Copyright Statement

Cytogenetics in AML & ALL: capturing disease biology to translate into Med-A

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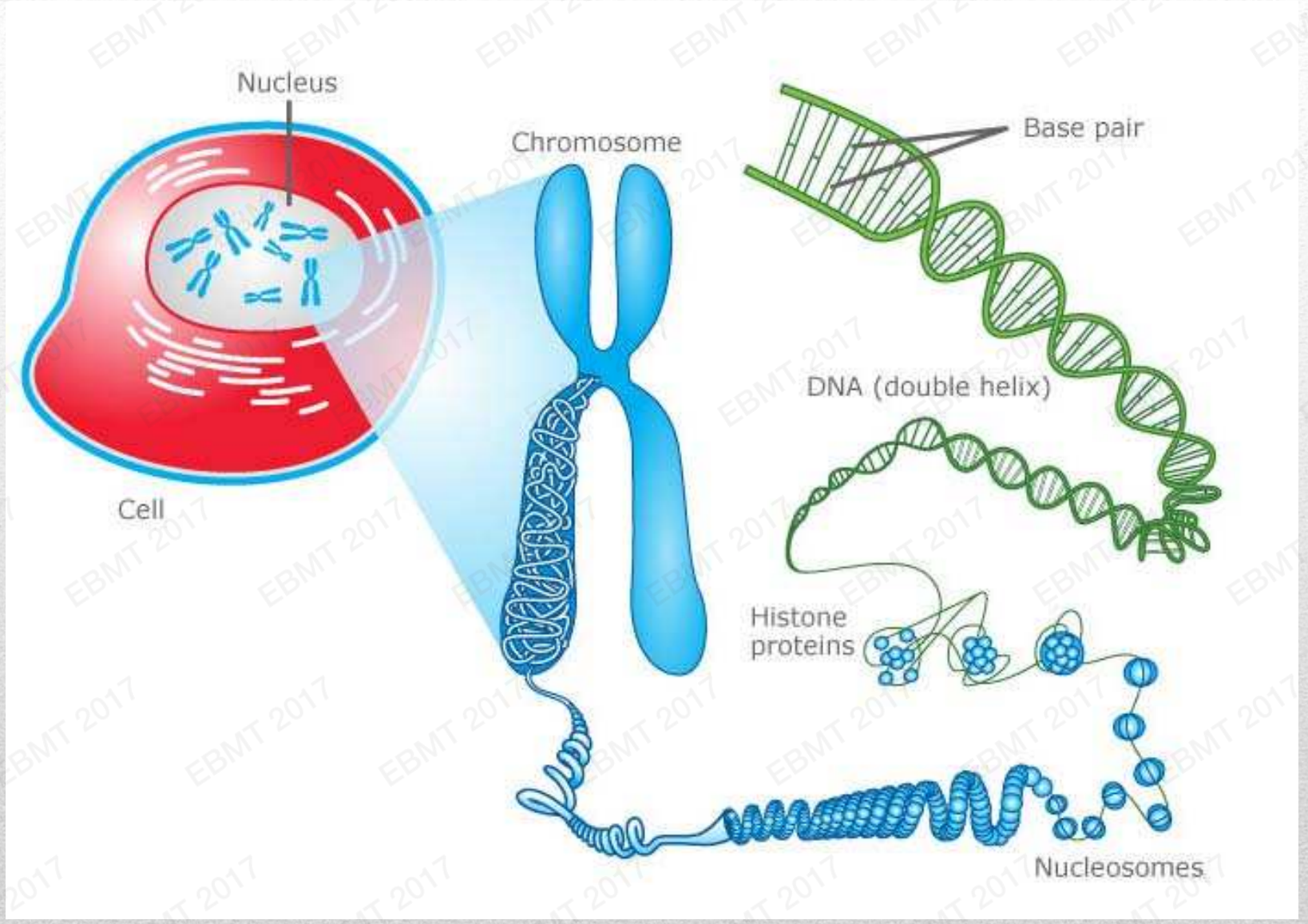

Nucleus
Cell
Chromosome
Base pair
DNA (double helix)
Histone
proteins
Nucleosomes

Cytogenetics in AML/ALL: outline for an *educational* session

- Initial statements
- Principles of cytogenetics and methods of analysis
- AML subtypes
- Complex, monosomal, abn(5q), abn(17p)
- ALL subtypes
- *A complex example (Dutch karyotype)*
- Q&A

Initial statements (1): AML & ALL heterogeneity

- AML & ALL are biological heterogeneous diseases, comprising distinctive diverse entities that can recognized by defining (cyto)genetics features

Cancer Genome Project proposed genomic classification of AML

Genomic subgroup	*%*	*Mutated genes*
AML with *NPM1*mut	27	NPM1, DNMT3A, FLT3-ITD, NRAS,…
AML with mut chromatin &/or spliceosome gene	18	RUNX1, MLL-PTD, SRSF2, ASXL1, STATG2…
AML with TP53mut, aneuploidy	13	Complex kar, -5/5q, -7/7q, TP53, -17/17p, -12/12p,…
AML with CBF-r	5+4	CBFb-MYH11, RUNX1-RUNX1T1
APL	4	PML-RARA, FLT3-TD, WT1,…
AML with CEBPAdm	4	CEBPA, NRAS, WT1, GATA2
AML with MLL-x	3	t(x;11q23), NRAS
AML with inv(3)/t(3;3)	3	GATA2-MECOM(EVI1),K/N-RAS,…
AML with t(6;9)	1	DEK-NUP214, FLT3-ITD,...
AML with IDH2mut	1	IDH2, DNMT3A,+8/8q
Other (non class-defining driver mut, no driver mutation, ≥2 genomic subgroups)	19	FLT3-ITD, DNMT3A

Papaemmanuil E, et al, NEJM 2016

Initial statements (2): analysis of genetics abnormalities in AML & ALL

- Genomics of AML & ALL is analyzed in daily clinical routine with complementary cytogenetic and/or molecular techniques:

  – Cytogenetics is a discipline aimed to analyze chromosome structure (with a low/high resolution tool)
  – Molecular techniques analyze DNA sequence

Initial statements (3): information provided in a cytogenetic report

- Is our cytogenetic result (report) characteristic of any predefined (AML, ALL) entity?
- Is it providing prognostic information (cytogenetic prognostic category)?
- Is there any additional confirmation (FISH, molecular techniques) of the observed cytogenetic abnormality?

Initial statements (4): different cytogenetic techniques provide different type of information

- Conventional cytogenetics (karyotyping) is the basic level of cytogenetic analysis for identifying gross chromosomal abnormalities: aneuploidies & structural (gain/loss, rearrangement)

- FISH analysis is based on probes directed to specific regions (molecular cytogenetics)

Initial statements (4'): different cytogenetic techniques provide different type of information

- High resolution techniques (CGH, SNP arrays) provide a comprehensive & detailed report of chromosomal structure (submicroscopical gain/losses, rearrangement)

CONVENTIONAL CYTOGENETICS (G BANDING)

METAPHASE

Short arm= p

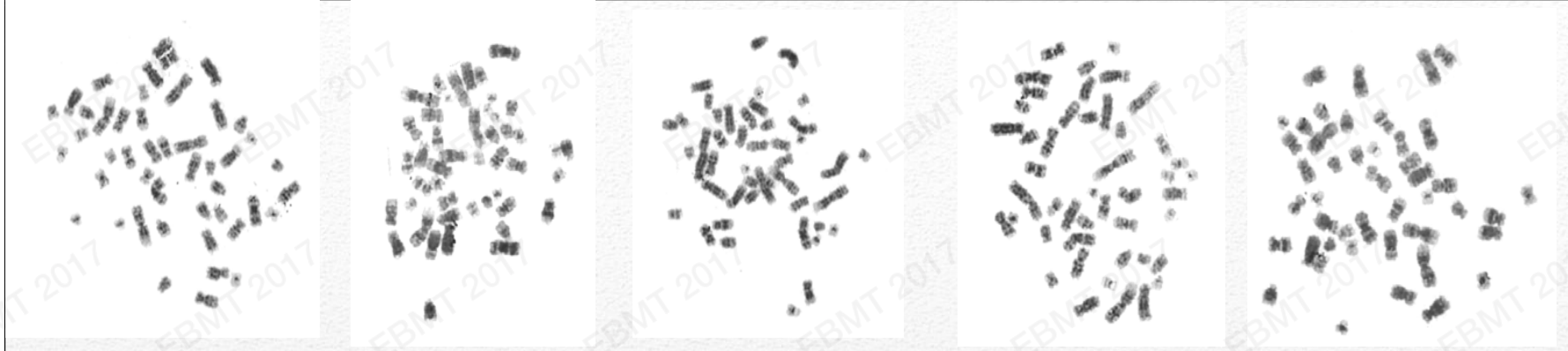
Long arm= q

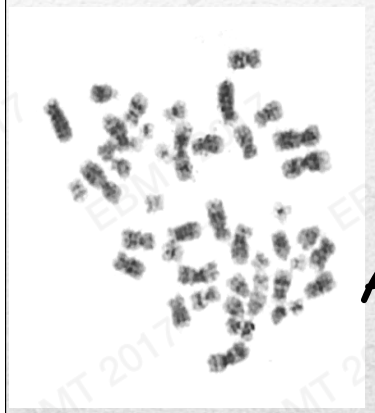
Telomere

Centromere

Telomere (end terminus)

Chromosome 5





Analysis of ≥20 metaphases

Cytogenetic report (ISCN)

Cytogenetic nomenclature

An International System for Human Cytogenetic Nomenclature (ISCN) (2013).

Eds.: **L.G. Shaffer, J. McGowan-Jordan, M. Schmid.**

Recomendations of the International Standing Committee on Human Cytogenetic Nomenclature

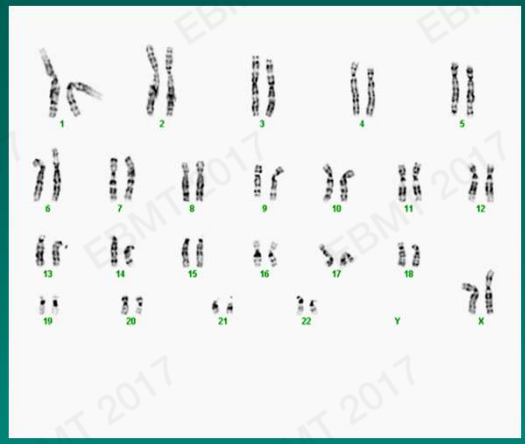
47,XX,+3,del(5)(q13q33)[15]/46,XX[5]

Conventional cytogenetics: limitations

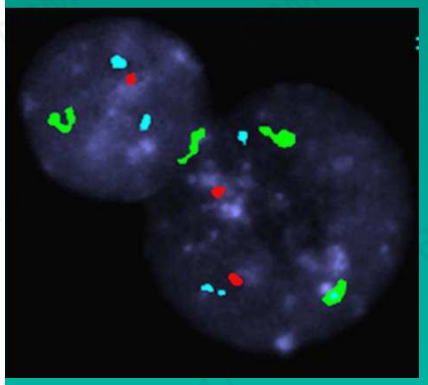
- Requires adequate metaphases (dividing cells) for analysis
- Detection of only large (*gross*) abnormalities (>5Mb)
- Complex karyotypes (=multiple aberrations) require additional studies for a proper characterization of involved chromosomes

CYTOGENETIC METHODS TO DETECT CHROMOSOME ABNORMALITIES

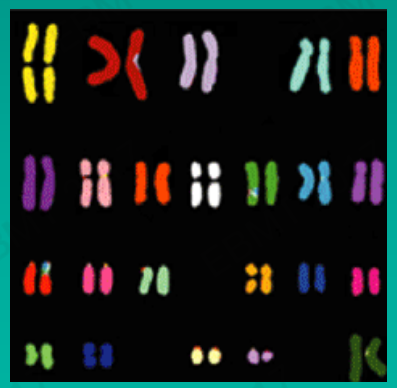
CONVENTIONAL CYTOGENETICS →



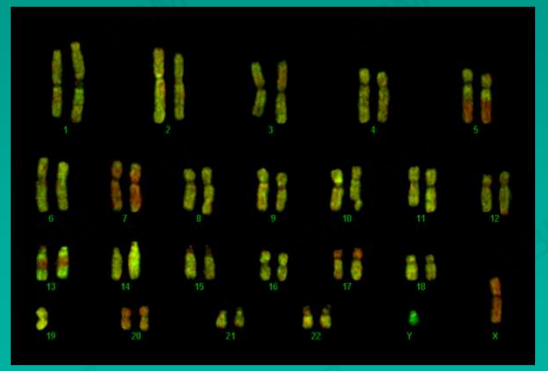
MOLECULAR CYTOGENETICS



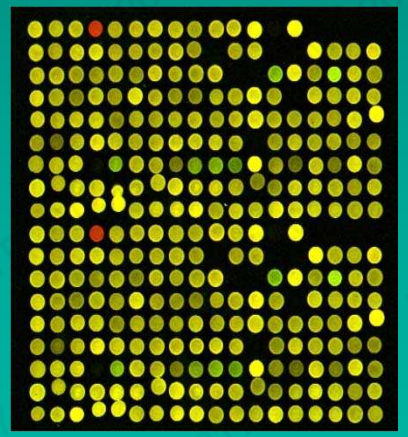
FISH



M-FISH

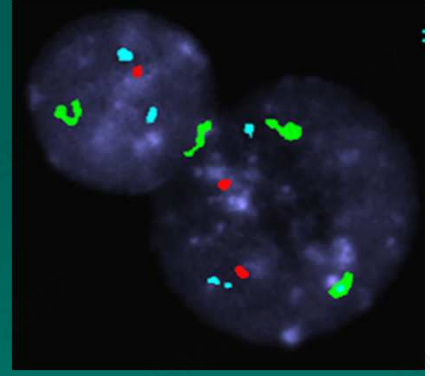


conventional CGH



CGH-array

FISH technique



- ✓ Introduced in the early '80s.
- ✓ Detection of specific regions of DNA at both chromosome and gene level. Detection of imbalances of less than 5Mb.
- ✓ Study of specific sequences of DNA in metaphases and interphase nuclei (interphase cytogenetics).
- ✓ Limitation of the FISH technique: we only obtain information about the tested chromosomes

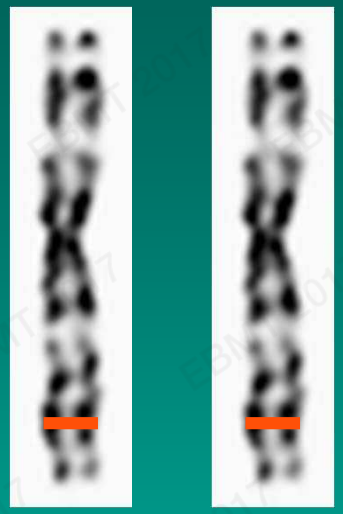
FISH probes

Study in nuclei and/or metaphases

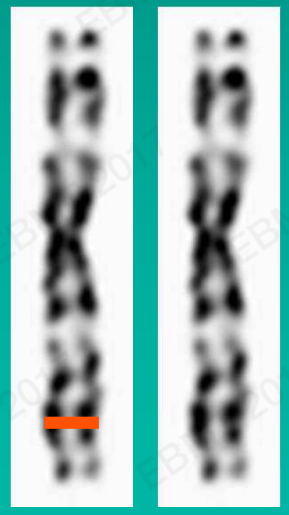
- **LSI:** locus specific probes (microdeletions). Nuclei and metaphases.
- **CEP:** centromeric repetitive sequences (numerical and structural abnormalities). Nuclei and metaphases.
- **WCP:** Whole chromosome painting. Metaphases.
- **TEL:** telomeric repetitive sequences. Nuclei and metaphases.

FISH LSI (locus specific probe)

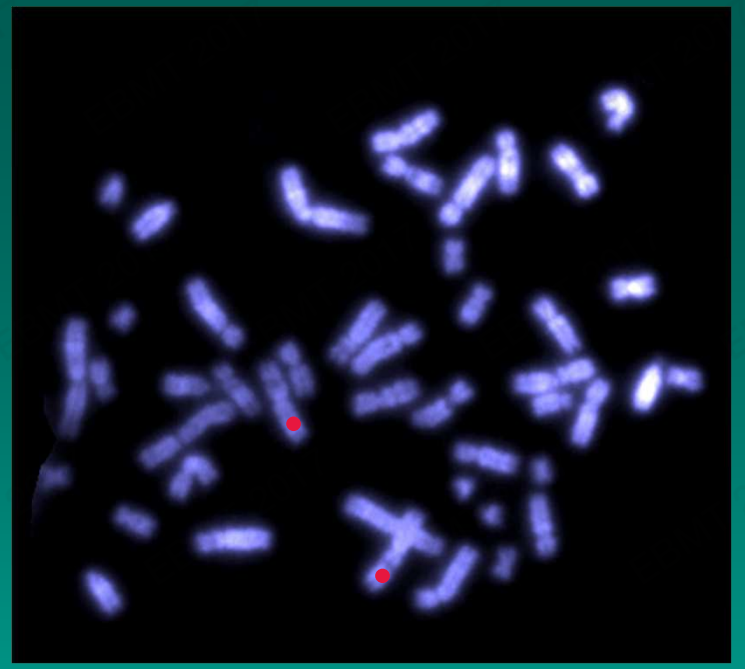
EVI 1
(3q26)

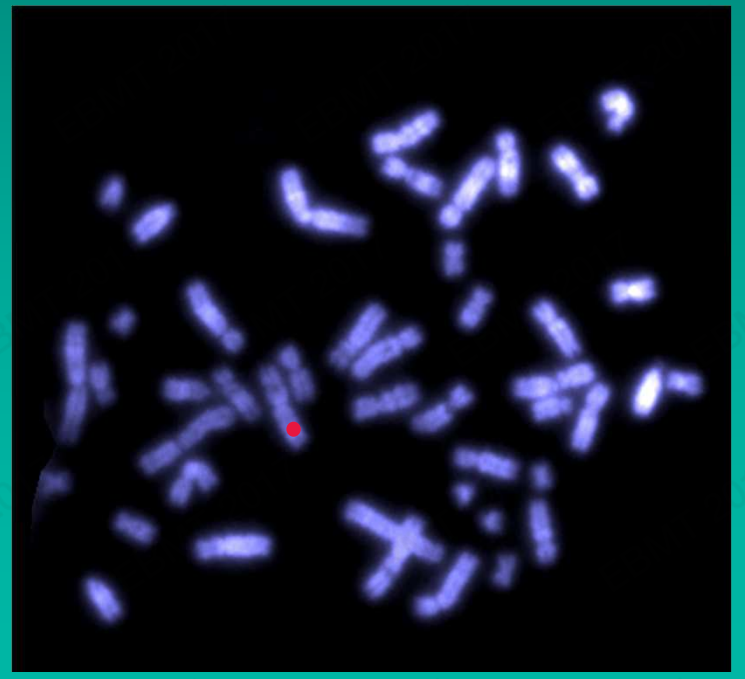


NORMAL



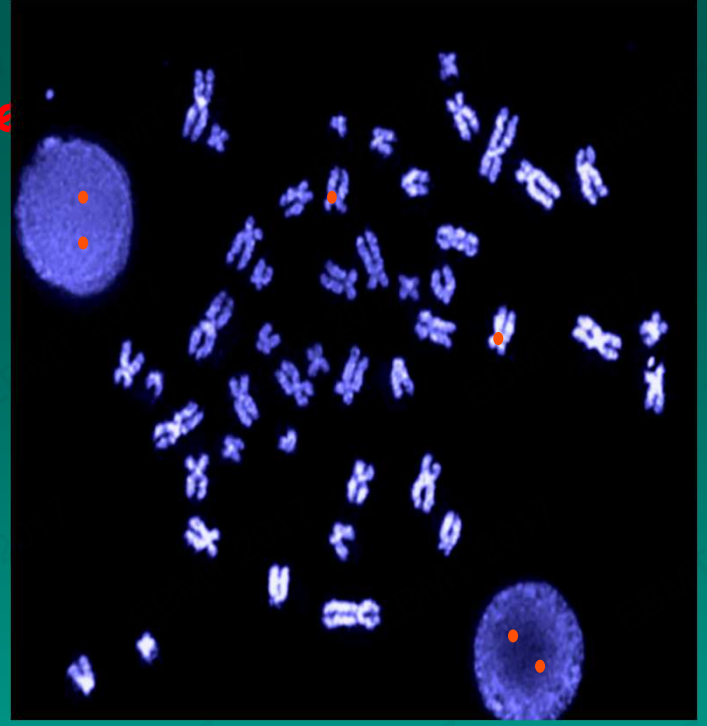
DELETION 3q26

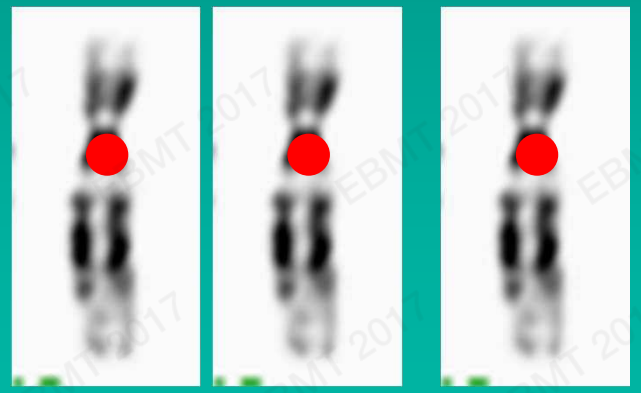




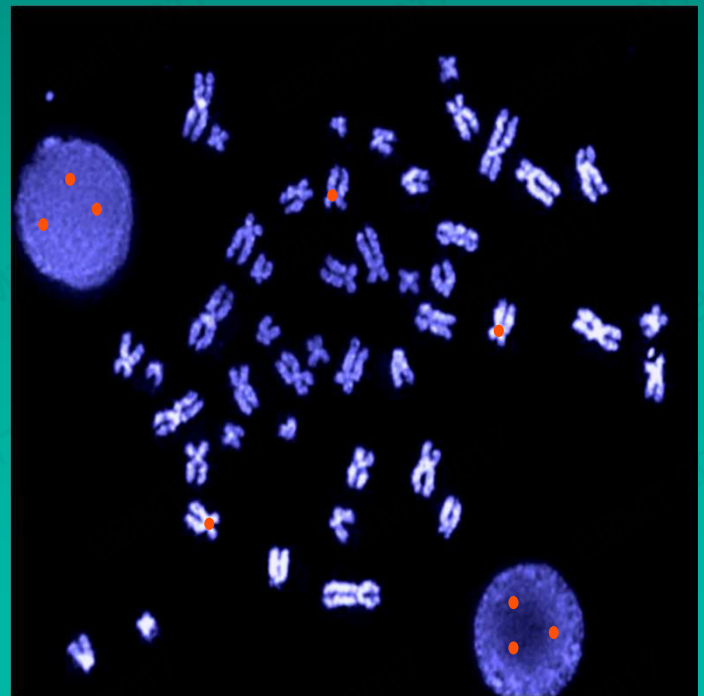
FISH CEP (centromeric repetitive sequence

NORMAL





TRISOMY 12



Initial statements (5): cytogenetics & molecular techniques give confirmatory and/or complementary information

- Many distinctive cytogenetic abnormalities in AML/ALL translate into a specific/leukemic DNA sequence that can be identified by molecular techniques:

APL=t(15;17)(q22;12)=*PML/RARA*

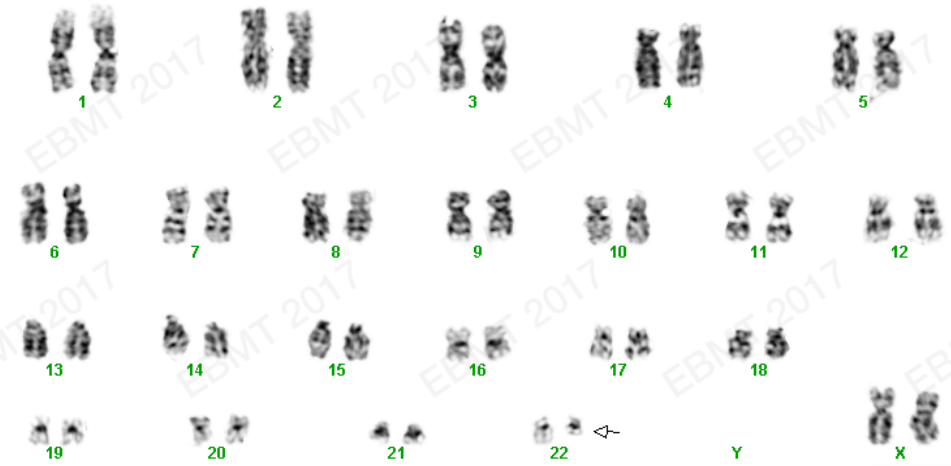
t(8;21)(q22;q22)=*RUNX1(AML1)/RUNX1T1*(*ETO*)

t(9;11)(p22;q23)=*MLLT3(AF9)/MLL(KMT2A)*

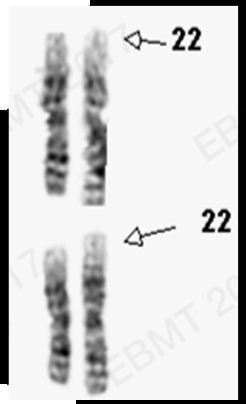
Initial statements (6): *hidden* cytogenetics

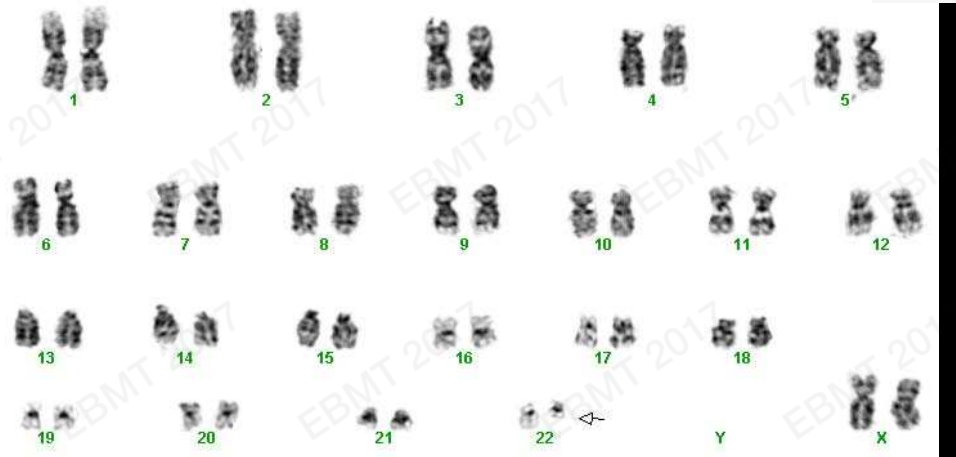
- Cytogenetic abnormalities can present in an *alternative* manner:

  - As a cryptic lesion (w/o *gross* chromosomal exchange)
  - As part of a complex karyotype, involving multiple chromosomes
  - As a variant form (variant APL)



46,XX,del(22)(q11)





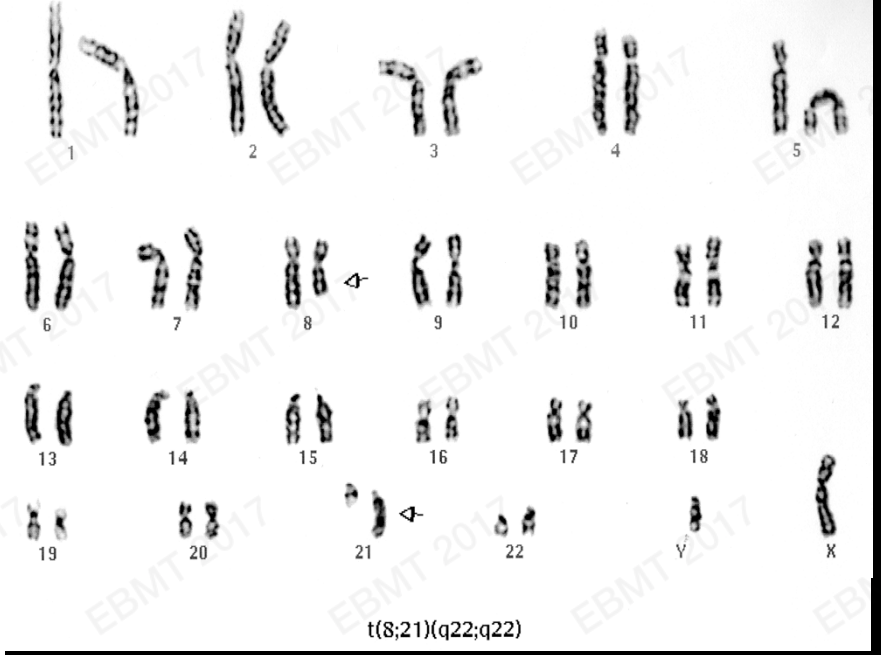
46,XX,t(1;9;22)(p36.1;q34;q11)

WHO classification of AML: AML with recurring genetic abnormalities

- AML with t(8;21)(q22;q22)/RUNX1-RUNXT1
- AML with inv(16) or t(16;16)(p13;q22)/CBFβ-MYH11
- Acute promyelocytic leukemia with PML-RARα
- AML with t(9;11)(p22;q23)/MLLT3(AF9)-KMT2A(MLL)
- AML with t(6;9)(p23;q34)/DEK-NUP214(CAN)
- AML with inv(3) or t(3;3)(q21;q26)/GATA2,MECOM
- Megakaryoblastic AML with t(1;22)(p13;q13)/RBM15-MKL1
- AML with mutated *NPM1*
- AML with biallelic *CEBPA* mutation
- AML with *BCR-ABL* rearrangement
- AML with *RUNX1* mutation

Daniel Arber, et al. Blood 2016;127:2391-405

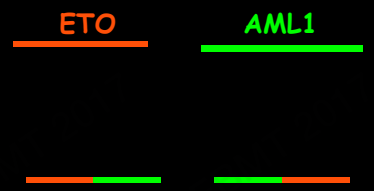
WHO Classification of Myeloid Neoplasms, 2016

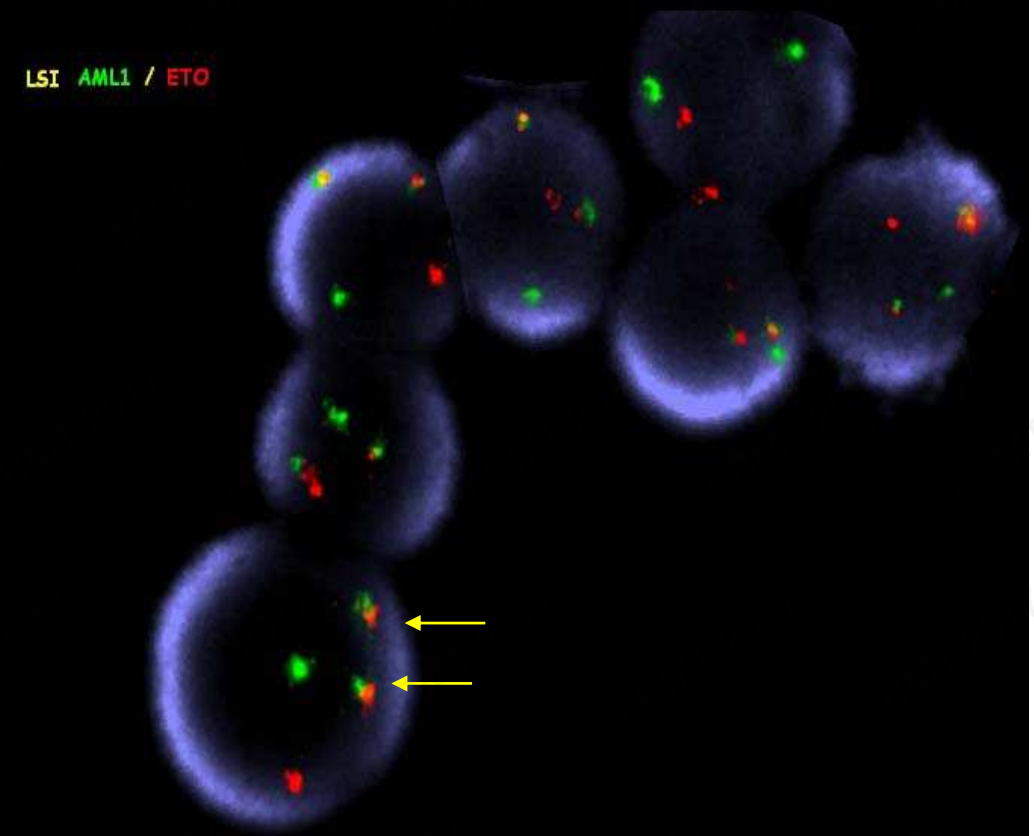


t(8;21)(q22;q22)

t(8;21)(q22;q22), (AML/ETO1

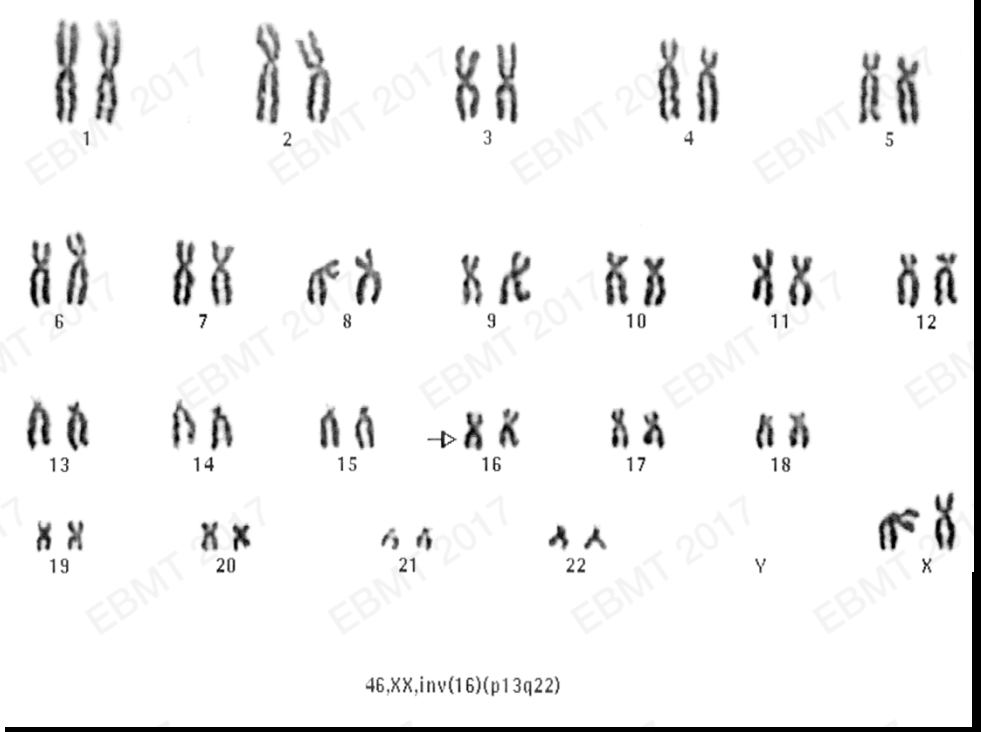
Dual color, Dual fusion





AML with t(8;21)(q22;q22): final tricks

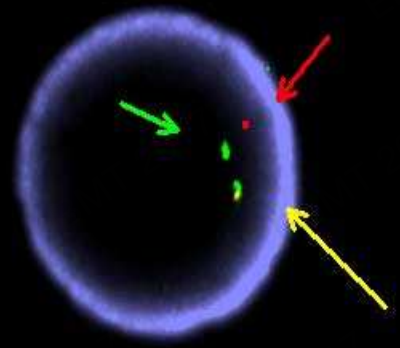
- One third of pts have additional cytogenetic abnormalities: +8, del(9p), -Y,…
- t(8;21) + 2 additional abn ≠ complex karyotype
- In principle mutually exclusive with other major recurrent cytogenetic abn: t(15;17), inv(6), inv(3q26), t(6;9)
- t(8;21) AML can harbor additional molecular common mutations: FLT3-ITD/TKD, KIT, RAS,…



46,XX,inv(16)(p13q22)

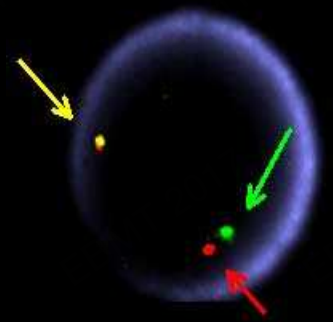
t/inv(16)(p13q22),(CBFβ/MYH11)

inv(16): POSITIU



Dual color, Break apart

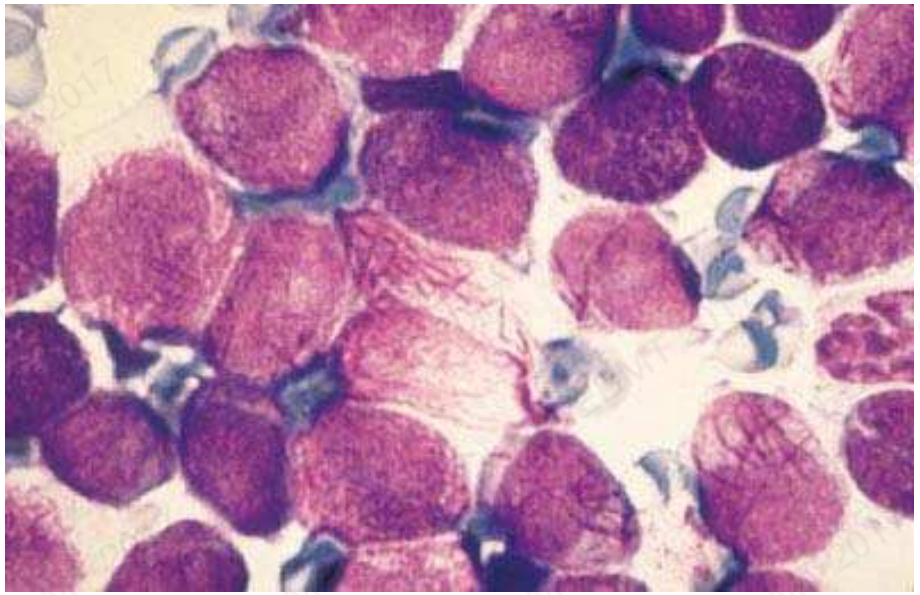
CBFB

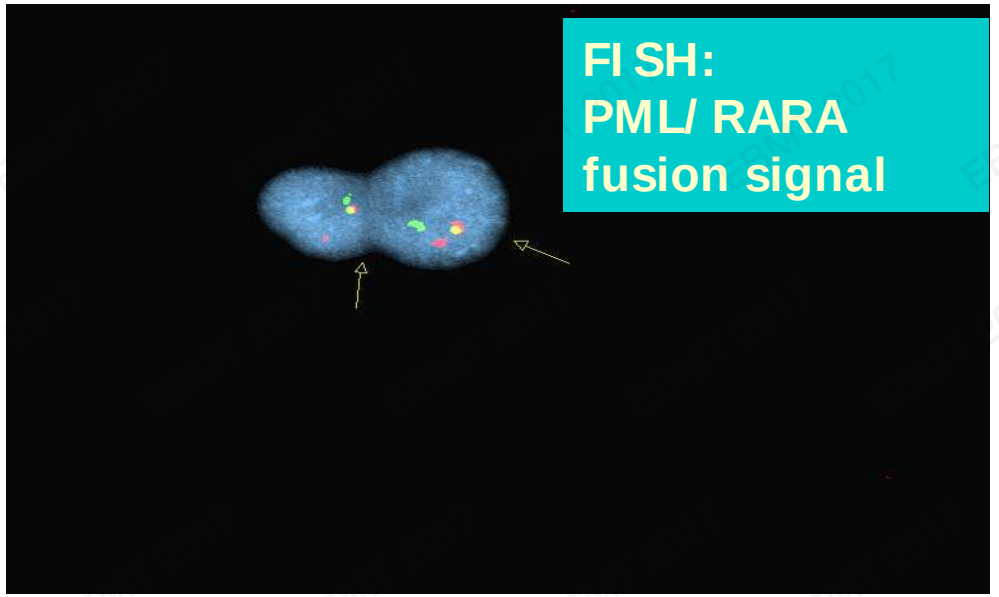


Molecular AML characterization, an integrated diagnosis: example - is this case a true inv(16)?

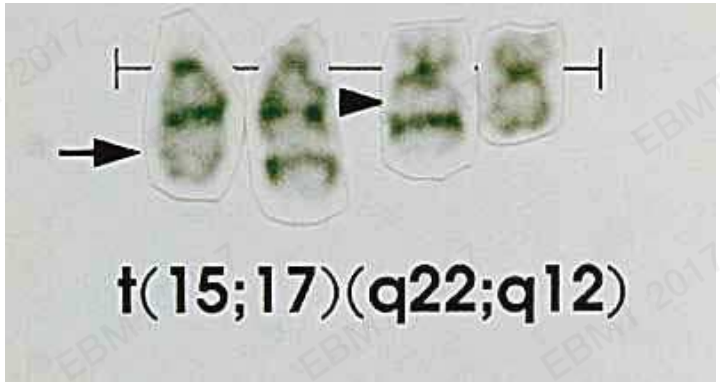
Conventional cytogenetics	*FISH*	*PCR*	*Conclusion*
+	+	+	AML with inv(16)
Normal	+	+	AML with inv(16) (cryptic rearrangement)
+	-	-	Other AML subtype (false + cytogenetics)
+	+	-	AML with inv(16) (false – PCR)

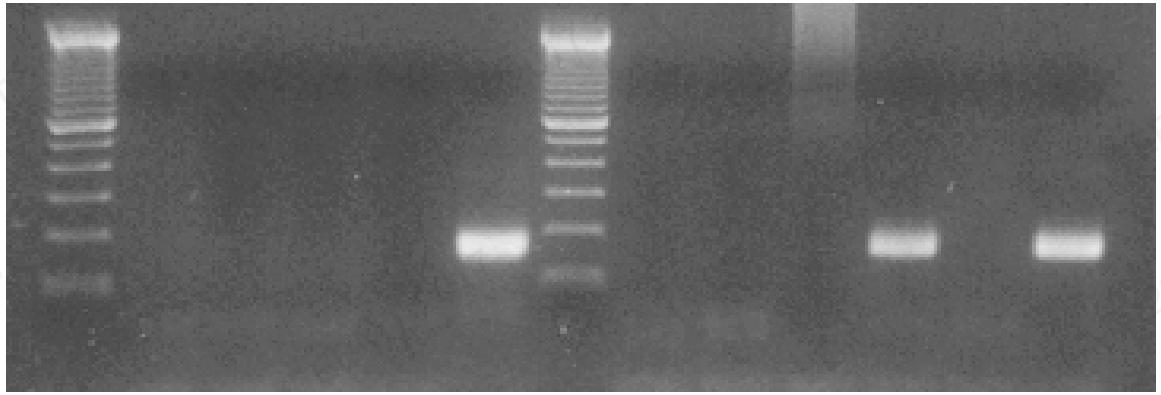
Acute promyelocytic leukemia (APL): an example of integrated diagnosis





bcr1 bcr3



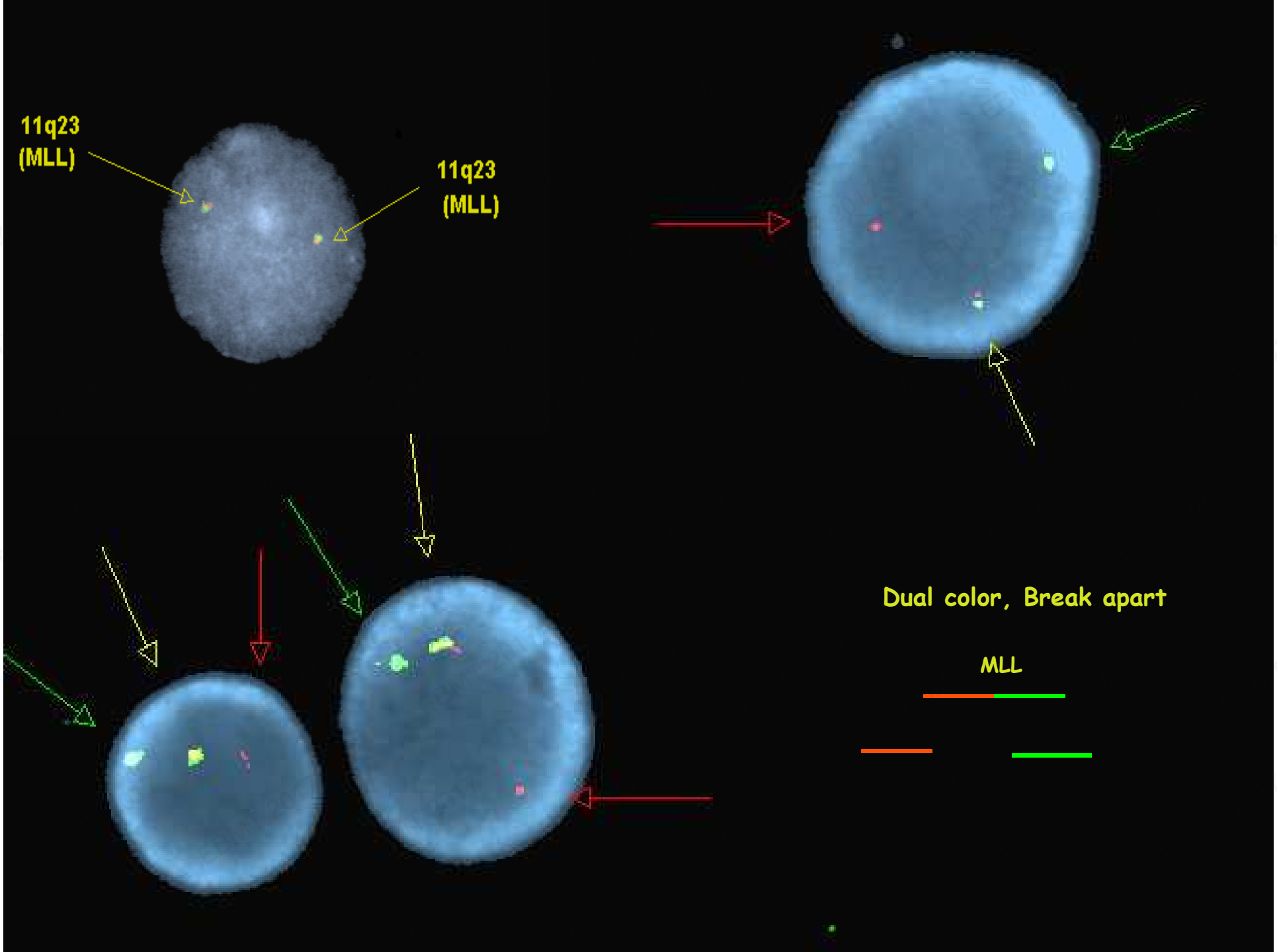


RT-PCR PML/RAR-alpha

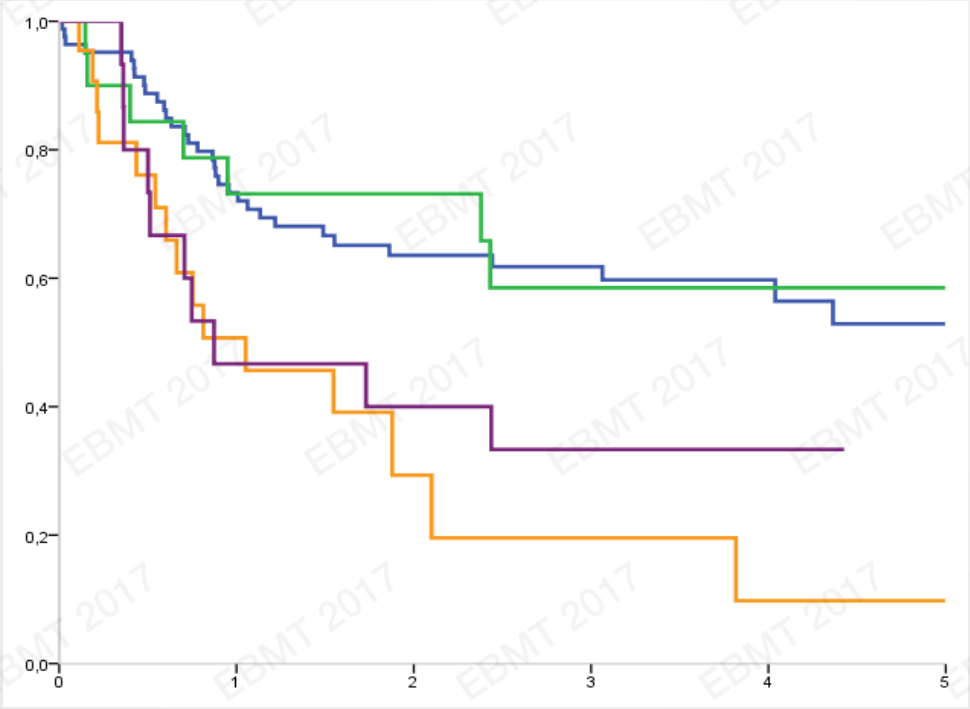
AML with 11q23(MLL)-rearrangement

- *MLL* (*KMT2A*) can fuse to >100 different partners:
  - t(9;11)(p22;q23)/MLLT3-MLL fusion transcript is the most frequent & only recognized as an individualized entity in WHO 2016 classification

- *KMT2A* is the current denomination of *MLL* gene

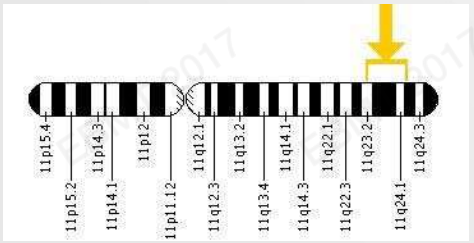

11q23
(MLL)
11q23
(MLL)
Dual color, Break apart
MLL

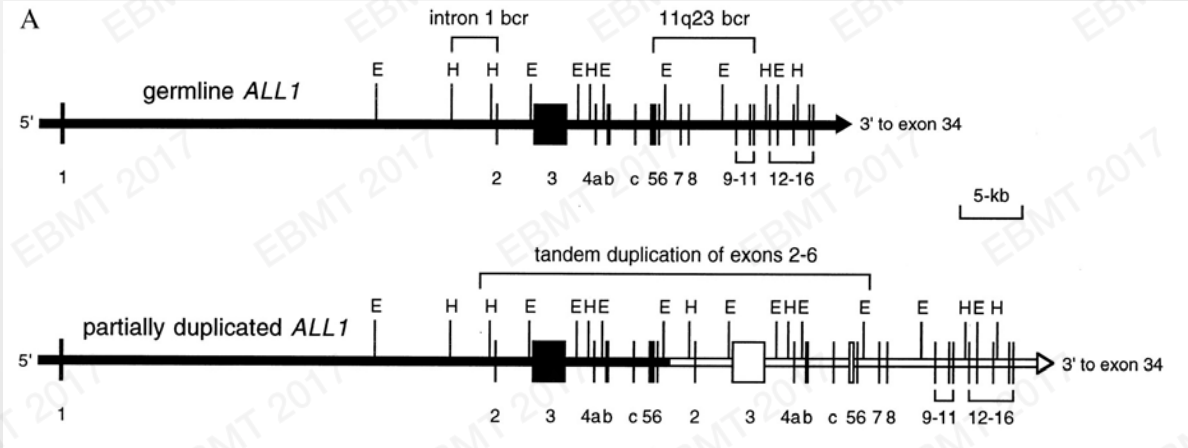
AlloHCT for AML with 11q23(MLL) rearrangement: different outcome according to the rearranged partner



KMT2A(*MLL*) gene can experience partial tandem duplication*: MLL*-PTD AML

- Only identified by molecular methods (PCR, Southern blot)
- Conventional cytogenetics & FISH (*MLL* probe) analysis usually yield a normal result

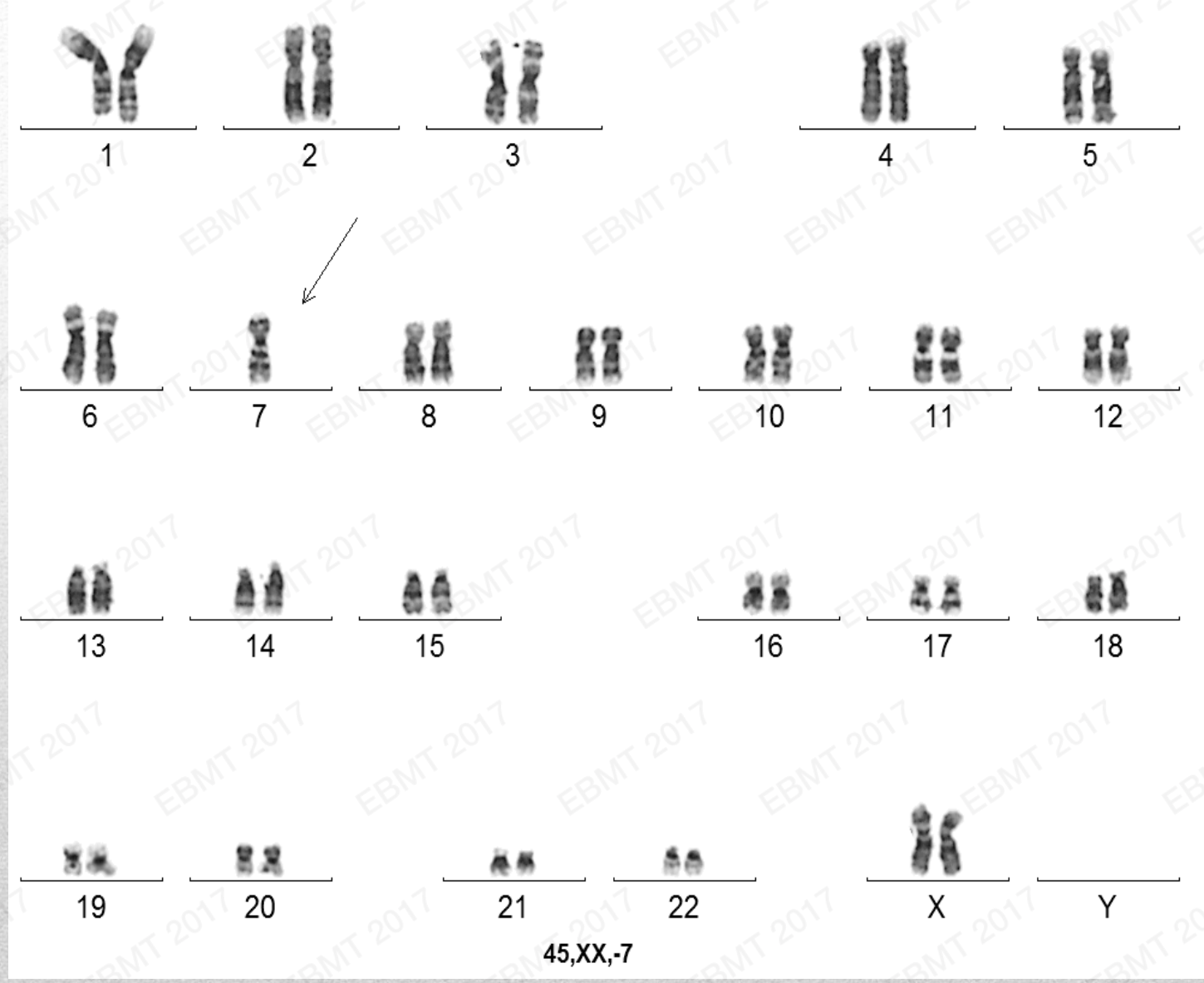




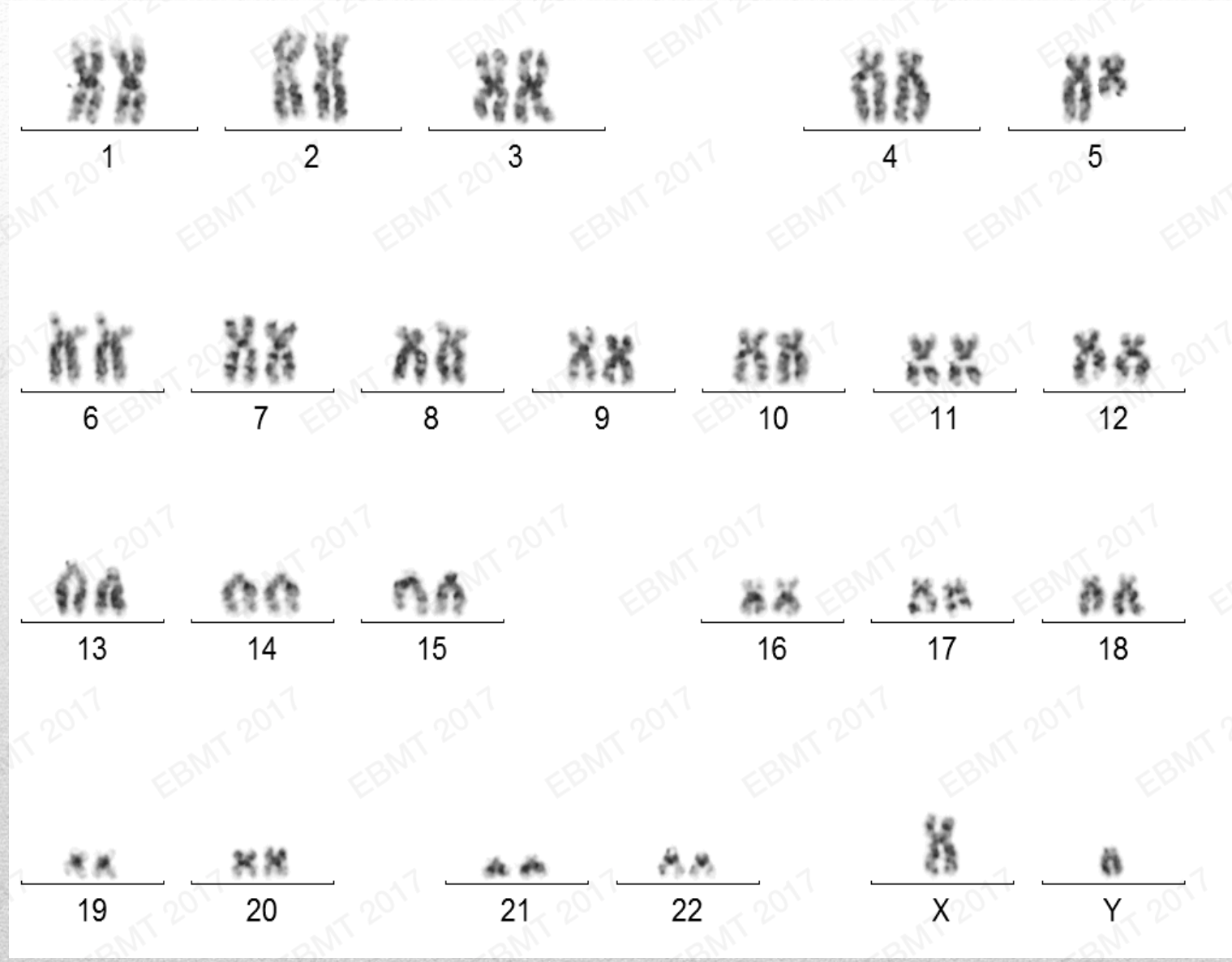
AML with myelodysplasia-related changes: an AML subtype defined by cytogenetic findings

Non-balanced changes	Translocations	Complex karyotype
-7/del(7) -5/del(5q) i(17q)/t(17p) -13/del(13q) del(11q) del(9q) del(12p)/t(12p) Idic(X)(q13)	5q32: t(5;12) / t(5;7) / t(5;17) / t(5;10)	>3

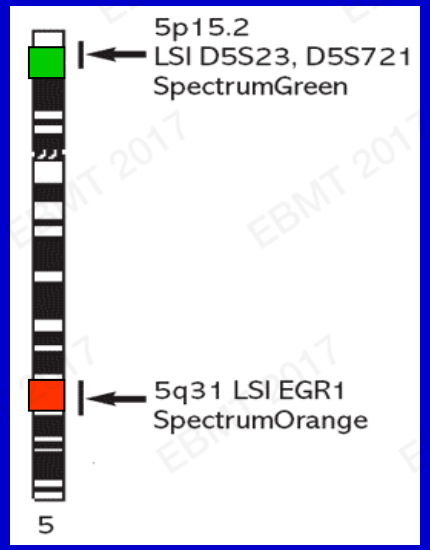
MONOSOMY 7

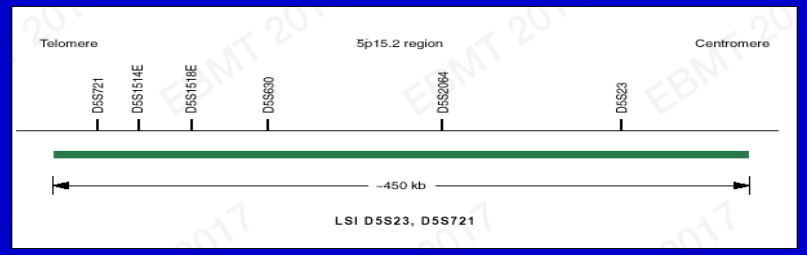


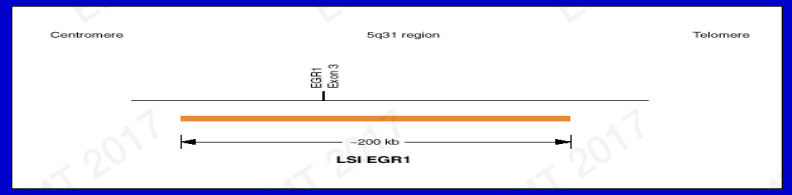
del(5)(q13q33)

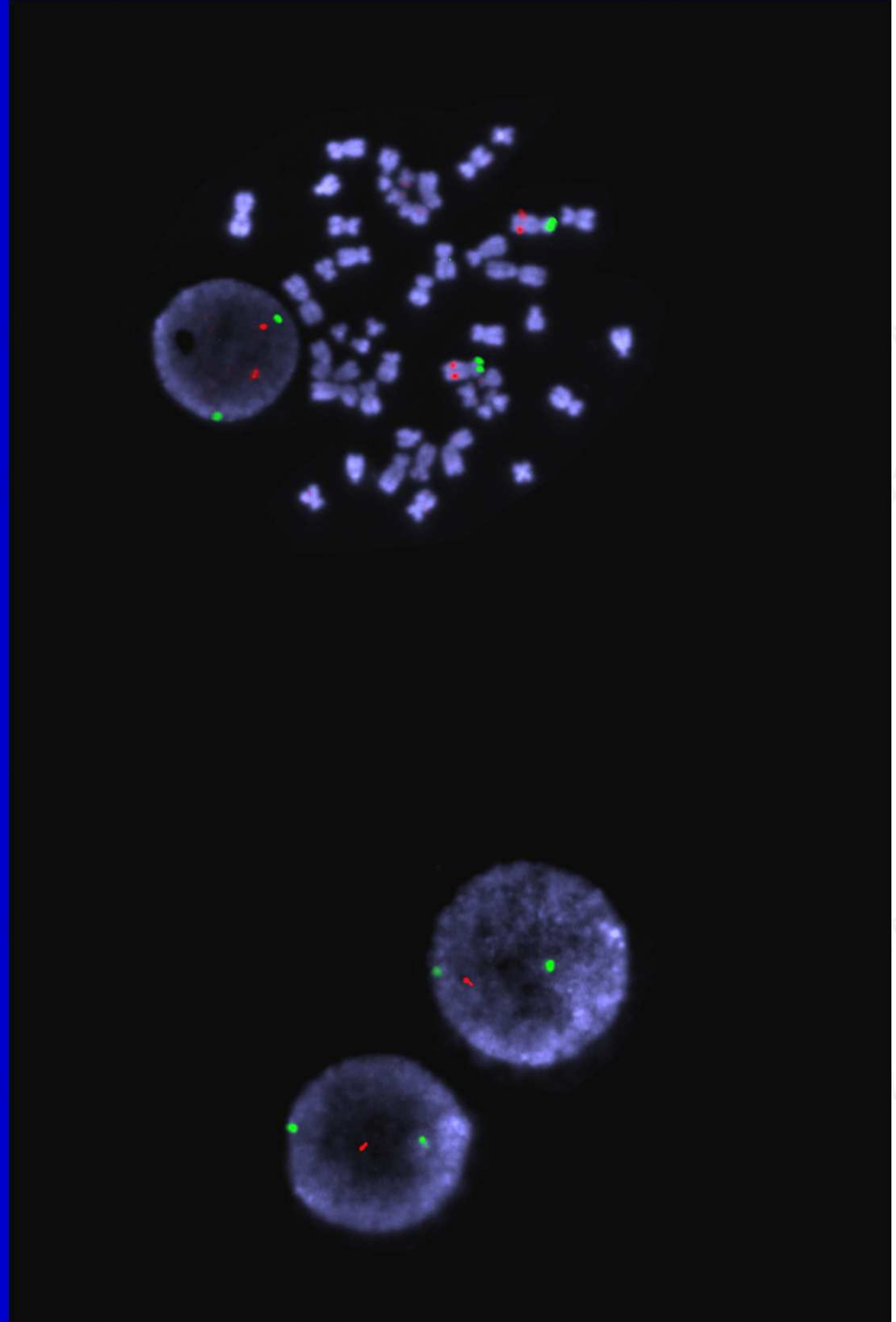


del(5)(q31)


5p15.2
LSI D5S23, D5S721
SpectrumGreen
5q31 LSI EGR1
SpectrumOrange
5


Telomere
5p15.2 region
Centromere
D5S721
D5S1514E
D5S1518E
D5S630
D5S2064
D5S23
~450 kb
LSI D5S23, D5S721


Centromere
5q31 region
Telomere
EGR1
Exon 3
~200 kb
LSI EGR1



Definition of monosomal karyotype

Breems et al, 2008

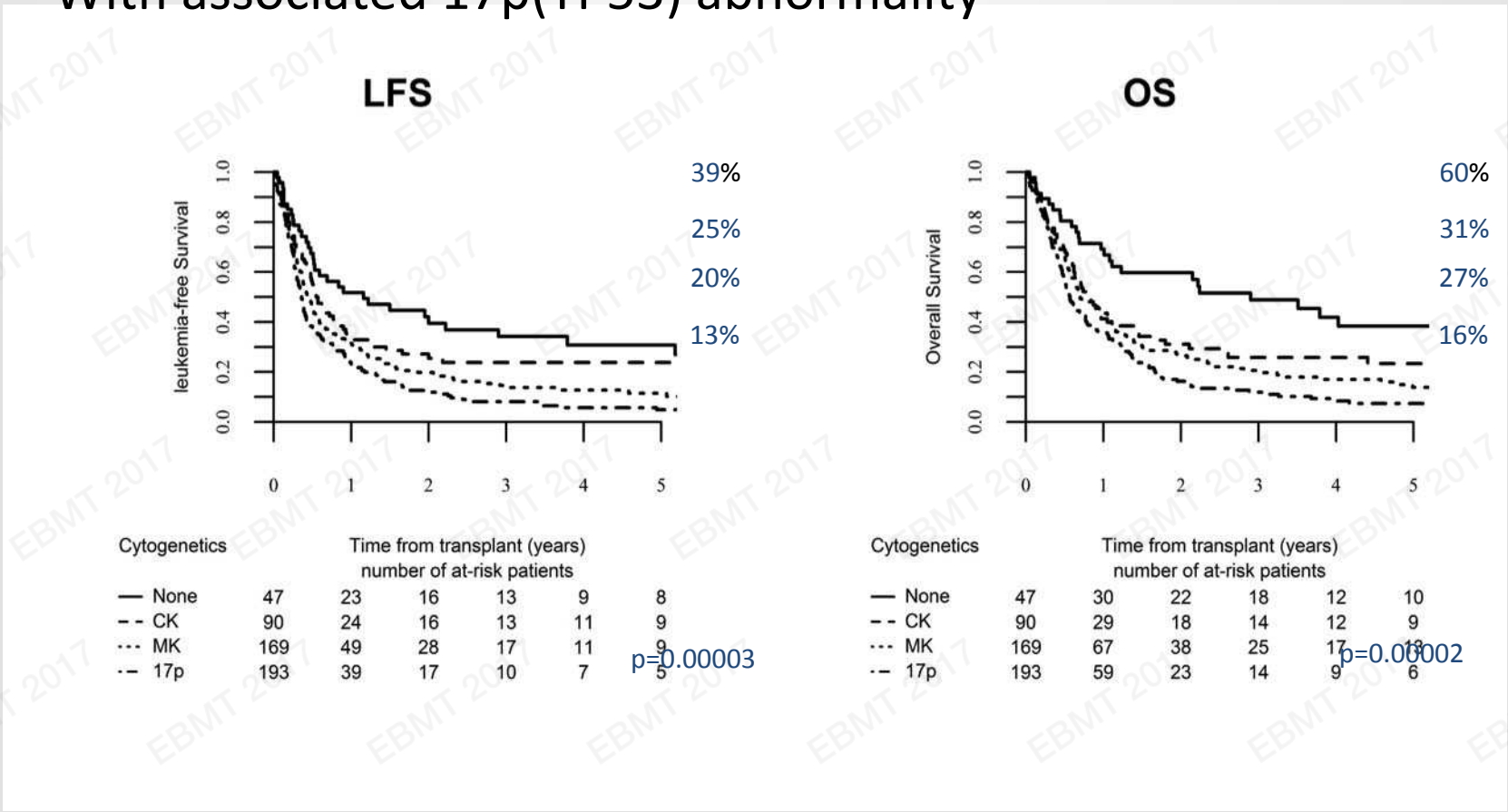
- ✓ >1 monosomy of an autosomal chromosome or
- ✓ 1 monosomy + 1 structural cytogenetical abnormality

Structural abnormalities

- Deletion
- Addition
- Translocation
- Inversion

Prognosis of AML with del(5q) according to additional cytogenetic abnormalities

- Del(5q)/-5 as a sole abnormality
- As part of a complex karyotype : del(5q)-5 + ≥2abn
- As part of a monosomal karyotype
- With associated 17p(TP53) abnormality



WHO classification (2016) of B-(A)LL

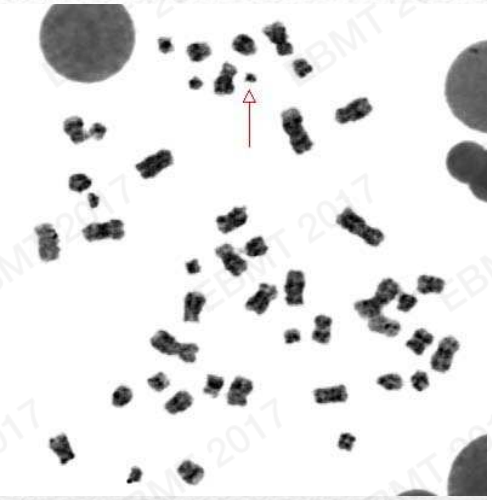
I. B-lymphoblastic leukemia/lymphoma (B-LL)
- B-LL with t(9;22)(q34;11.2); *BCR-ABL1*
- B-LL with t(v;11q23); *MLL*-rearranged (*KMT2A* gene)
- B-LL with t(12;21)(p13;q22); *TEL-AML1* (ETV6-RUNX1)
- B-LL with hyperdiploidy (>50,<66)
- B-LL with hypodiploidy (<44-45)
- B-LL with t(5;14); *IL3-IGH*
- B-LL with t(1;19)(q23;p13.3); *E2A-PBX1* (*TCF3-PBX1*)
- B-lymphoblastic leukemia/lymphoma, NOS
- *B-LL BCR-ABL1-like*
- *B-LL with iAMP21*

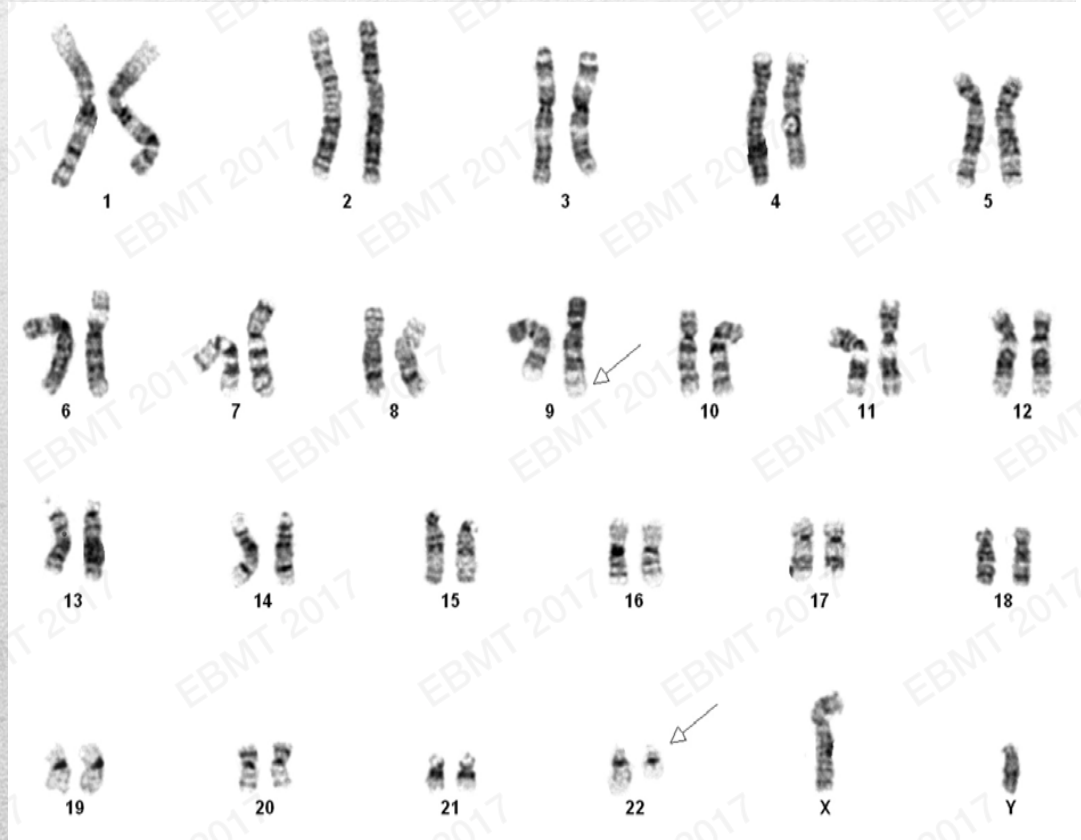
CML, Ph-pos ALL

1960. Nowell & Hungerford: Gq- (21) (Ph')

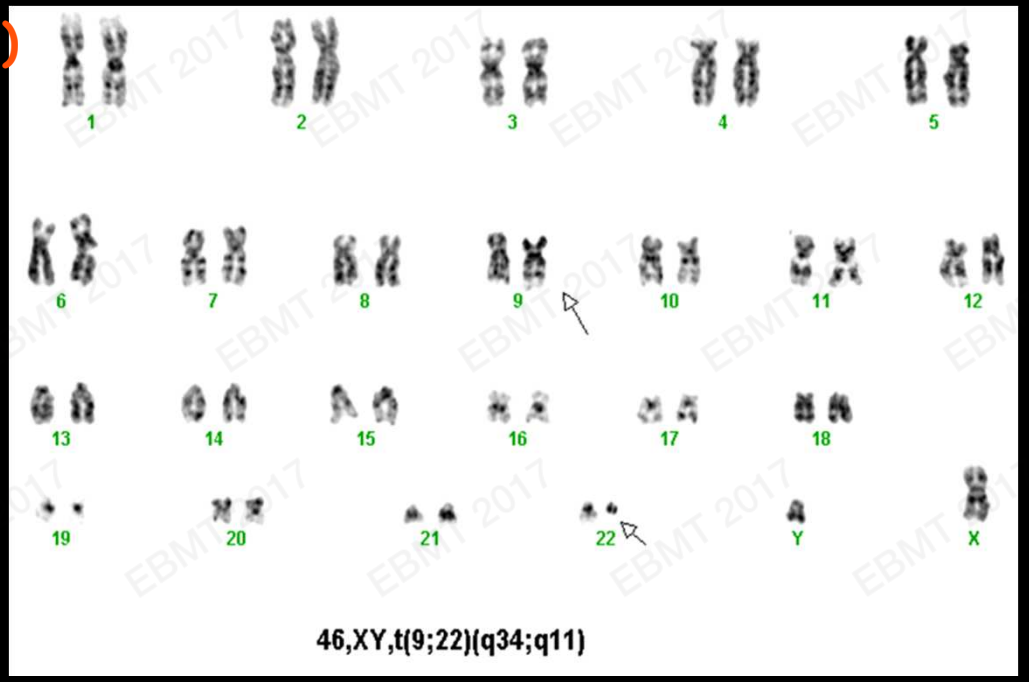
1970. G banding: Ph' was a chromosome 22!

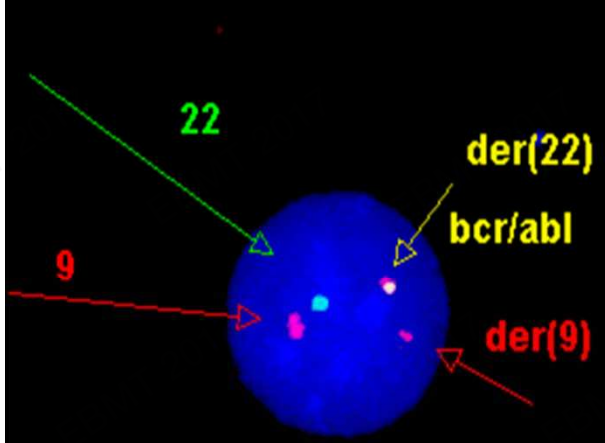
1973. Rowley et al. Description of t(9;22)(q34;q11.2)

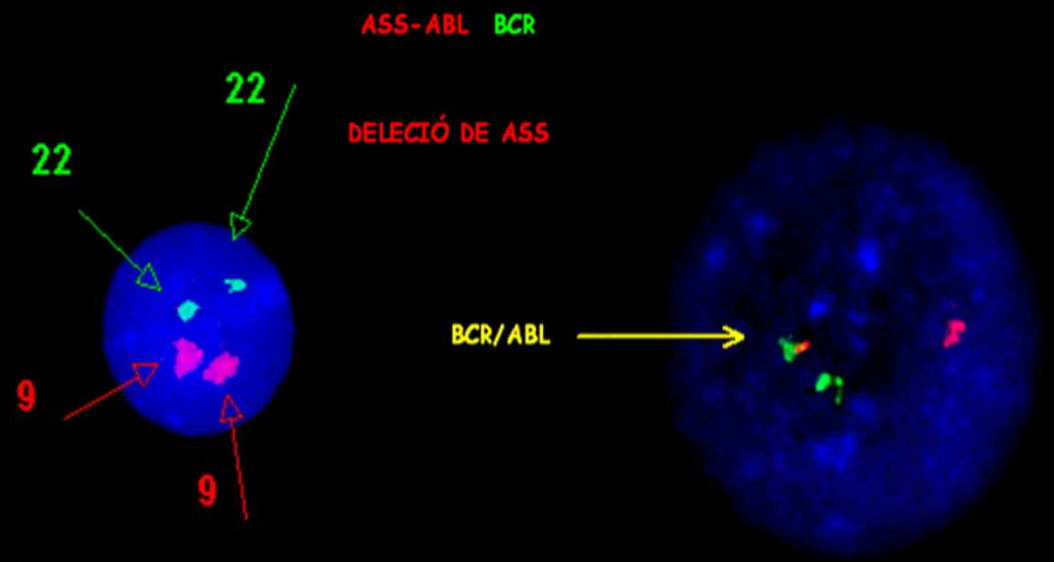




t(9;22)(q34;q11.2)







How can we classify this karyotype? (the complex “Dutch karyotype”)

Acute Myeloid Leukemia type M2

46,XY,?der(9)[2]/46,XY[18].

ish der(9)(pter->p10::p10->pter::9q34.13)(wcp9+,43N6+,CDKN2A/B+,cen9+,CDKN2A/B+,43N6+,NUP214+),ins(?17;9)(q?q34q34)(NUP214+).

Nuc ish(MECOMx2)[200],(DEKx2,NUP214x3)[87/200],(KMT2Ax2)[200],(MYH11,CBFB)x2[200]

.arr[hg19] 3q25.1q29(150,369,151-197,581,147)x2 hmz,9p24.3p13.1(46,587-39,179,289)x2~3, 9q21.11q34.13(70,984,372-134,032,544)x1~2,9q34.3(140,133,600-141,066,491)x1~2

How can we classify this karyotype? (the complex “Dutch karyotype”)

Acute Myeloid Leukemia type M2 (FAB)

Conventional karyotype: **46,XY,?der(9)[2]/46,XY[18].**

In situ hybridization: **ish der(9)(pter->p10::p10->pter::9q34.13) (wcp9+,43N6+,CDKN2A/B+,cen9+,CDKN2A/B+,43N6+,NUP214+), ins(?17;9)(q?q34q34)(NUP214+).**

FISH analysis of common AML abn:
Nucish(MECOMx2)[200],(DEKx2,NUP214x3)[87/200],(KMT2Ax2)[200], (MYH11,CBFB)x2[200]

SNP arrays:

.arr[hg19] 3q25.1q29(150,369,151-197,581,147)x2 hmz,9p24.3p13.1(46,587-39,179,289)x2~3,9q21.11q34.13(70,984,372-134,032,544)x1~2,9q34.3(140,133,600-141,066,491)x1~2

•

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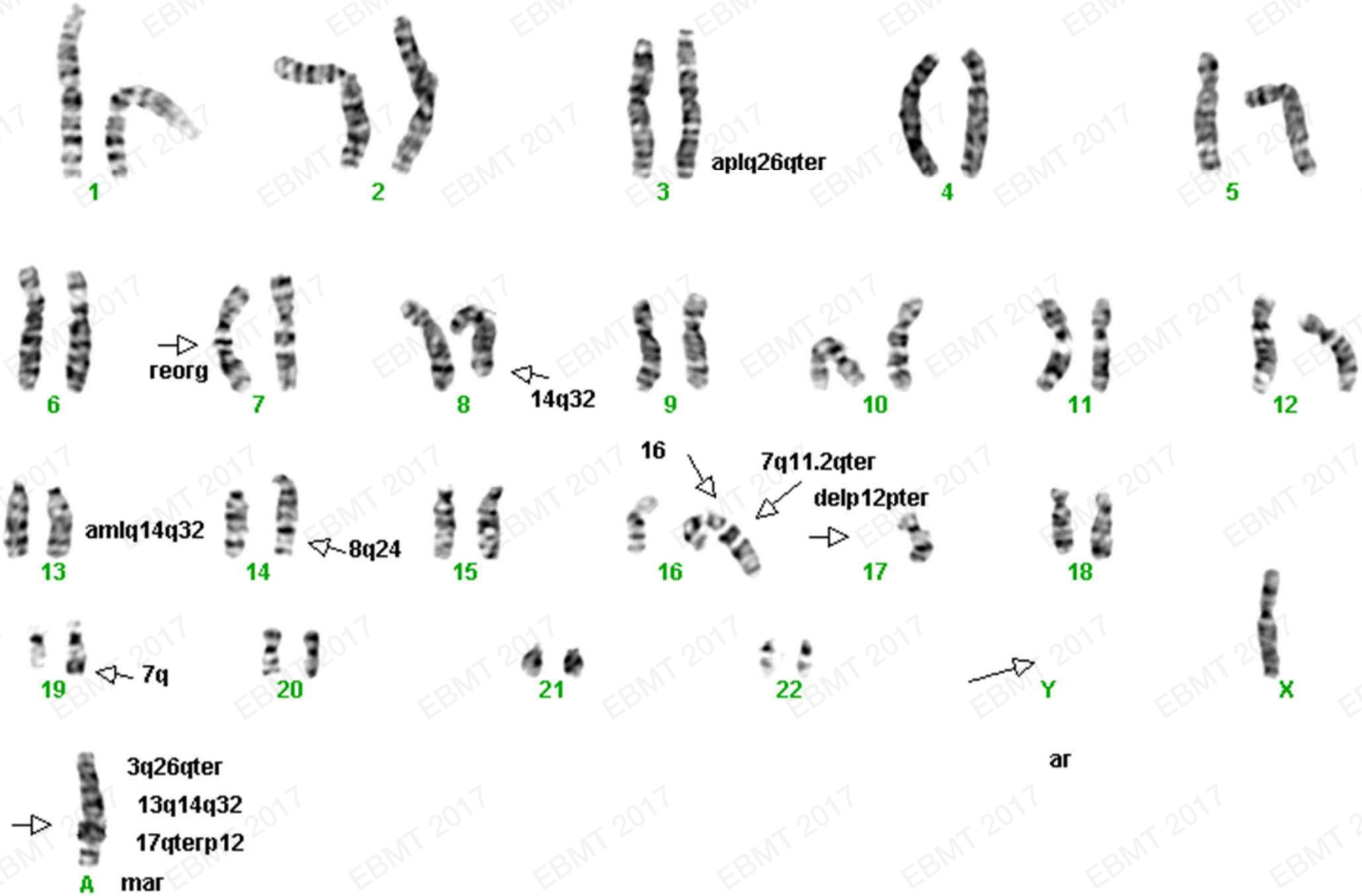
This cytogenetic analysis includes a high-throughput, non-routine technique such as SNP-array. In fact, the conventional karyotype (**46,XY,?der(9)[2]/46,XY[18])** informs us of **a normal karyotype in 18 metaphases and, in two additional metaphases, a chromosomal abnormality at chromosome 9**. Different analyses performed at chromosome 9 (including FISF, WCP and SNP arrays) unravel an abnormal chromosome 9 resembling an i(9p) structure and, more interestingly, a possible insertion of chromosome 9 region containing NUP214 gene in chromosome 17, possibly leading to a novel fusion gene of NUP214 with an unknown partner. EVI1(MECOM), MLL, CBFb-MYH11 and t(7;11) translocattion/rearrangement were discarded by FISH analysis

Of course, this is a non-recurrent abnormality, not “recognized”/interrogated in MedA questionnaire!

Cytogenetics in Med-A: final considerations

- Can we identify a defining-cytogenetic abnormality in the karyotype (formula)?
- Do we have molecular confirmation (FISH, PCR?
- Part of a complex, monosomal karyotype?
- Molecular complementary information?

- Do not hesitate (never!) to ask an expert or *expert-like*



45,X,-Y,der(7),t(8;14)(q24;q32),der(16)t(7;16)(q11.2;p13),der(17)t(3;13;17)(q26;q14q32;p12),der(19)t(7;19)(q?;q13.3)

Acknowledgments

- Dolors Costa, Ana Carrió, Joan Colomer (cytogeneticists & cytogenetic *advisors*)

- All people involved in the data entry process (reporting physicians, data-managers, ALWP office)

- Emmanuelle Polge