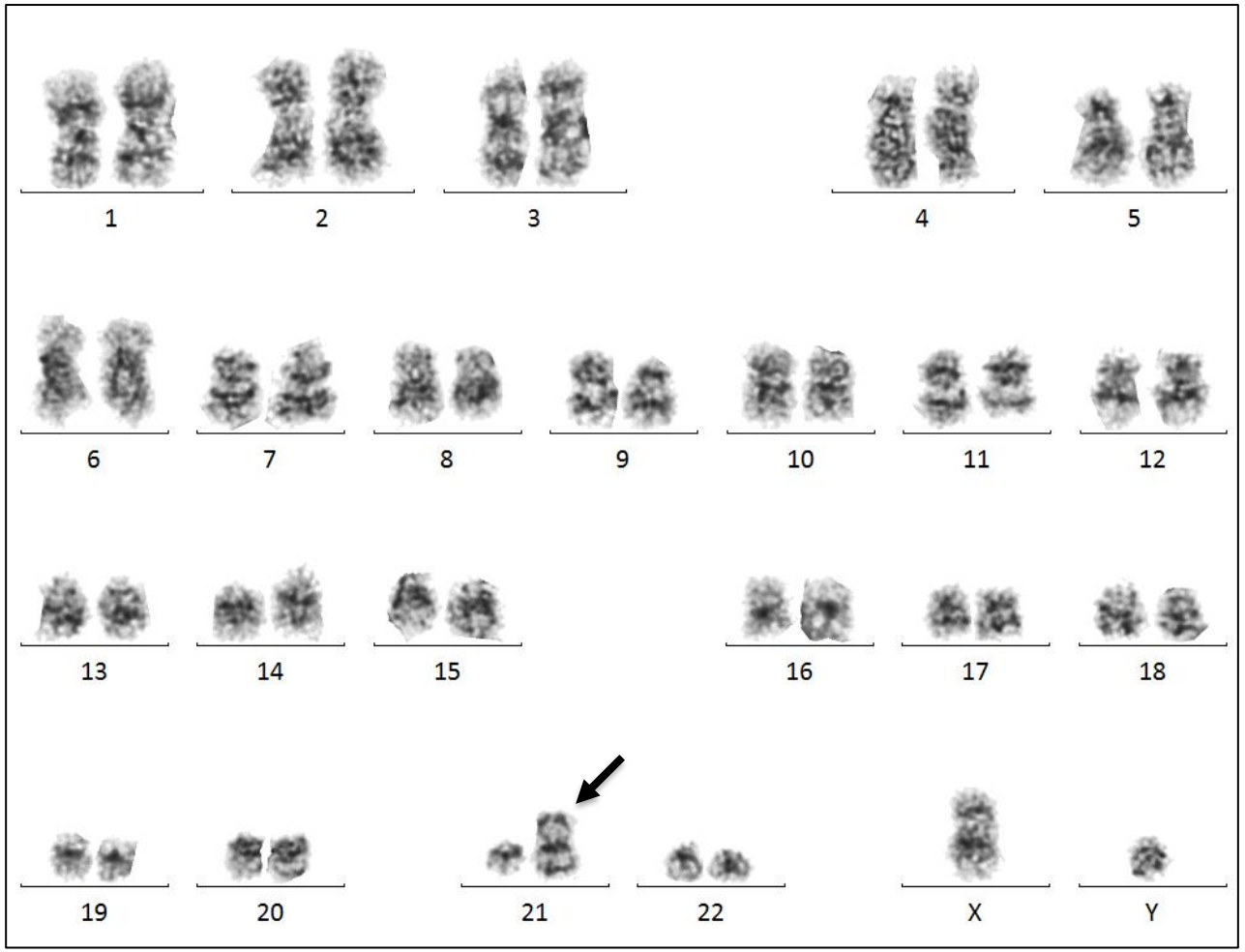




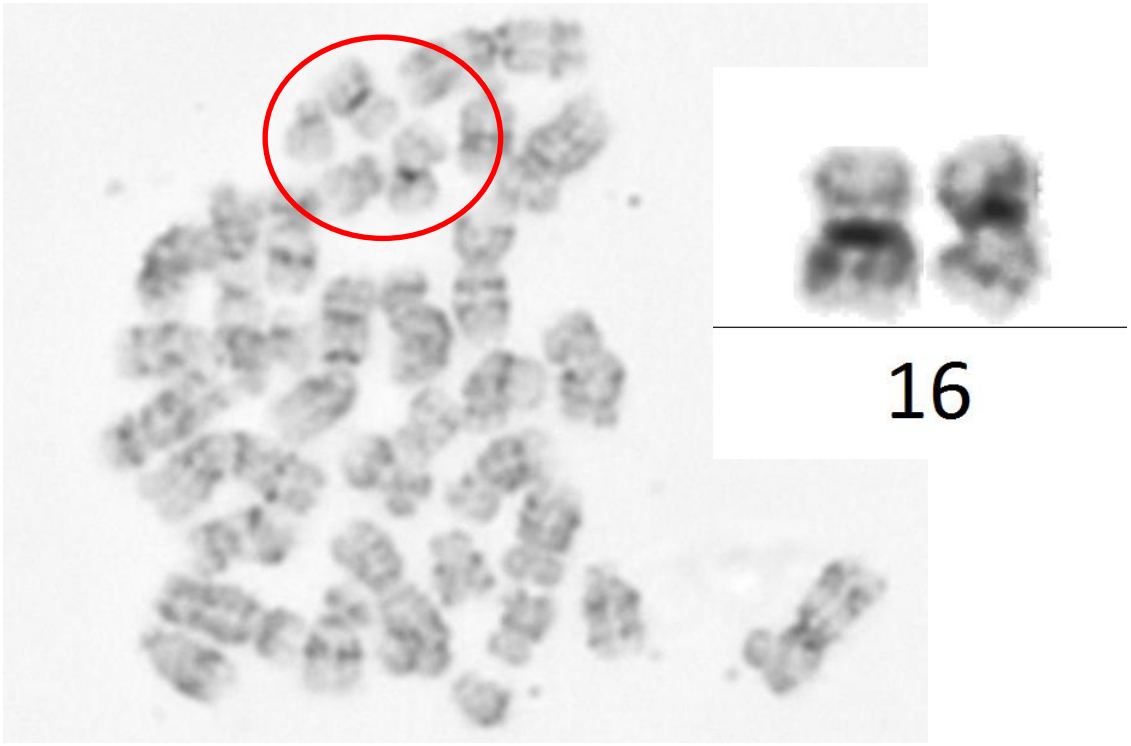
Utility of FISH + Karyotype in B-ALL

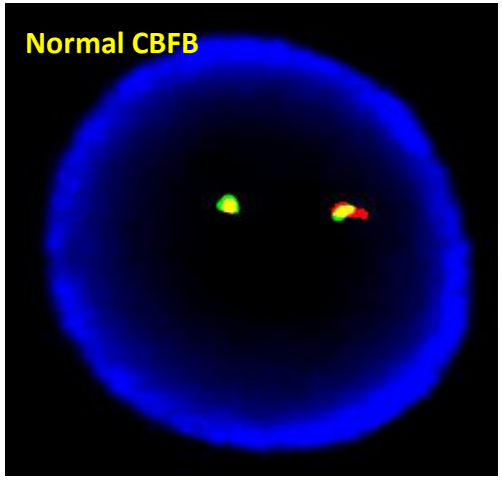


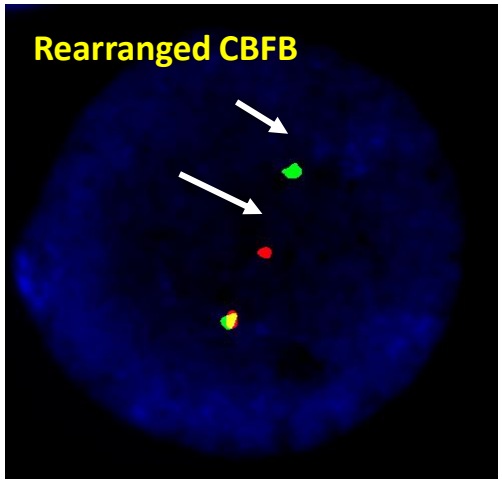




Utility of FISH in *de novo* AML





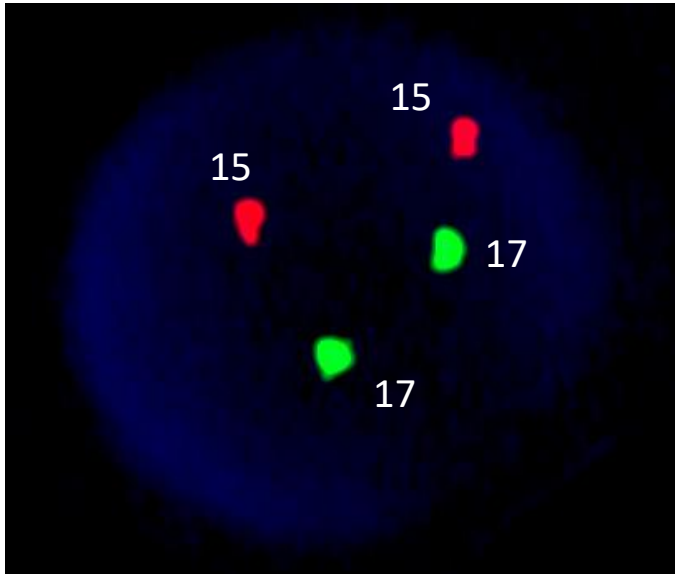


- The inv(16) *CBFB-MYH11* fusion is a cytogenetically subtle rearrangement associated with a favorable prognosis

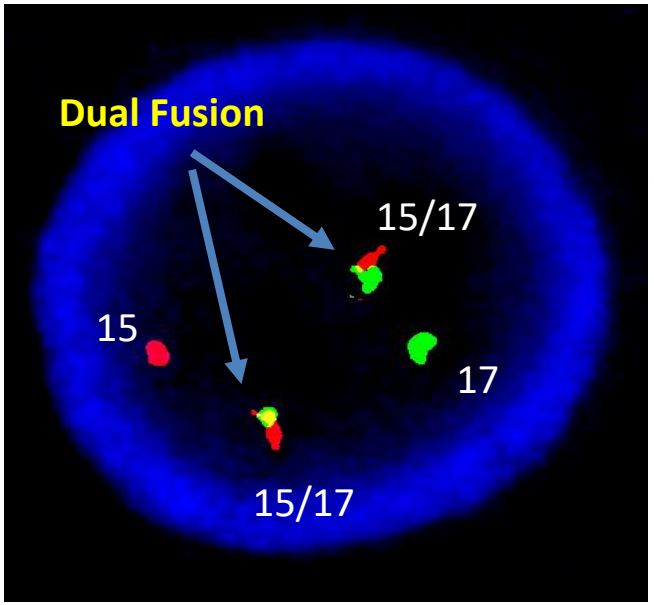
➢ FISH is useful for confirmation at diagnosis and for monitoring

Utility of FISH in *de novo* AML

Normal



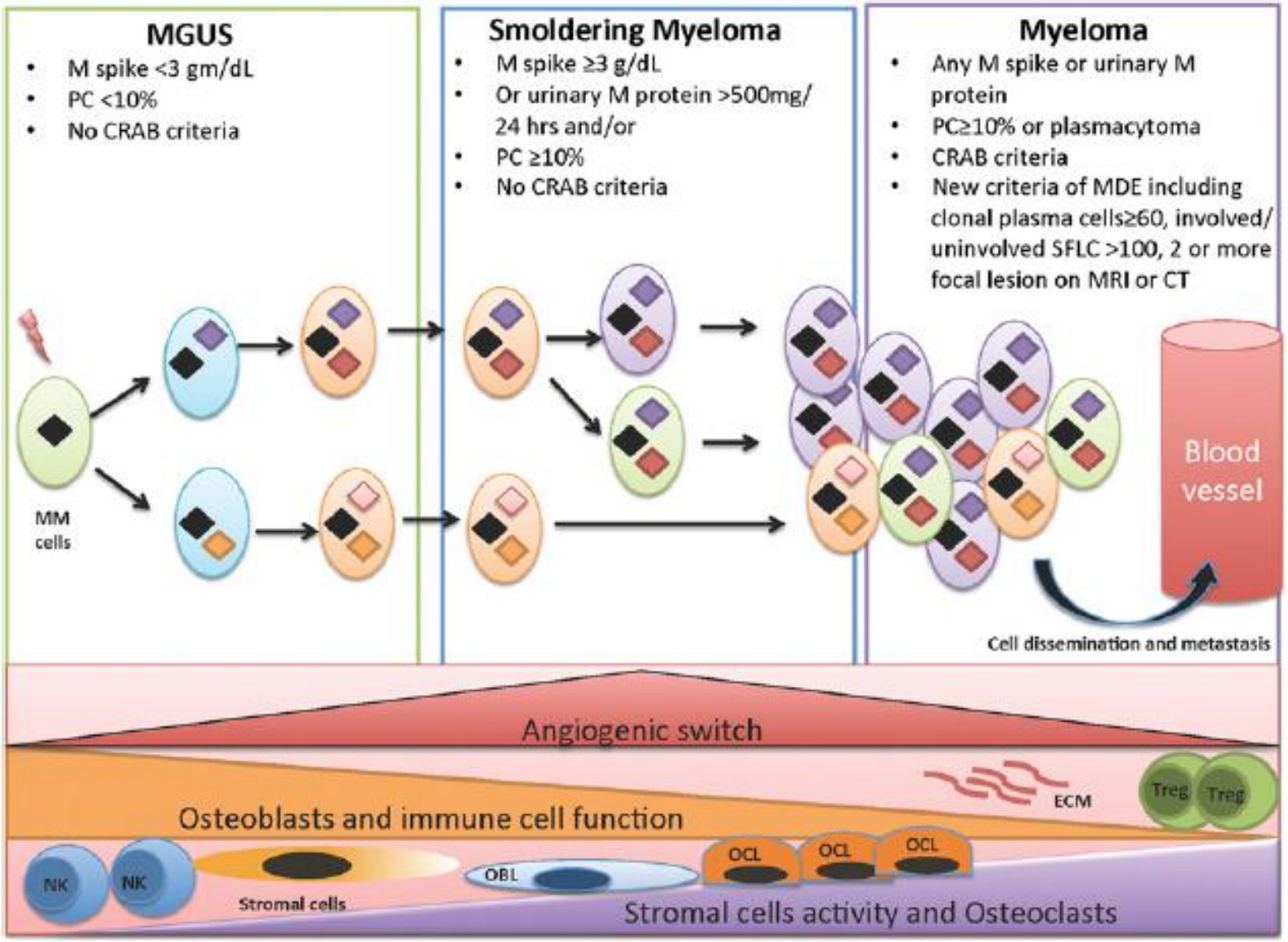
Abnormal



- The t(15;17) *PML-RARA* fusion is diagnostic for APL, which can lead to disseminated intravascular coagulopathy, a medical emergency, treatable with ATRA

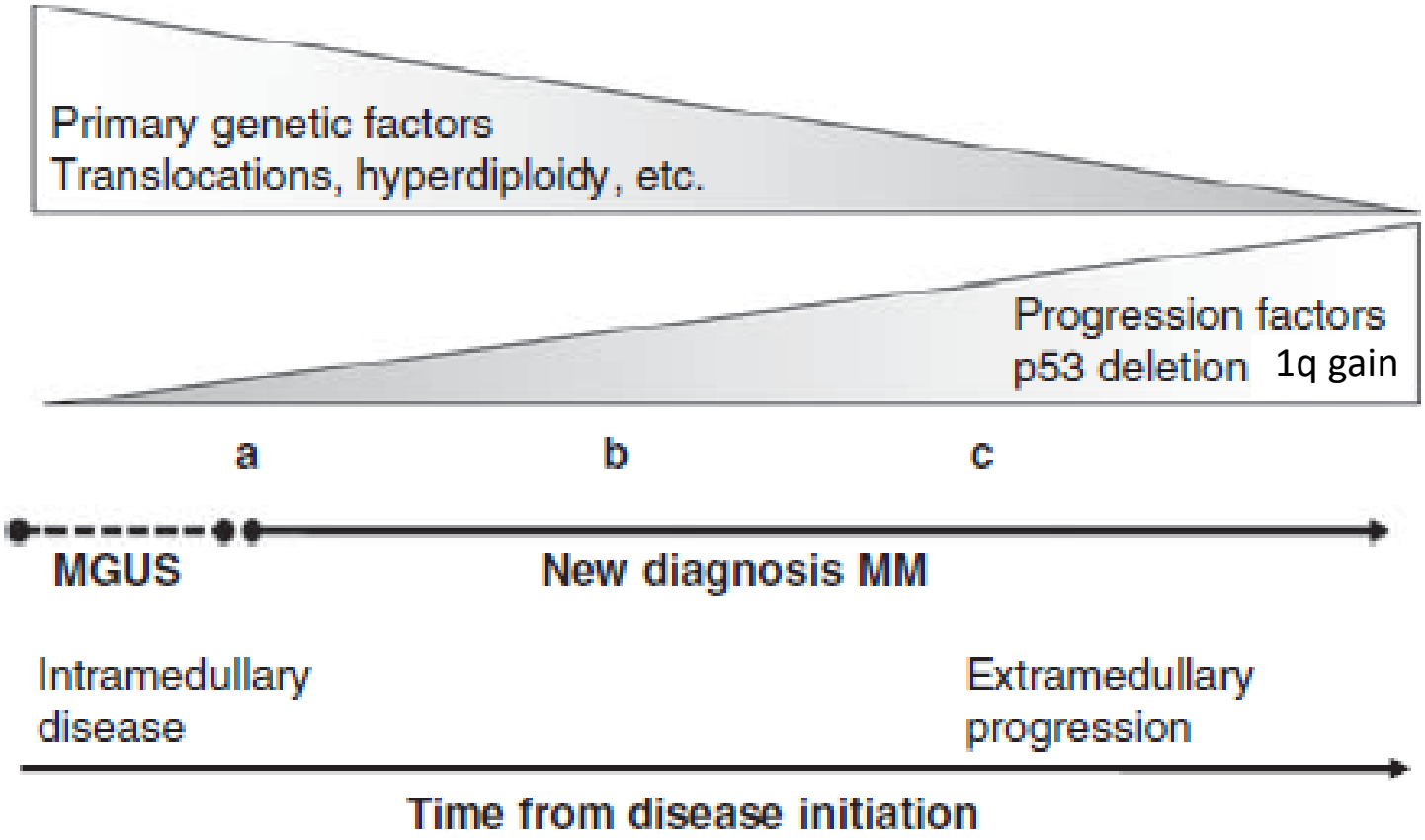
➢ FISH (or RT-PCR) is recommended at diagnosis for quick turn-around time

Plasma cell neoplasms (PCNs)



Ghobrial and Landgren; *Blood*. 2014

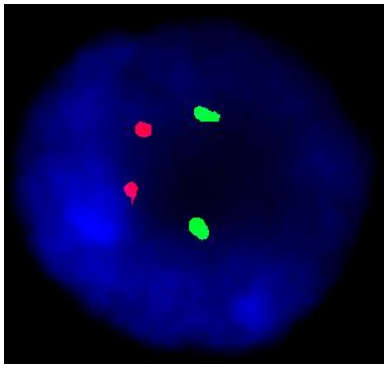
Genetic profiles of PCN across diagnostic time points



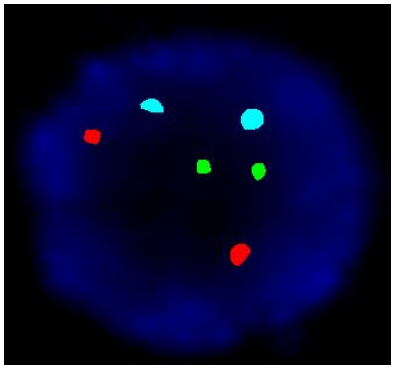
Modified from Fonseca et al. (IMWG) *Leukemia*. 2009

Utility of FISH in PCN

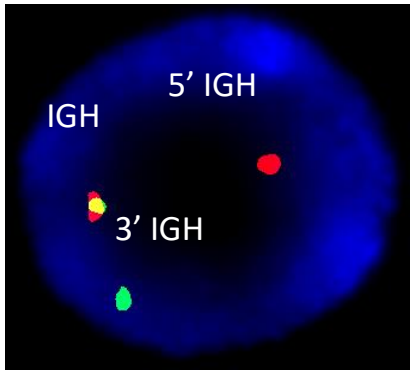
1q21/17p13.1



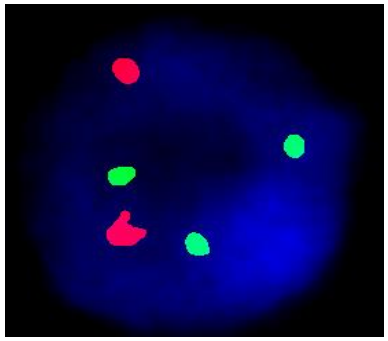
9q34/15q22/17q21.2



14q32

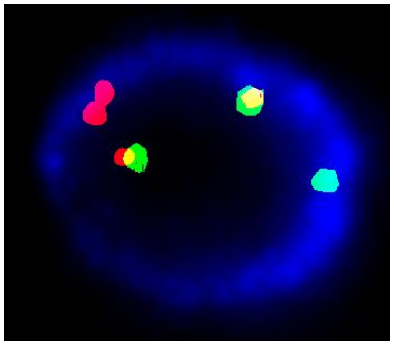


11q13/14q32



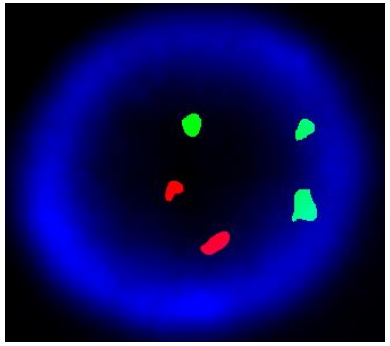
CCND1/
IGH

4p16.3/14q32



FGFR3
/IGH

14q32/16q23

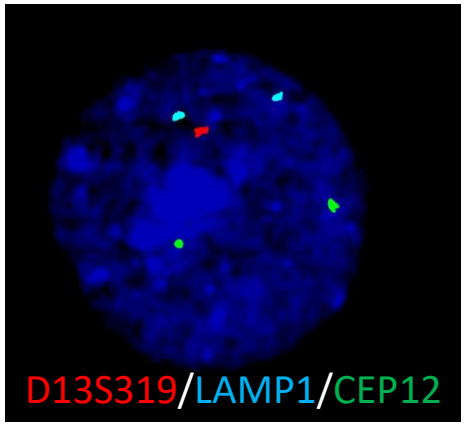


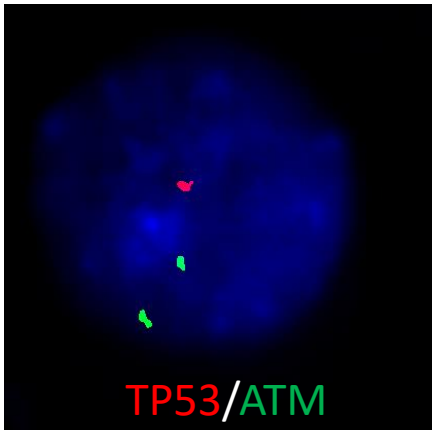
MAF/
IGH

- Use of CD138+ isolation and PCN labeling techniques has significantly improved the diagnostic yield (from 25-40% to >90%)

Cytogenetic risk stratification in CLL/SLL

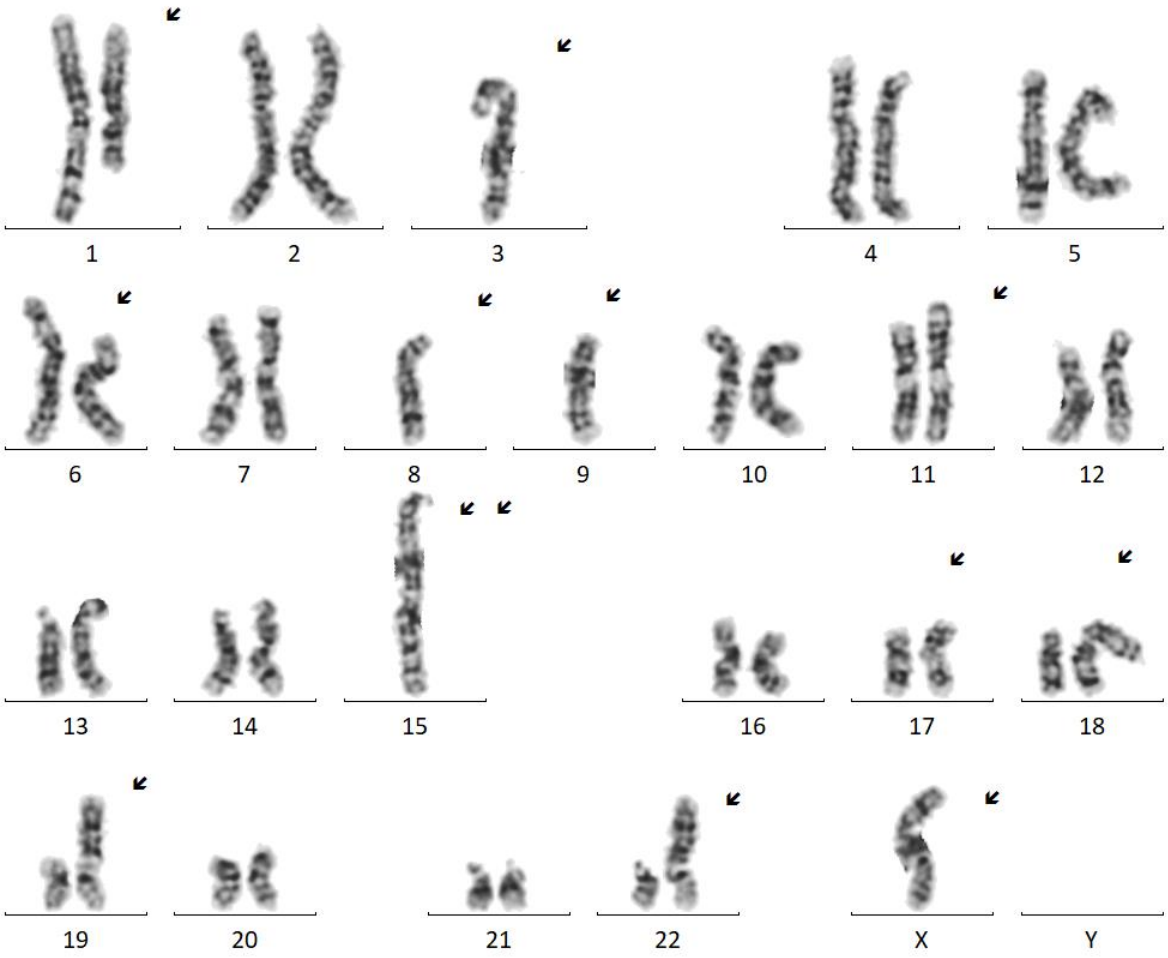
Risk Category	Genetic Entity	Proportion of cases by iFISH	Oncogene/ TSG
Unfavorable	17p deletion	7-20%	TP53
	11q deletion	15-20%	ATM, BIRC3
	Complex karyotype (≥3 abnormalities)	n/a	Multiple, incl. TP53
Intermediate/ Neutral	Trisomy 12/12p13	15-20%	MDM2, others
Favorable	13q deletion (sole)	50-55%	miR15a/16





Sources, modified from: WHO 2018; NCCN; Dohner et al *N Engl J Med.* 2000; Malek *Oncogene* 2013

Don’t forget your chromosomes!



41,X,-X,
add(1)(q12),
-3,
del(6)(p23),
-8,
-9,
add(11)(p15),
-15,
der(15)?t(1;15)(q12;q26.1),
add(17)(p13),
der(18)t(15;18)(p11.32;q24),
der(19)t(8;19)(q13;p13.3),
der(22)t(9;22)(q12;p11.2)

Targeted del/dup detection: FISH

SYNDROMES		
SUSPECTED DIAGNOSIS	**PROBE TARGET**	**GENE(S)/UNIQUE SEQUENCE**
Aneuploidy, common	13/18/21/X/Y	
4p-	4p16.3	*WHSC1*
5p-	5p15.2	D5S23-D5S721
15q11.2-13 duplication	15q11.2-13	D15S11, D15S10
22qter deletion	22q13.3	22qtel (*SHANK3*)
Angelman	15q11.2-13	D15S10
Cri-du-chat	5p15.2	D5S23-D5S721
DiGeorge	22q11.2	TUPLE-1 (HIRA)
Kallman	Xp22.3	*KAL1*
Male detection (*SRY*)	Yp11.3	*SRY*
Miller-Dieker (Lisencephaly)	17p13.3	*LIS1*
Phelan McDermid	22q13.3	22qtel (*SHANK3*)
Prader-Willi	15q11.2-13	D15S10
SHOX	Xp22.3	*SHOX*
Smith-Magenis	17p11.2	*SHMT1-TOP3-FL11-LLGL1*
SRY	Yp11.3	*SRY*
Steroid sulfatase deficiency (*STS*)	Xp22.3	*STS*
Velocardiofacial (VCF)	22q11.2	TUPLE-1 (HIRA)
Williams (elastin)	7q11.23	*ELN-LIMK1*-D7S613
Wolf-Hirschhorn	4p16.3	*WHSC1*

http://www.aruplab.com/Testing-Information/resources/FISH_probes/fish_probes_constitutional.pdf

Genomic Microarray

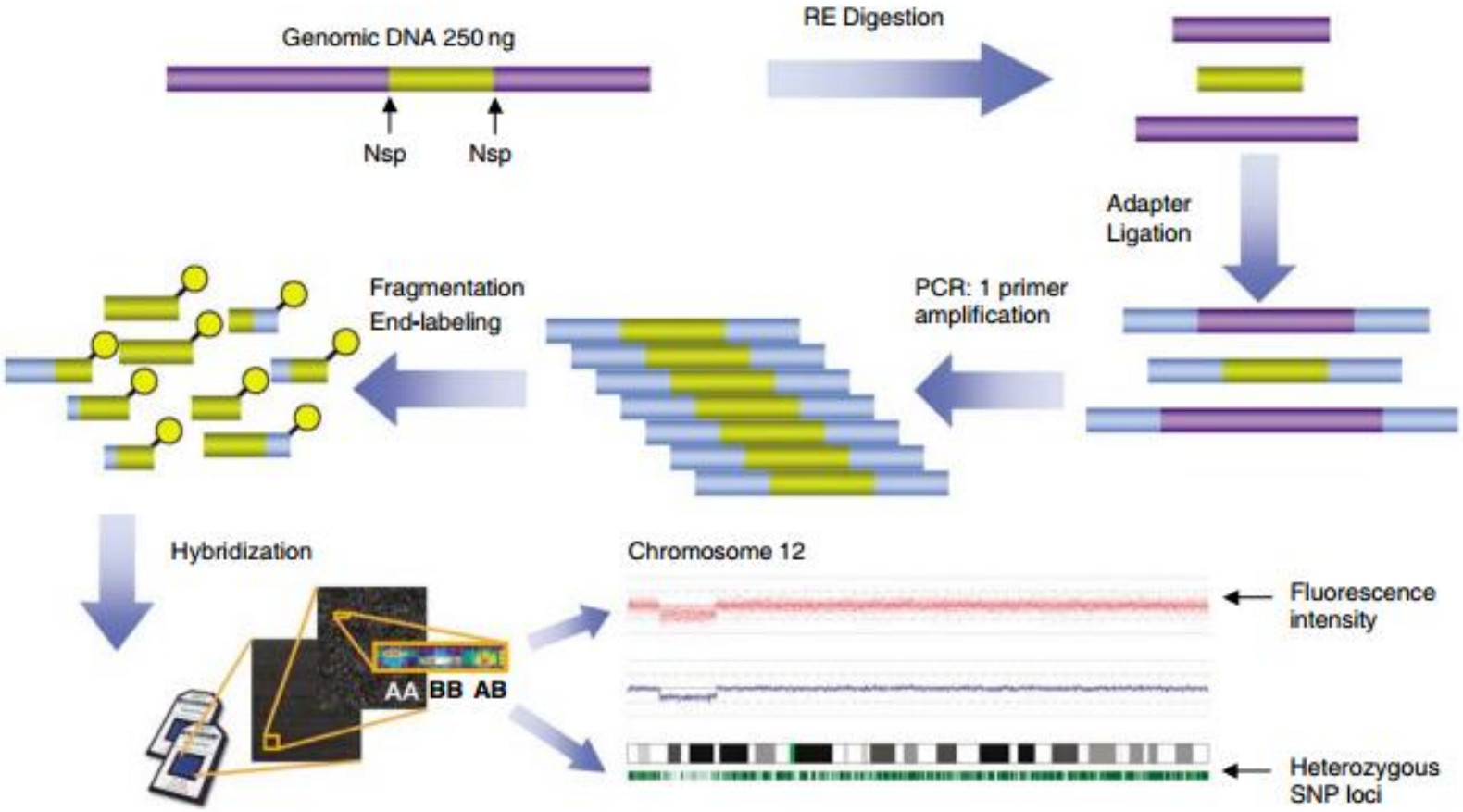
Definition: A genome-wide analysis technology used to assess DNA copy number, and in some cases genotype, in a sample

- Copy number variants (CNVs): gains (duplications) and losses (deletions) of genomic material
- Copy-neutral alterations: absence- or loss-of-heterozygosity (AOH/LOH)
  - Absence of heterozygosity is the preferred term for describing constitutional copy neutral changes (does not impose a mechanism of origin onto the change)

Synonyms

- Cytogenomic microarray
- Chromosomal microarray
- Array CGH (unlikely to interrogate genotype)
- SNP array (implied this includes interrogation of the genotype = copy neutral alterations)
- Cytogenetic microarray
- DNA microarray
- Microarray (too generic)

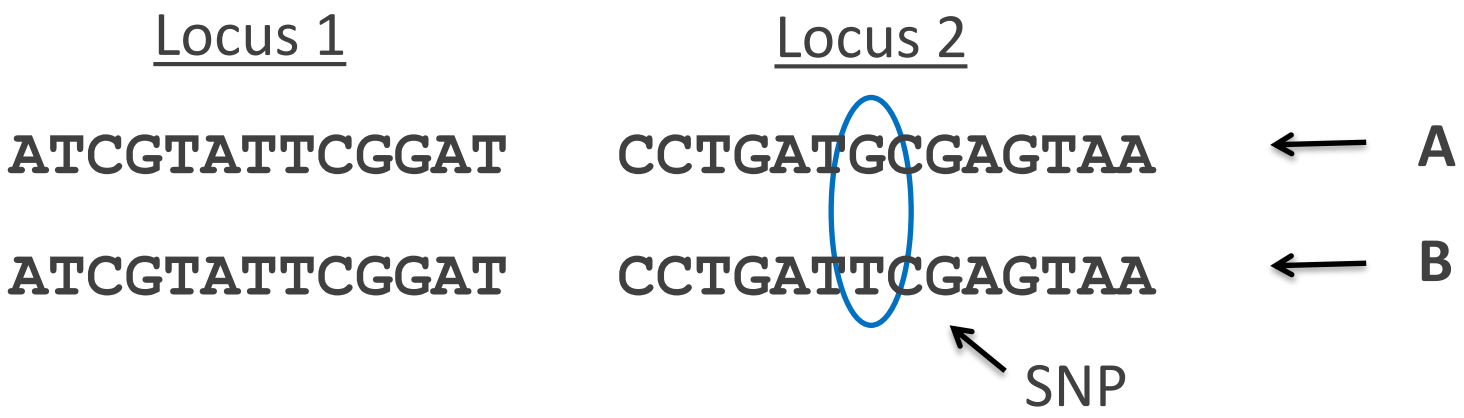
Genomic SNP Microarray (SNP-A) Process



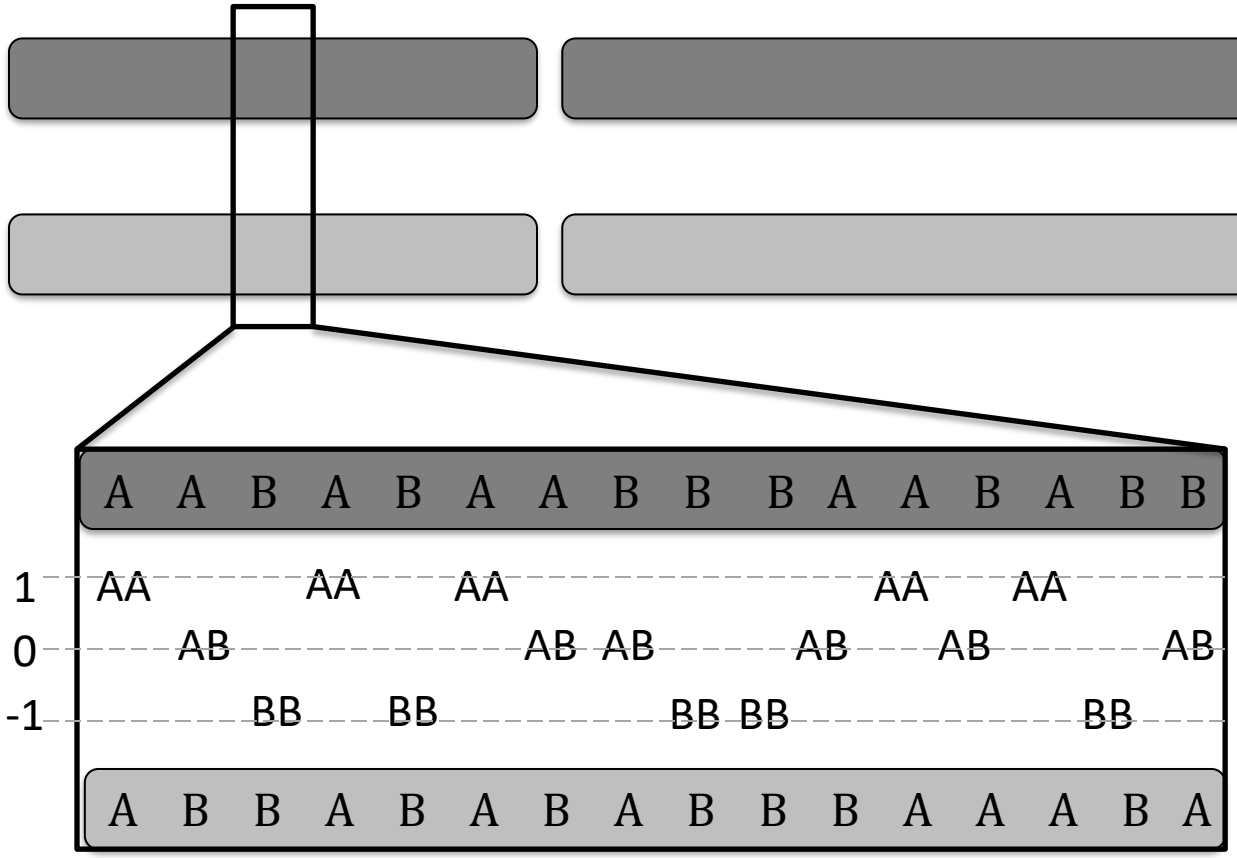
Tiu et al., Leukemia, 2007

SNP array design

- Copy number probes
  - Used to increase density of coverage genome-wide, within genes
- Polymorphic probes (contain SNPs)
  - Detect copy number and genotype
  - Used to interrogate genotype (generally two relatively frequent alleles, A or B) at select loci across the genome
  - SNP probes are not evenly distributed and are lower in density



Even distribution of AA, AB and BB genotypes generates a balanced allele pattern



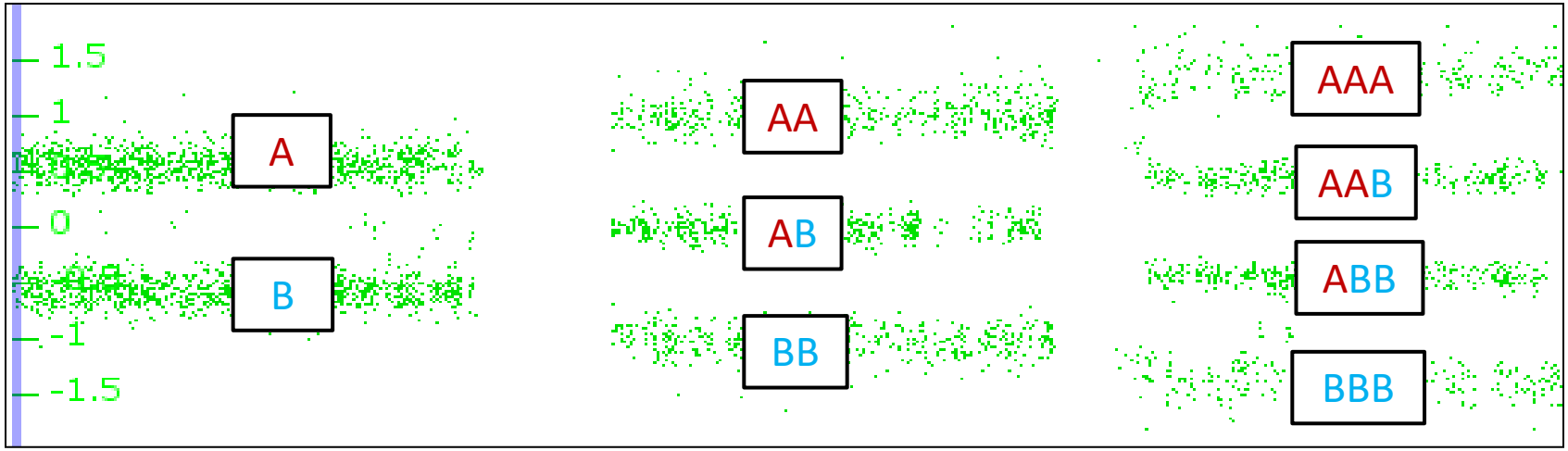
- Set A,B = 0.5
- Subtract #B from #A
- Plot (A-B) on y-axis

SNP probes can also show copy number changes

Deletion
(1 allele, 2 tracks)

Normal Diploid
(2 alleles, 3 tracks)

Duplication
(3 alleles, 4 tracks)



A: 0.5 – 0 = 0.5
B: 0 – 0.5 = -0.5

AA: (0.5+0.5) – 0 = 1
AB: 0.5 – 0.5 = 0
BB: 0 – (0.5+0.5) = -1

AAA: (0.5+0.5+0.5)–0 = 1.5
AAB: (0.5+0.5)–0.5 = 0.5
ABB: 0.5–(0.5+0.5) = -0.5
BBB: 0–(0.5+0.5+0.5) = -1.5

Pros and Cons of Genomic Microarray

Advantages

- High resolution technology
  - Down to 10's of kb range (compared to 3-5 Mb by 550-band chromosomes, 100's kb by FISH)
- No cell culturing or cell preparation required
  - Can use on archived tissues: frozen or formalin-fixed paraffin-embedded (FFPE)
- Detection of absence or loss of heterozygosity (AOH/LOH) if SNP genotyping is incorporated

Limitations

- Cannot detect balanced structural abnormalities (i.e. translocations, inversions)
- Cannot interrogate repetitive DNA sequence

Considerations

- May uncover findings unrelated to the indication for testing (incidental findings)

Clinical Utility of GMA in Postnatal Studies

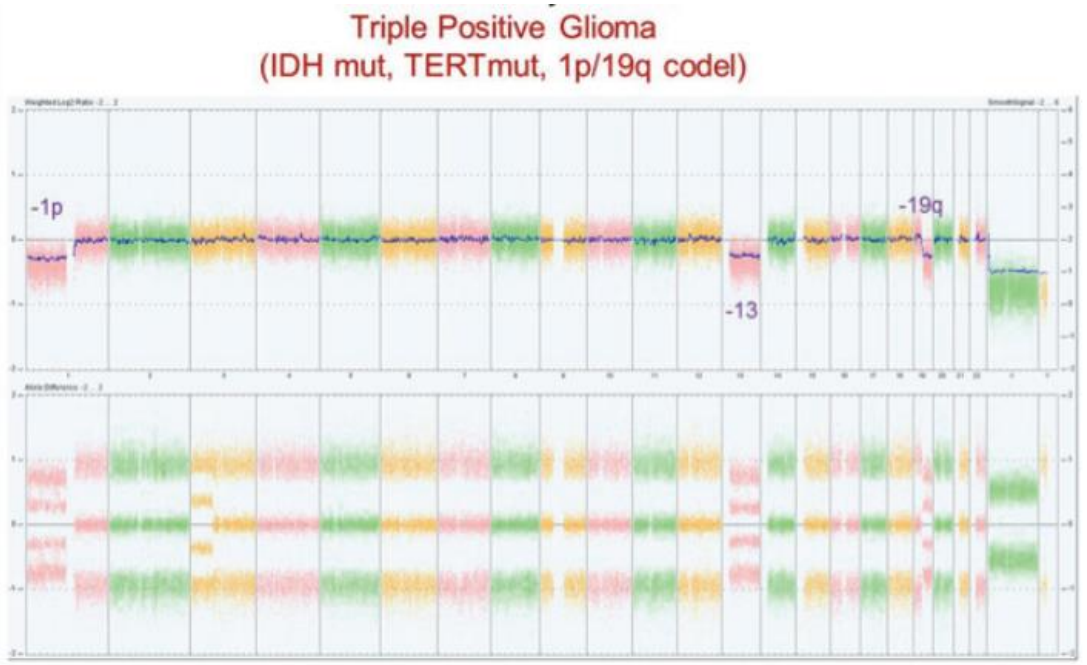
Consensus Statement: Chromosomal Microarray Is a First-Tier Clinical Diagnostic Test for Individuals with Developmental Disabilities or Congenital Anomalies

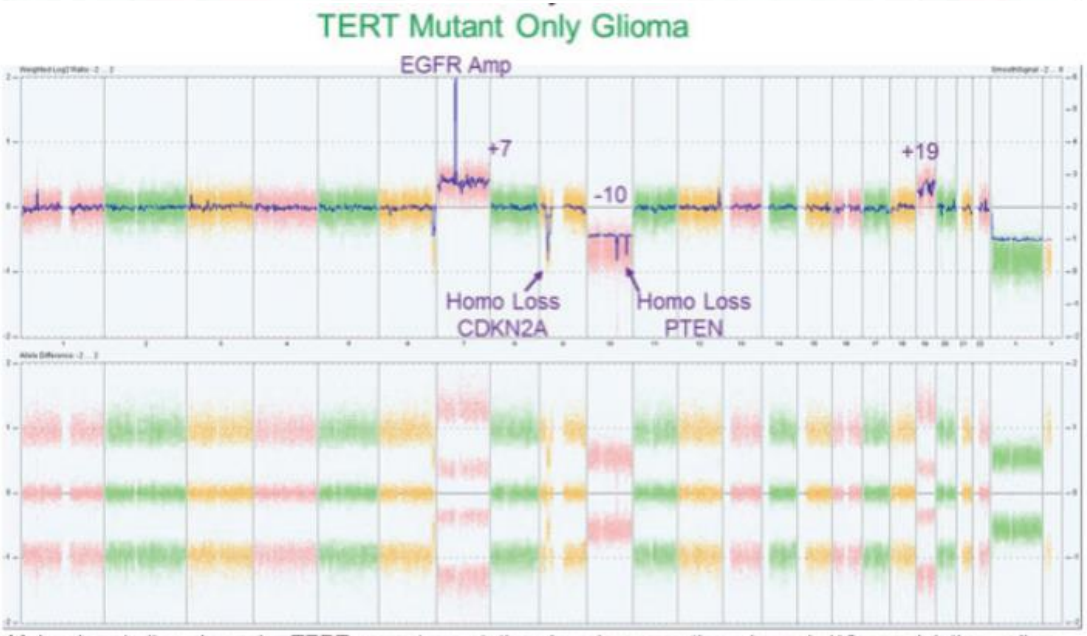
Miller et al., *The American Journal of Human Genetics* 86, 749–764, May 14, 2010

- International standards for cytogenomic arrays (ISCA) consortium: reviewed evidence from 33 studies, including >21,000 patients tested by GMA

➢ For genetic testing of individuals with unexplained developmental delay, intellectual disability, autism or multiple congenital anomalies, this technology offers a much higher dx yield (between 15-20%) compared to ~3% by karyotype and excluding other recognizable chromosome syndromes

Which cancers should be studied by GMA?

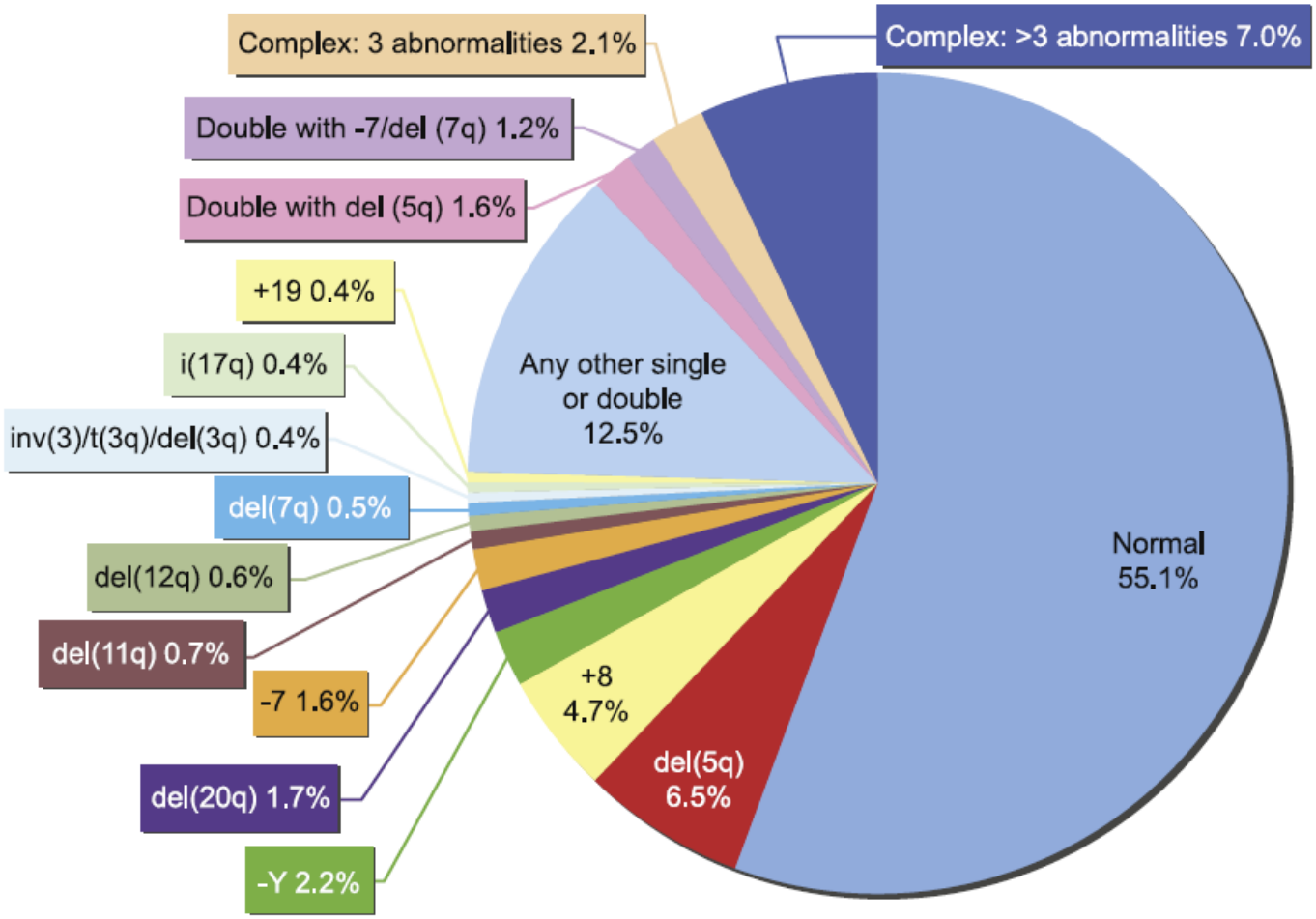




- Those characterized by recurrent copy number changes (whole/segmental aneuploidy and microdeletions/duplications) and/or loss of heterozygosity
- Those that do not grow well in culture or have poor mitotic activity compared to nonmalignant cells (typically have a normal karyotype)

From R.B.Jenkins., Mayo Clinic

Recurrent cytogenetic findings in MDS

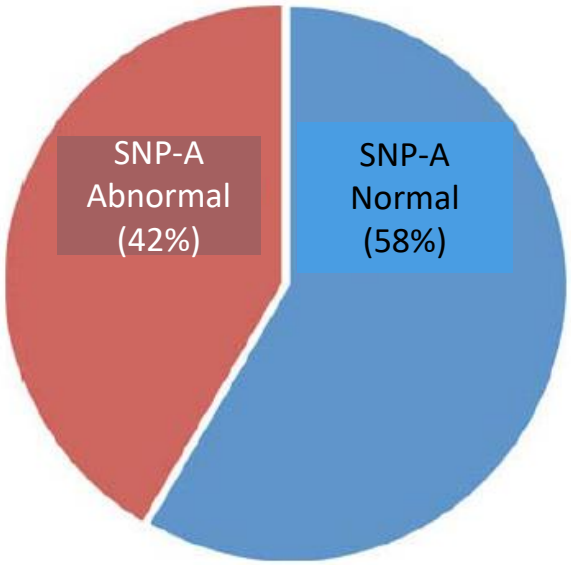


Schanz et al., 2012 J Clin Oncol (Table 2)

Image source: Nybakken and Bagg, JMD 2014

SNP-A increases the diagnostic yield in MDS from 50% to 70-80%

Normal karyotype (n=296, composite of multiple studies)



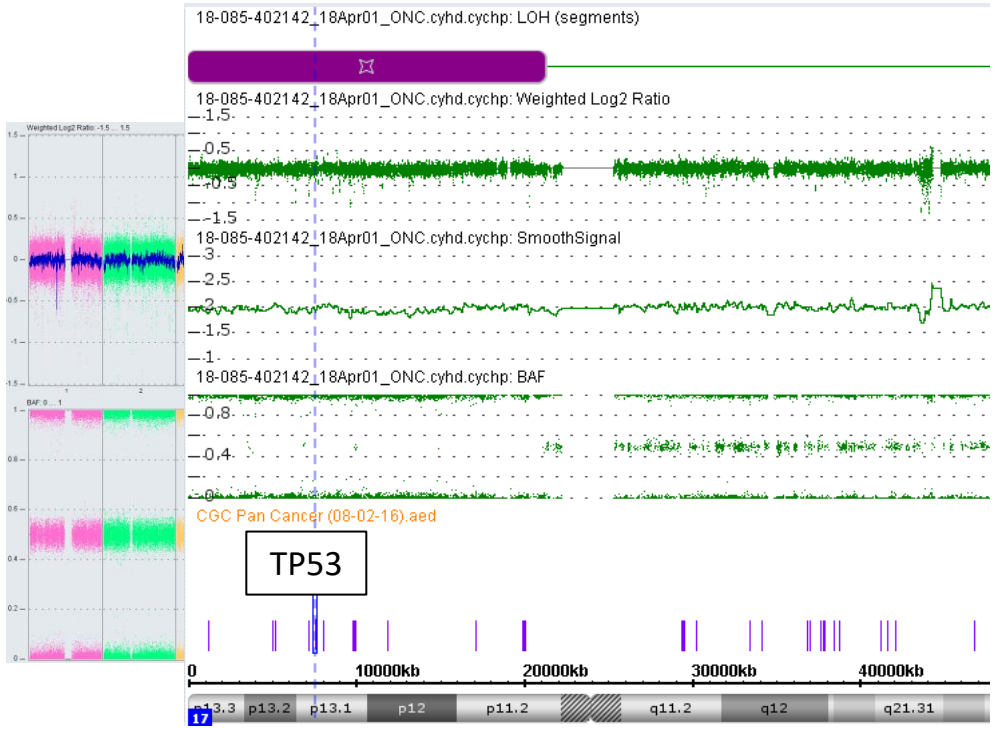
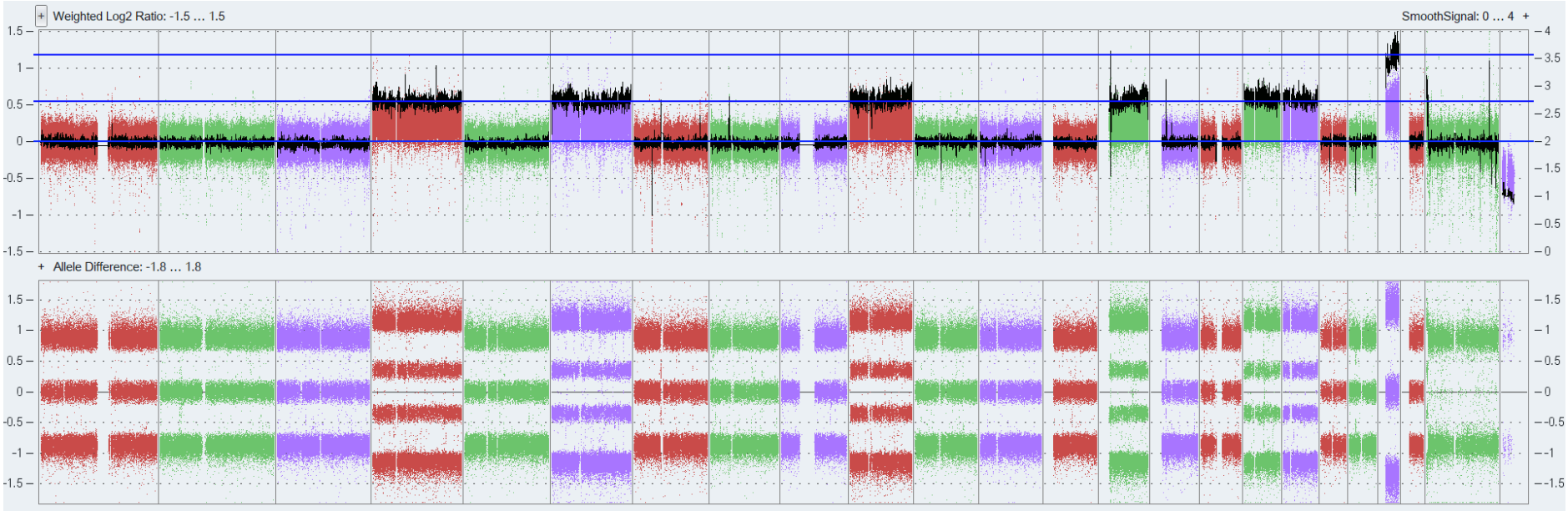


Image source: modified from Kulasekararaj, Br J Haematol 2013

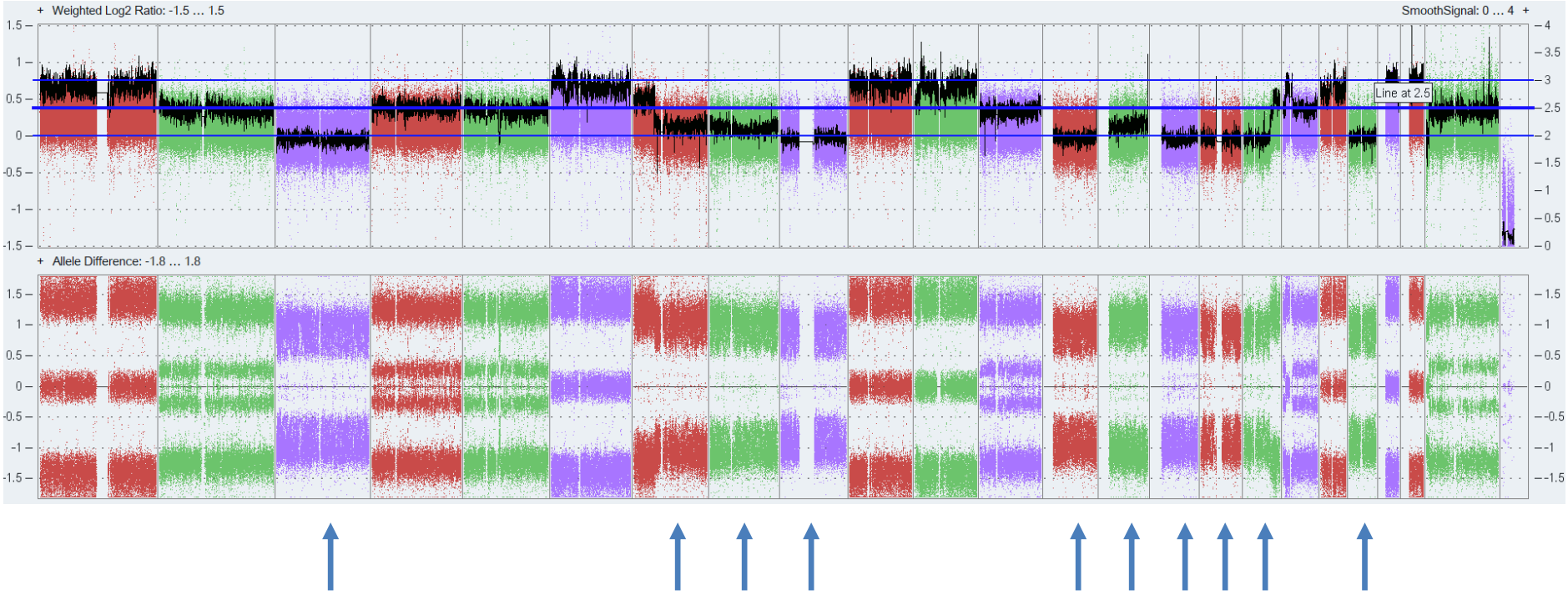
See references: Gondek et al., 2008; Heinrichs et al., 2009; Tiu et al., 2011; others

Utility SNP-A in B-ALL: hyperdiploidy



Chromosome Results:
55,XY,+X,+4,+6,+10,+14,+17,+18,der(19)t(1;19)(q2?3;p13),+21,+21,inc[1]
/46,XY[7] *Suboptimal Mitotic Index

Utility SNP-A in B-ALL: masked hypodiploidy



Incidental or secondary findings from GMA testing

- Constitutional
  - Genome-wide AOH, suggestive of consanguinity
  - Alteration (usually deletion) of dosage sensitive gene/region associated with adult-onset or hereditary cancer predisposition
    - May or may not be associated with indication for testing
  - Mosaicism associated with hematologic disease (rare)
- Oncology
  - Genome-wide AOH, suggestive of consanguinity
  - Constitutional pathogenic/likely pathogenic CNVs

➢ Pre-test counseling is generally recommended to inform individuals about the capabilities of this test, and what could be uncovered by genome-wide analysis

Multiple techniques are employed for the detection of different cytogenetic abnormalities

Technique	Resolution	Sensitivity (mosaicism)	Culturing required?	Global?	Unbalanced abns?	Balanced abns? Structural info?
G-banded chromosomes	3-5 Mb (550 bands)	10-15%	Yes	Yes	Yes	Yes
Metaphase FISH	100's kb	n/a	Yes	No	Yes	Yes
Interphase FISH	100's kb	1-5%	No	No	Yes	Yes
GMA	10-100's kb	10-20%	No	Yes	Yes	No

- Sizes: kb=$1x10^3$, Mb=$1x10^6$