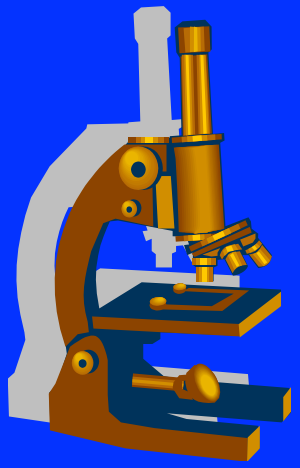
Role of FISH in Hematological Cancers

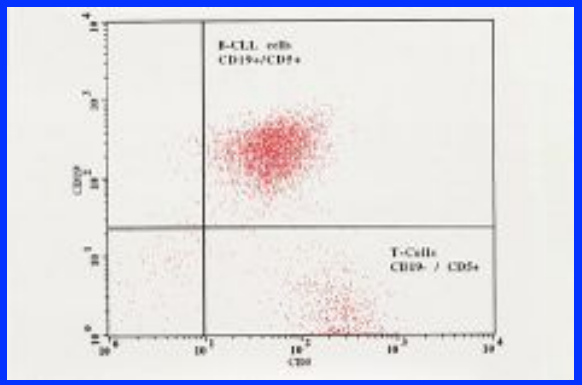
Thomas S.K. Wan

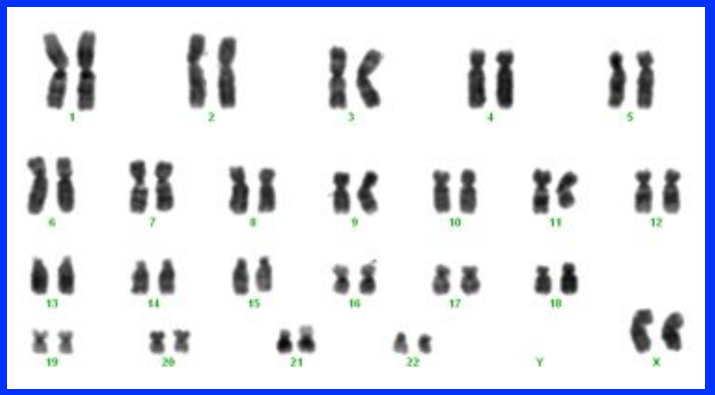
PhD,FRCPath,FFSc(RCPA)

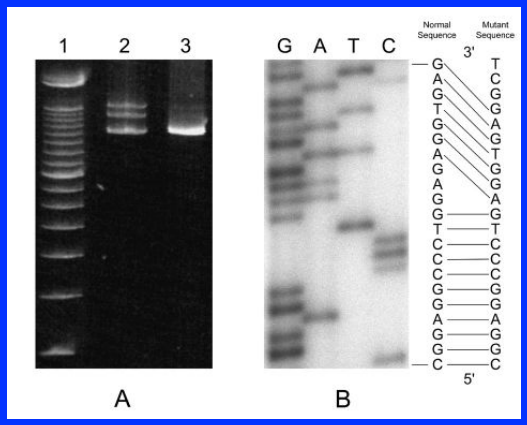
Honorary Professor,
Department of Pathology & Clinical Biochemistry,
Queen Mary Hospital, University of Hong Kong.
e-mail: wantsk@hku.hk

Leukemia diagnosis

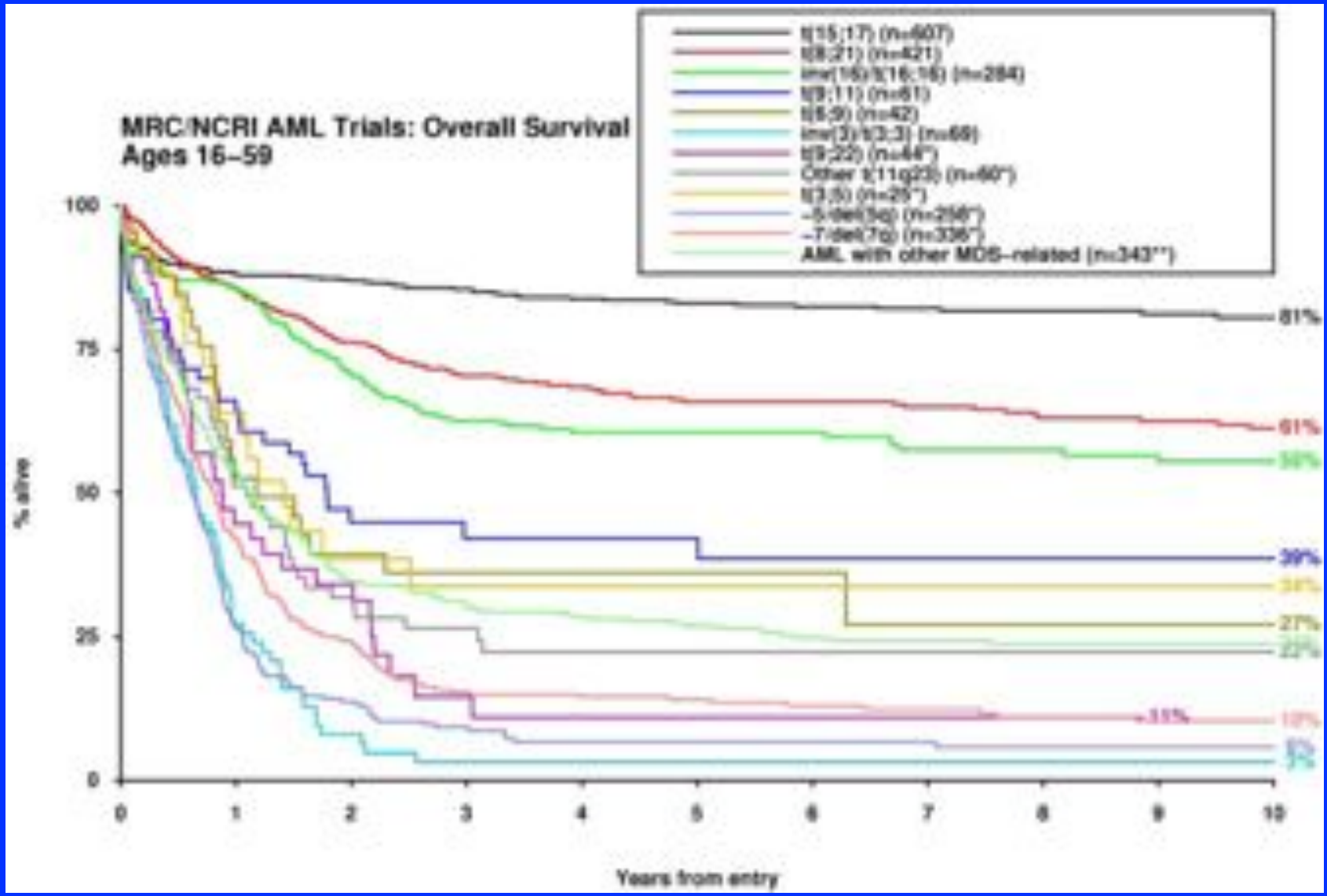








Acute myeloid leukemia



UK MRC AML data. Blood 116: 354-65, 2010

Limitations of conventional cytogenetic analysis

- requires living cells
- metaphase size
- resolution of chromosome bands
- requires considerable expertise
- labour intensive
- expensive

Molecular Cytogenetics:

FISH

Fluorescence in-situ hybridization

螢光原位雜交

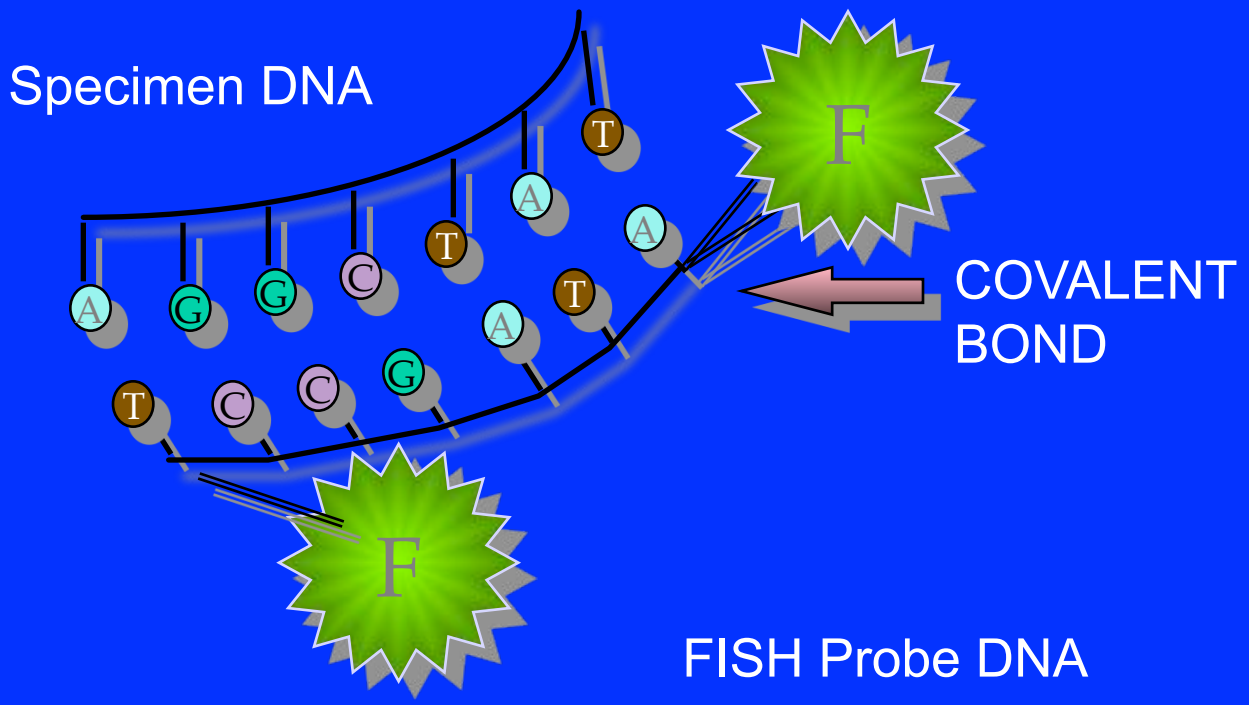
荧光原位杂交

การผสมพันธุ์ในแหล่งกำเนิด

형광에 원위치 하이브리드화

--- Use of DNA probes to detect changes in human chromosomes.

Direct Fluorescent-Labeled Probe



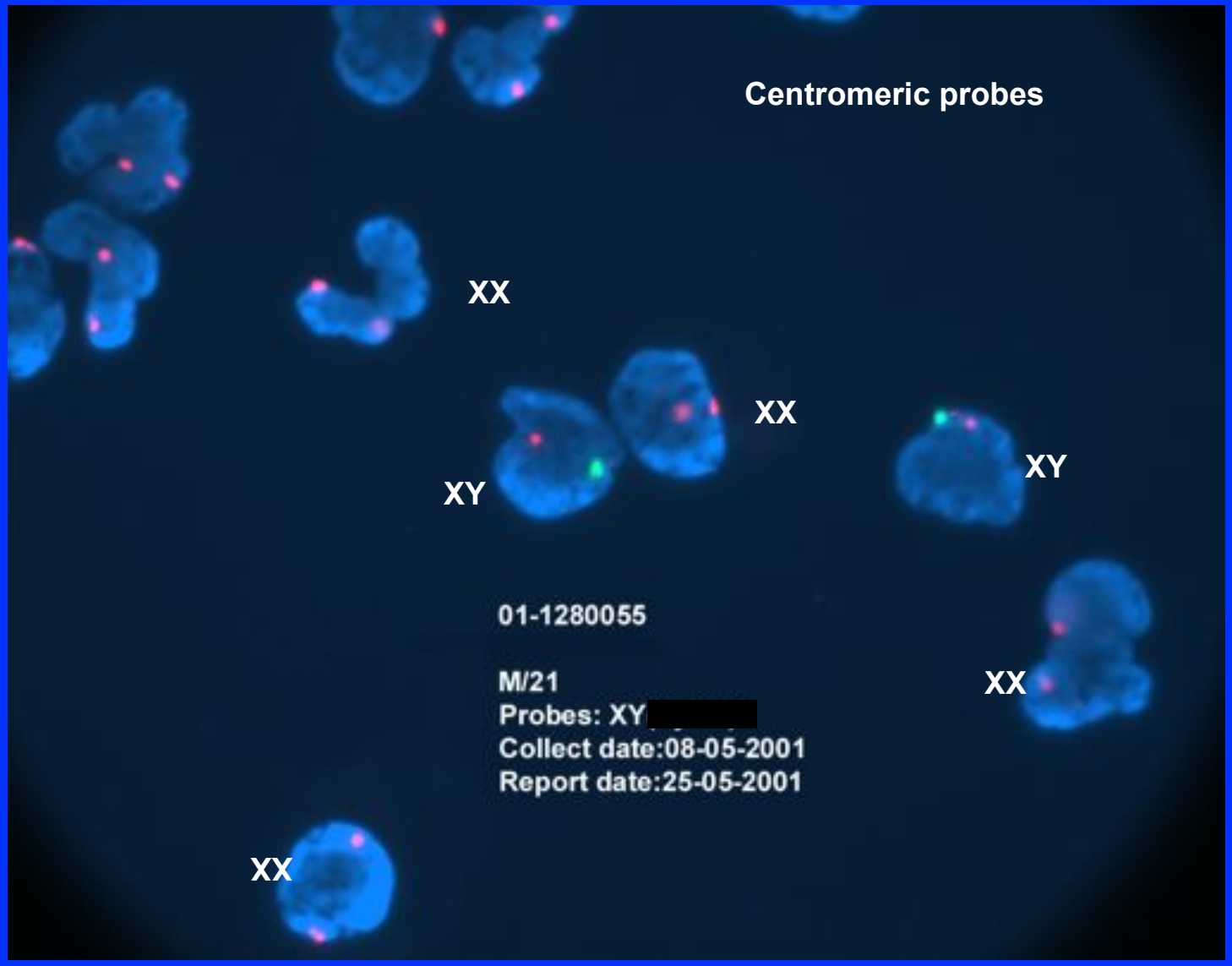
Advantages of FISH analysis

- Genetic abnormality measurable in dividing and non-dividing cells
- Applicable to many specimen types
- Direct correlation with cytologic features and immunophenotype
- Rapid
- Sensitivity & specificity

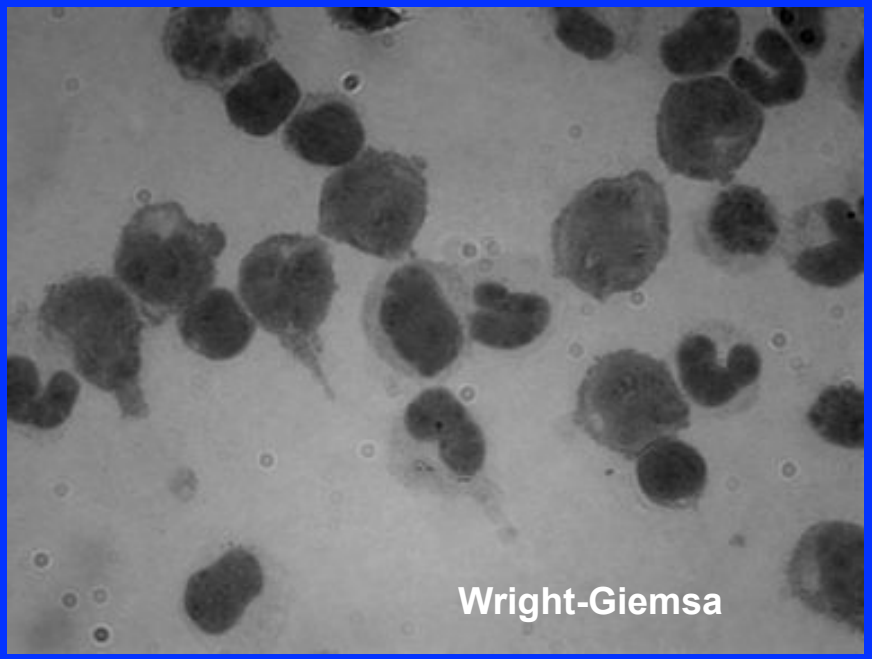
FISH as an investigative tool in haematological malignancies

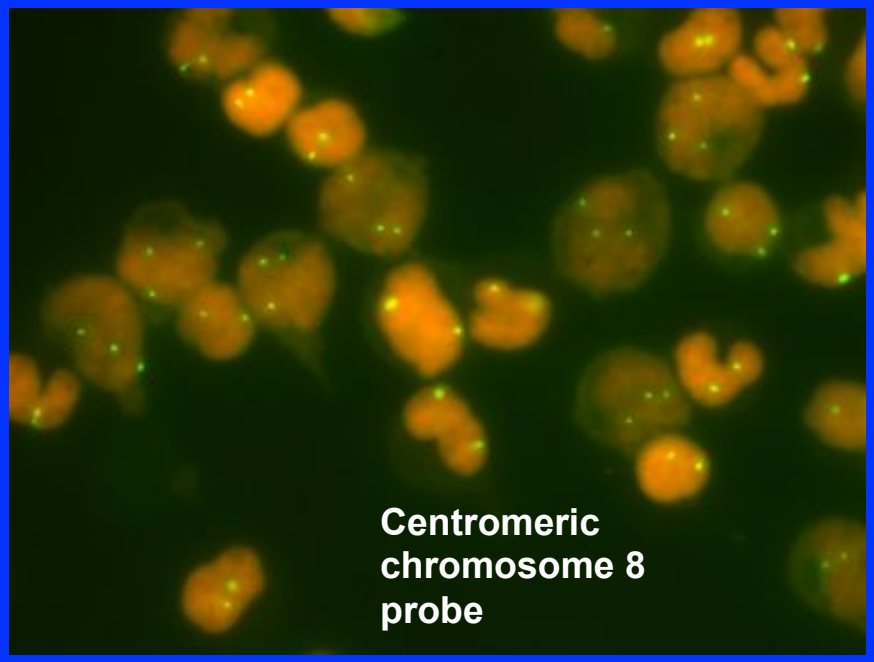
- Detection of numerical and structural anomalies in interphase and metaphase cells
- Characterization of marker chromosome
- Detection of cryptic translocation
- Lineage involvement by the neoplastic clone
- Detection of gene amplification
- Disease monitoring after treatment
- Chimerism study post-sex-mismatched BMT

Detection of chimerism of post bone marrow transplantation patients using centromeric XY FISH probes



Characterization cell lineage involvement using FISH and cell morphology



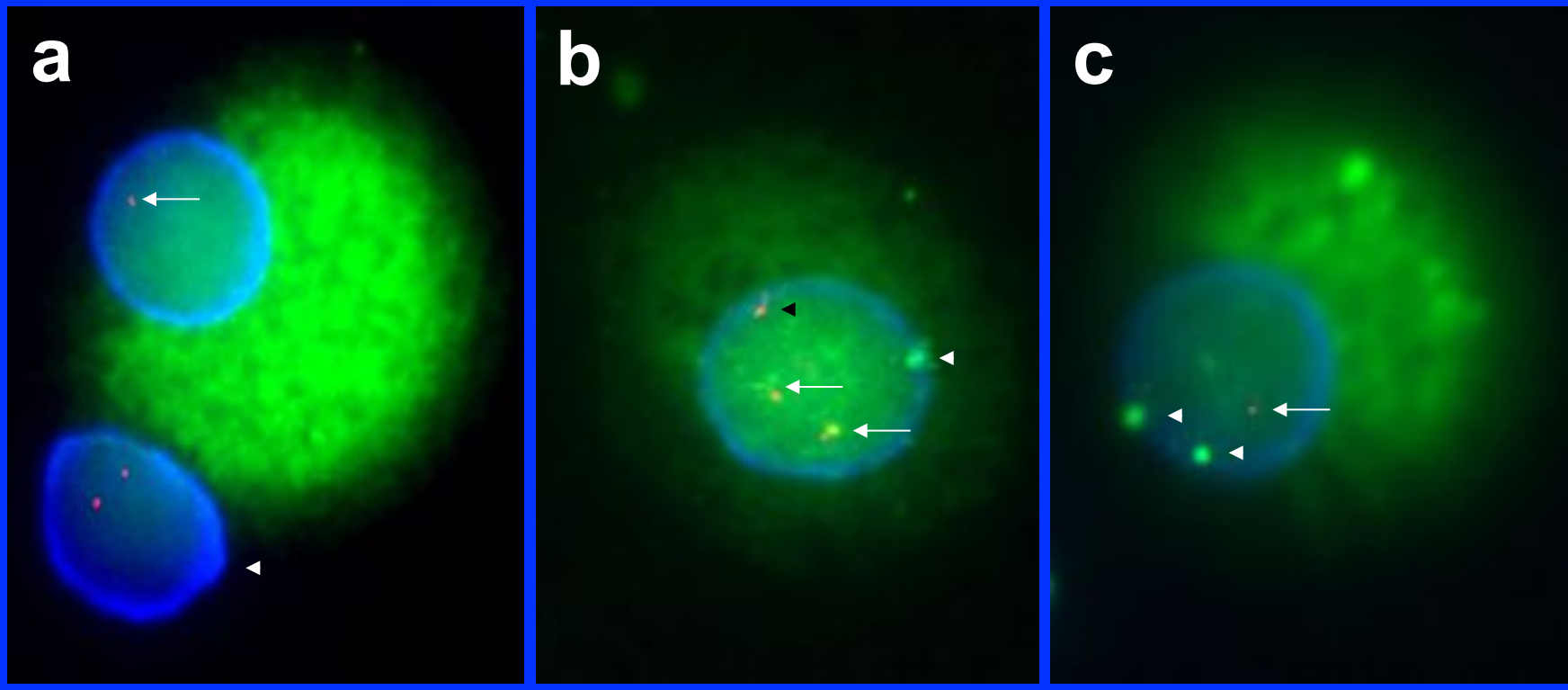


Acute megakaryoblastic leukemia (AMKL)
F/19m

Karyotype:
48,XX,+8,+21[2]/47,XX,+21c[10]

Ma, Wan et al (1999) Leukemia 13:491-492.

FISH + cytoplasmic Immunostaining



13q14.3 IgH-FGFR3 TP53

Choice of FISH probes for the detection of gene rearrangement:

1. **Dual color translocation probes**

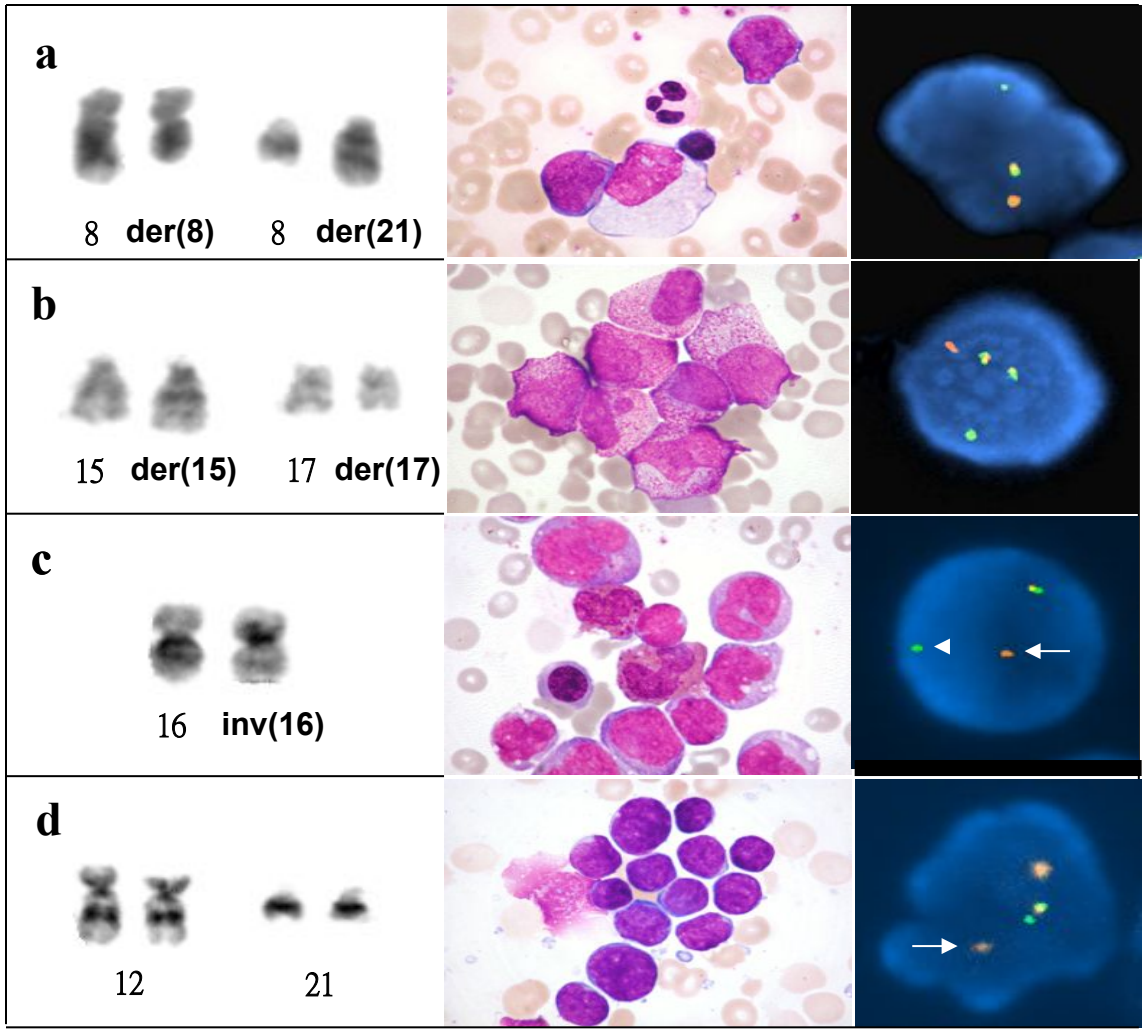
   - identifying translocation with known partners

   - *dual color signal fusion (S-FISH)*
   - *dual color extra-signal (ES-FISH)*
   - *dual color dual fusion (D-FISH)*
   - *tricolor color dual fusion (TD-FISH)*

2. **Dual color break-apart probes**

   - identifying translocation with unknown partners

Commonly used dual color translocation FISH probes for haematological disorders



Dual color single fusion probe

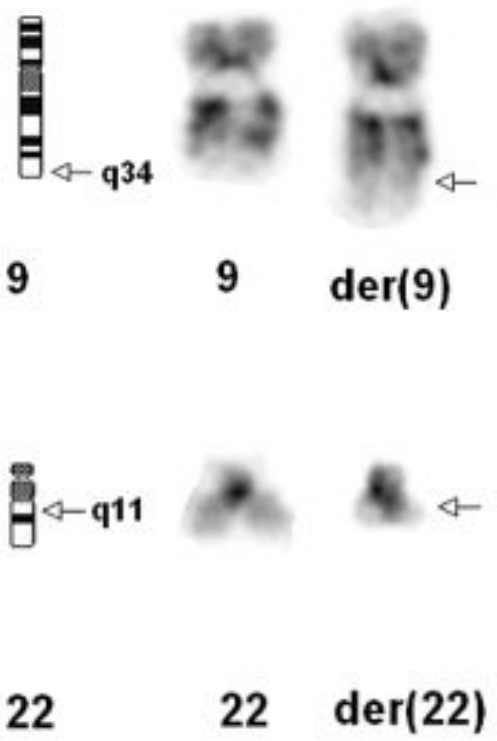
Dual color dual fusion probe

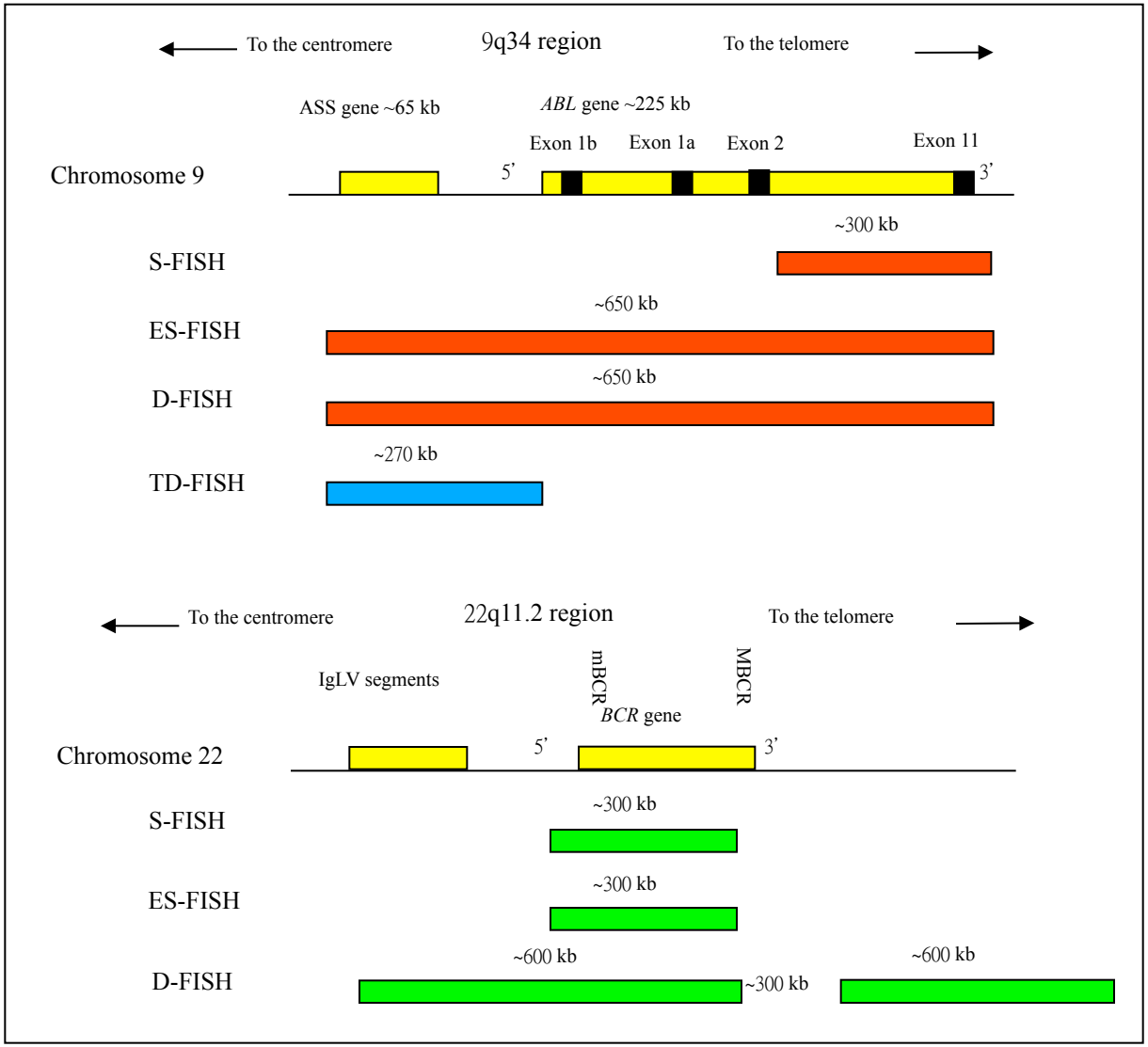
Dual color break apart probe

Dual color extra signal translocation probe

Ma & Wan (2004) Cancer Review: Asia-Pacific 2:131-141.

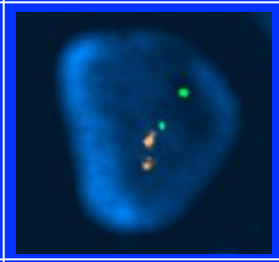
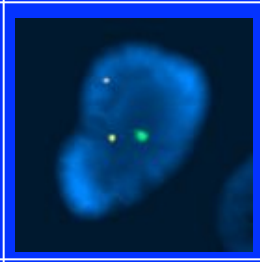
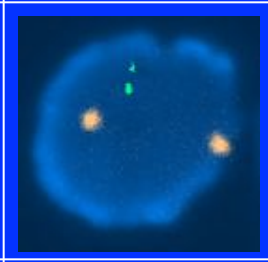
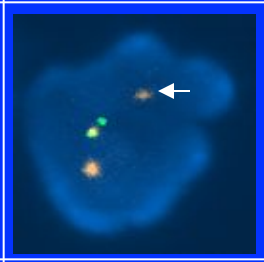
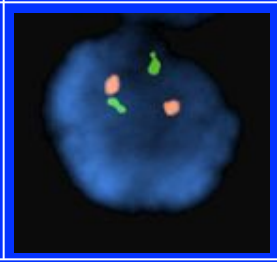
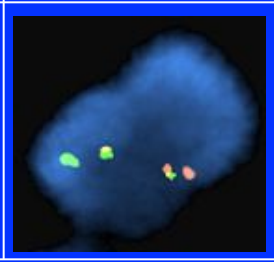
Development of BCR/ABL translocation probes





Wan et al. (2004) J Hong Kong Inst Med Lab Sci 9:1-12

Comparison of *BCR/ABL* translocation probes

Probes	Negative signal pattern	Positive signal pattern	False positive	Sensitivity
Single fusion	 2G 2O	 1G 1O 1F	2% - 10%	5% - 10%
Extra signal	 2G 2O	 1G 2O 1F	0% - 2.5%	< 1%
Dual fusion	 2G 2O	 1G 1O 2F	<1%	~0.2%

Derivative chromosome 9 deletions in CML:
Interpretation of atypical D-FISH pattern

- 4.3% (2/46) CML cases showed atypical interphase D-FISH pattern with 1O1G1F

- The presence of *BCR-ABL* gene fusion was documented by S-FISH at diagnostic marrow

- Submicroscopic deletion of *ABL-BCR* gene fusion on der(9) were characterized by metaphase FISH

- Occur at time of the Ph translocation

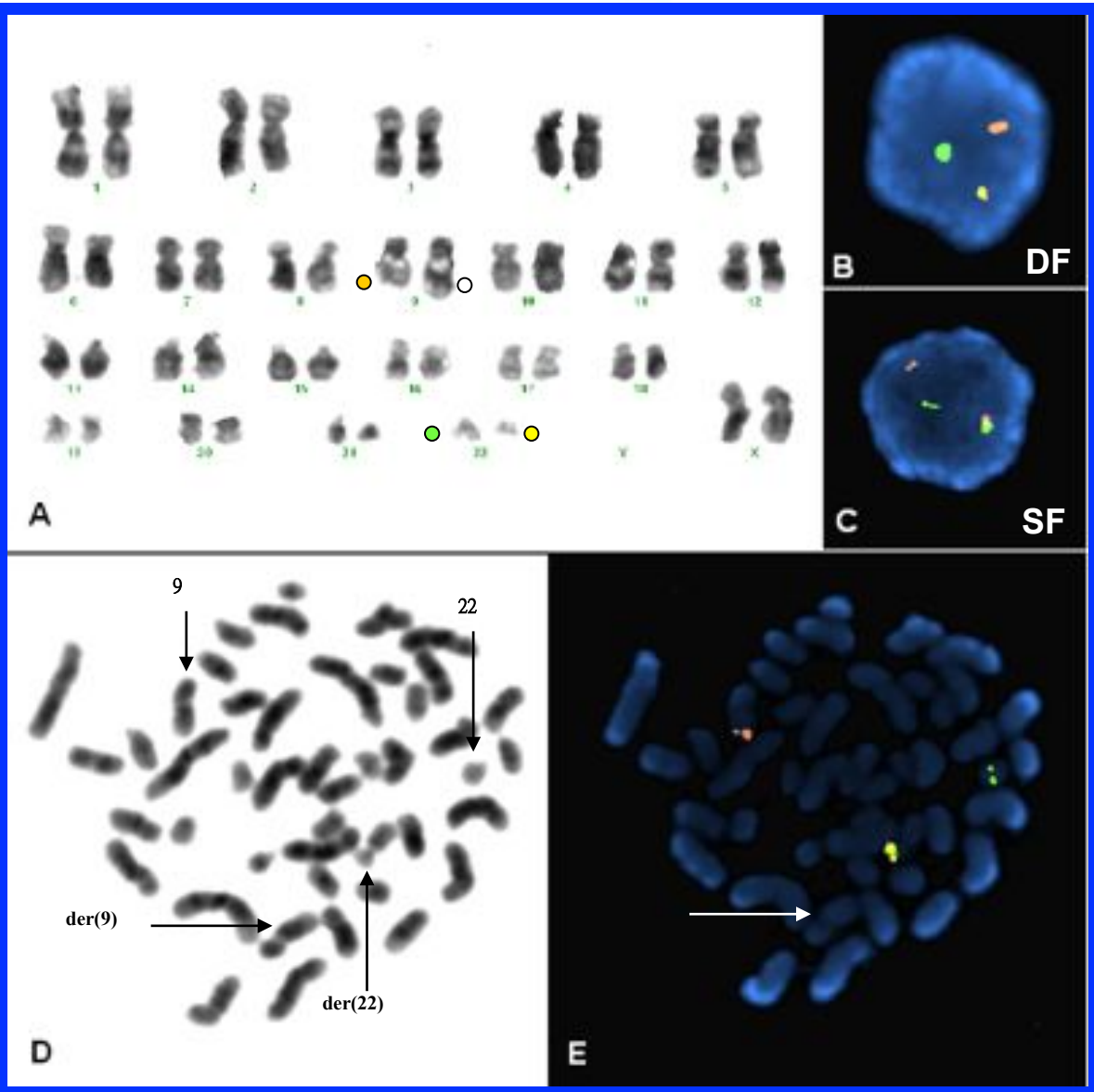
- Predict for a poor prognosis in CML and may be related to the loss of one or more genes with der(9) chromosome

Wan *et al.* (2003) J Clinical Pathology 56:471-474.

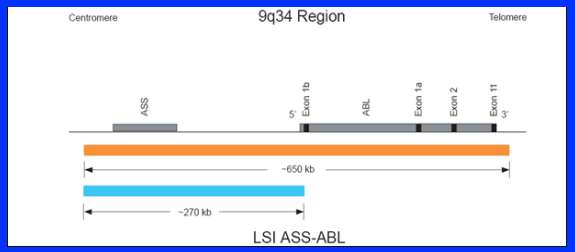
Derivative chromosome 9 deletions in chronic myeloid leukaemia: interpretation of atypical D-FISH pattern

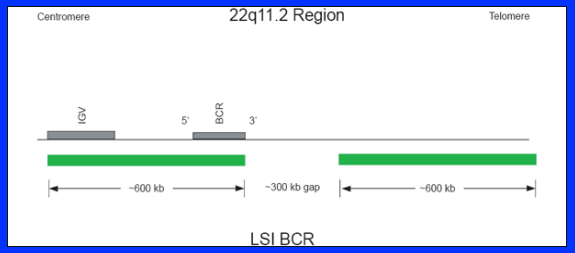
T S K Wan, S K Ma, W Y Au, L C Chan

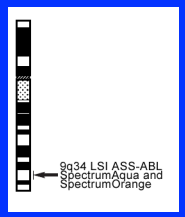
J Clin Pathol 2003;56:471–474



LSI BCR/ABL + 9q34 Tricolor, Dual Fusion Translocation Probe

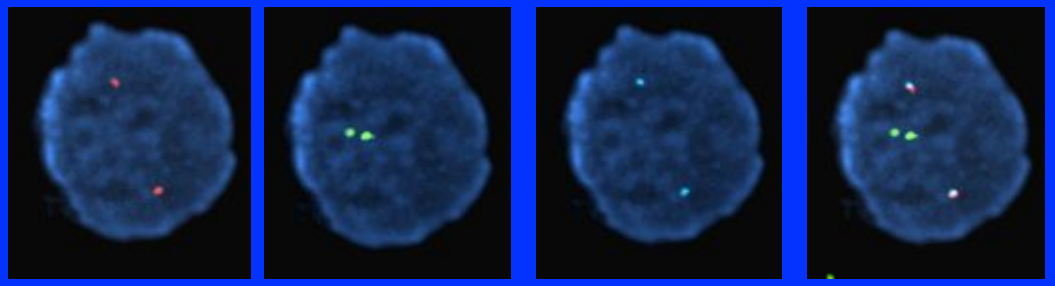




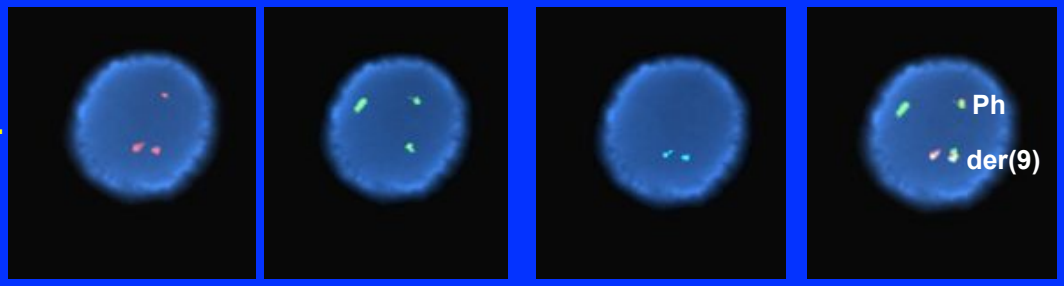


ABL BCR ASS

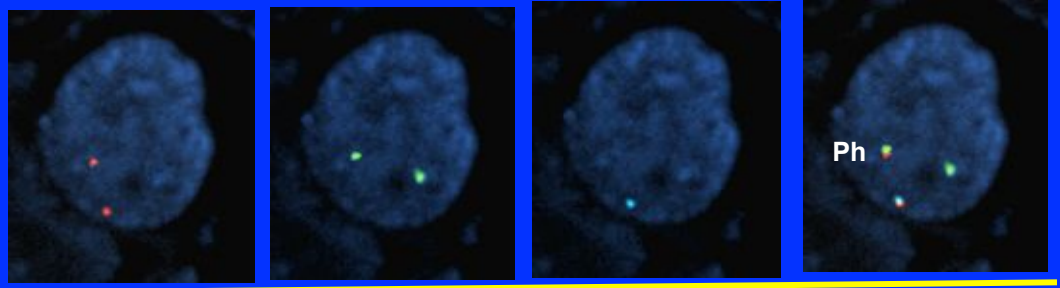
BCR/ABL negative



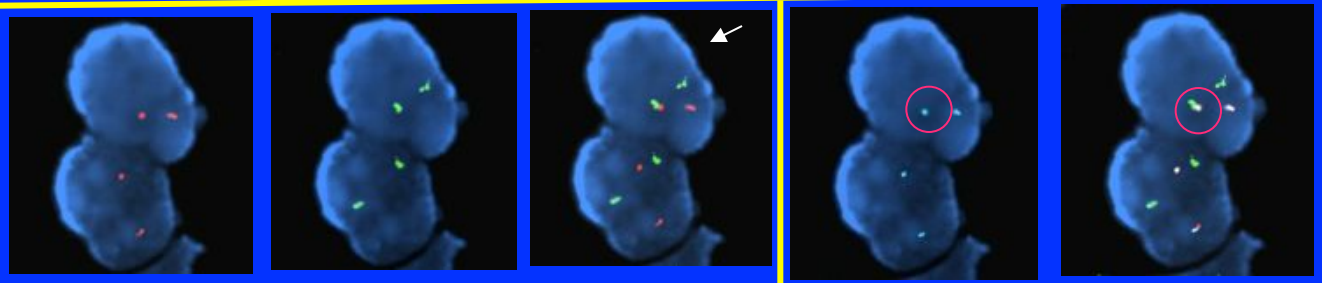
BCR/ABL fusion



BCR/ABL fusion & ABL/BCR deletion



? False positive of BCR/ABL fusion & ABL/BCR deletion

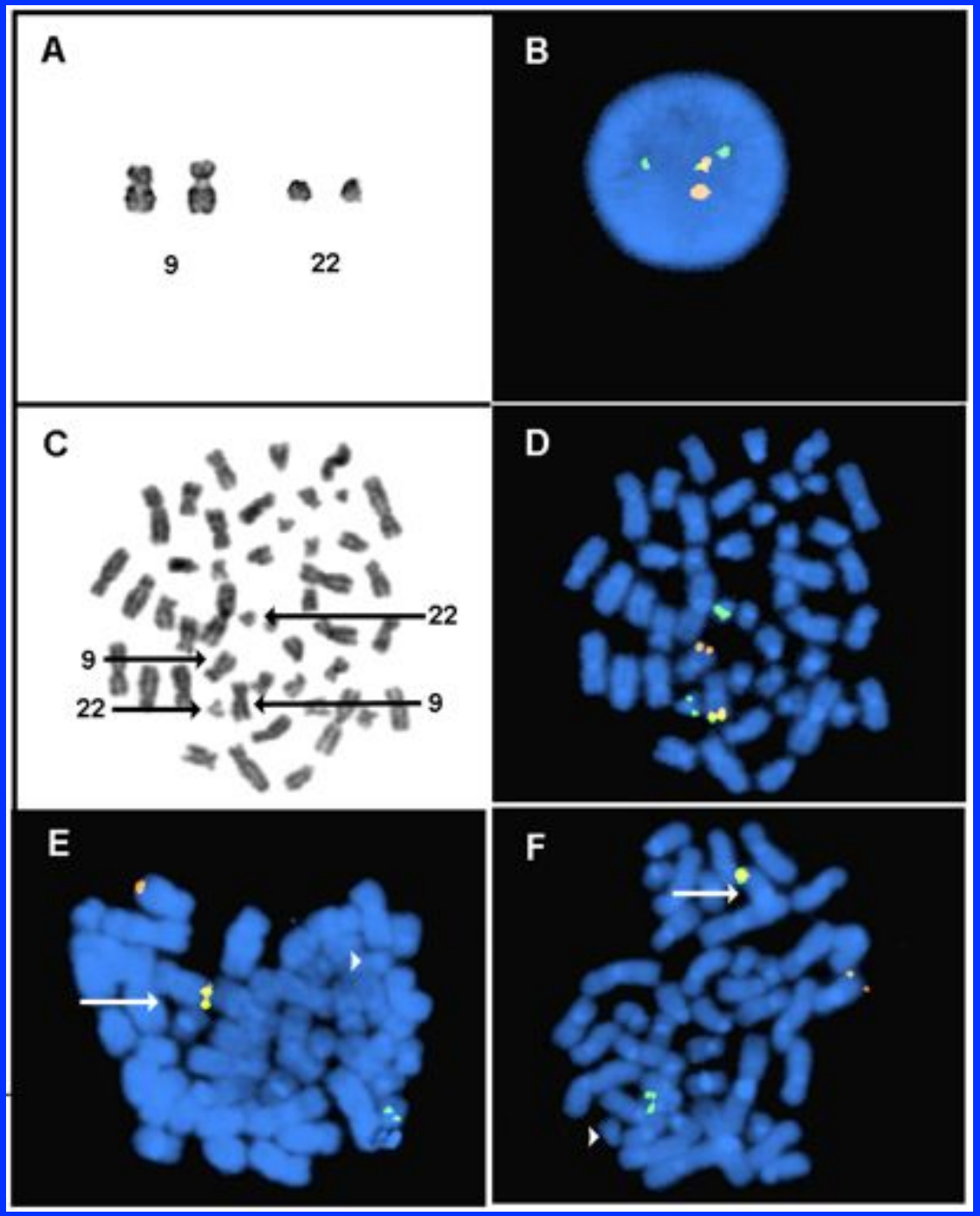


DF: ? 9q-

TDF: Ph negative cell

Prof TWan

Atypical FISH pattern in CML due to cryptic insertion of *BCR* at 9q34



Wan *et al* (2004) Leukemia 18, 161-162

Characterization of additional genetic events in childhood ALL with *TEL/AML1* gene fusion:
A molecular cytogenetics study

- 18.5% (12/65) childhood ALL harbored *TEL/AML1* fusion transcript i.e., t(12;21)(p13;q22)

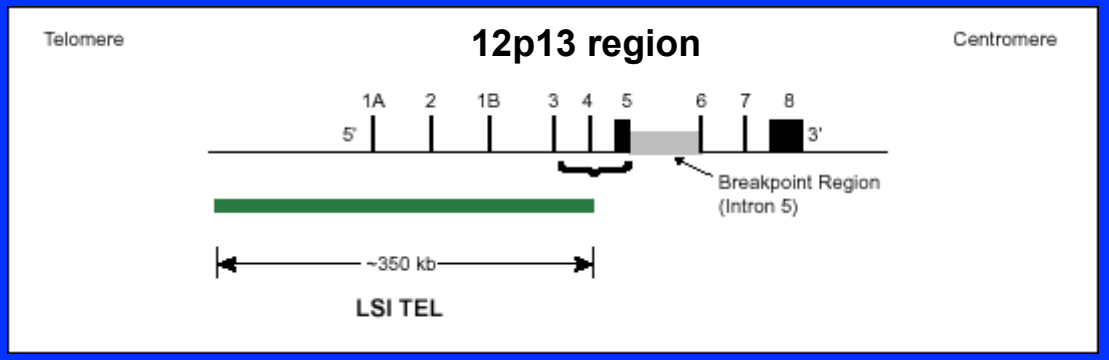
- 54.5% (7/12) with additional or secondary genetic changes:
  3 cases showed extra copies of chromosome 21
  1 cases showed amplification of the *AML1* gene
  1 cases showed deletion of the normal *TEL* gene
  1 cases showed duplication of the *TEL/AML1* fusion signal
  1 cases showed loss of chromosomes 12 together with duplication of der(12)t(12;21)(p13;q22)

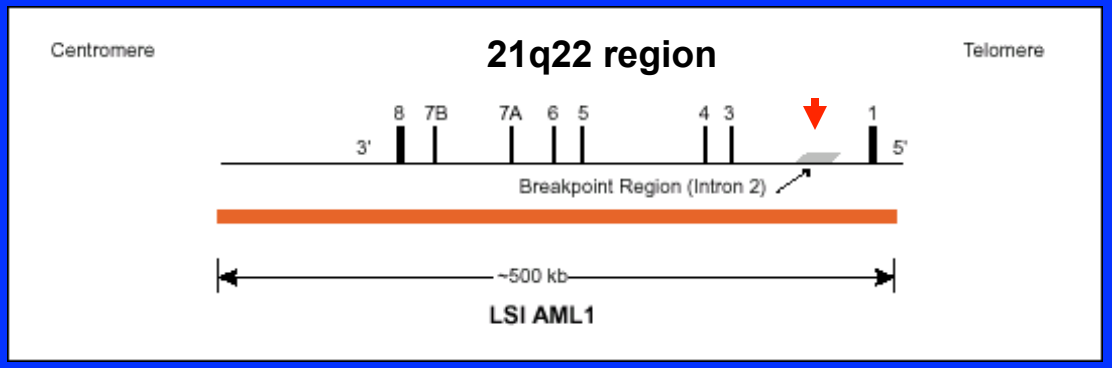
- Additional or secondary genetic changes including *AML1* amplification are commonly encountered in childhood ALL with *TEL/AML1* gene fusion

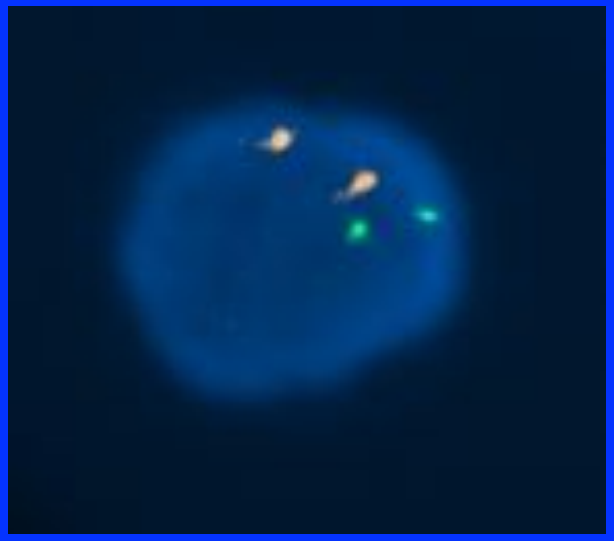
- These genetic changes are expected to play critical roles in disease progression

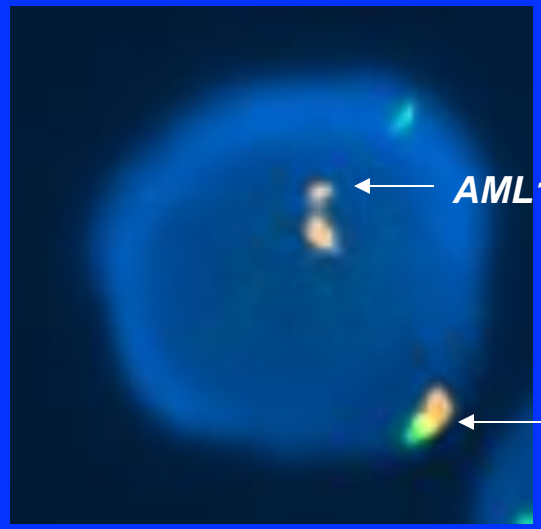
Ma, Wan *et al*. (2001) Leukemia 15, 1442-1447.

TEL/AML1
dual color
extra signal
translocation probe









AML1 residual

TEL/AML1 fusion

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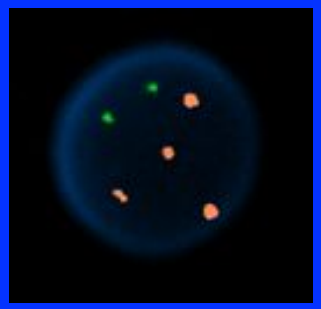
Case 1

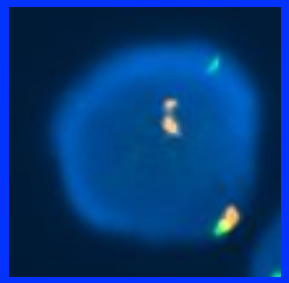
TEL/AML1 fusion, *+AML1*

AML1 x4

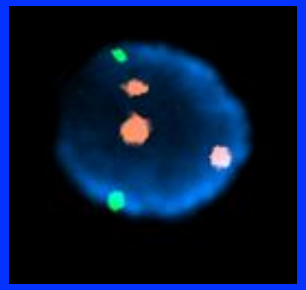


Case 2

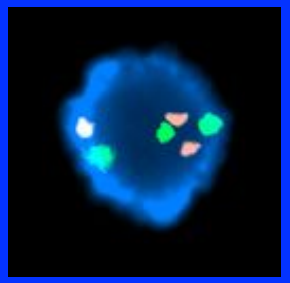
TEL/AML1 fusion



AML1 x3

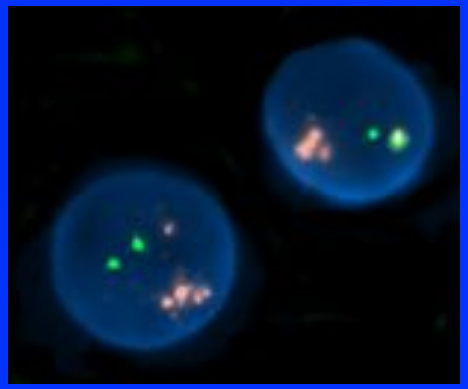


CEP 12 (green) x3,
LSI 21q22 (orange) x3

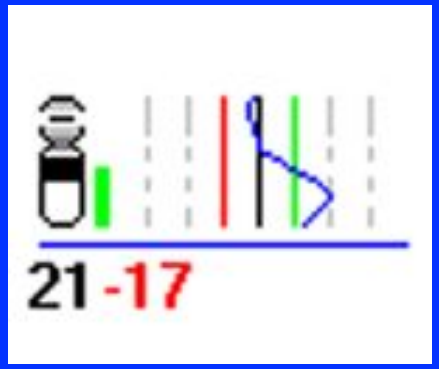


Case 4

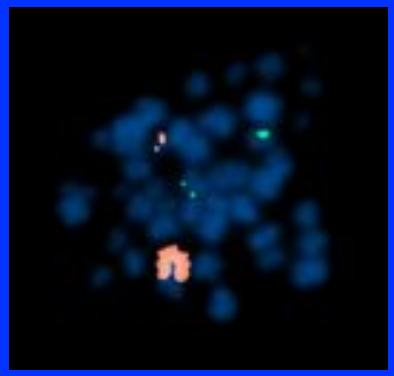
AML1 amplification



Amplification of 21q

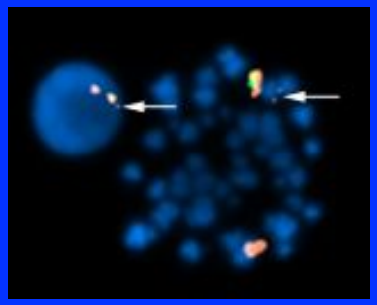


AML1 amplification

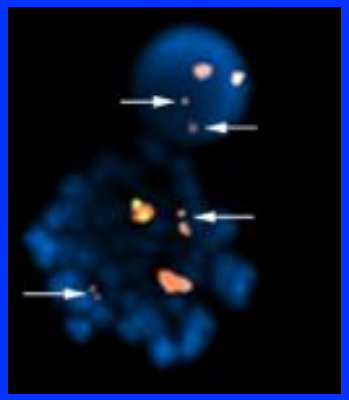


Case 5

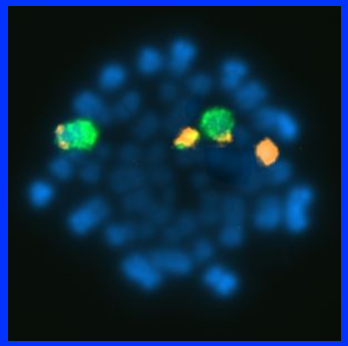
TEL/AML1 fusion x1,
AML1 residual x1,
Loss of normal *TEL*

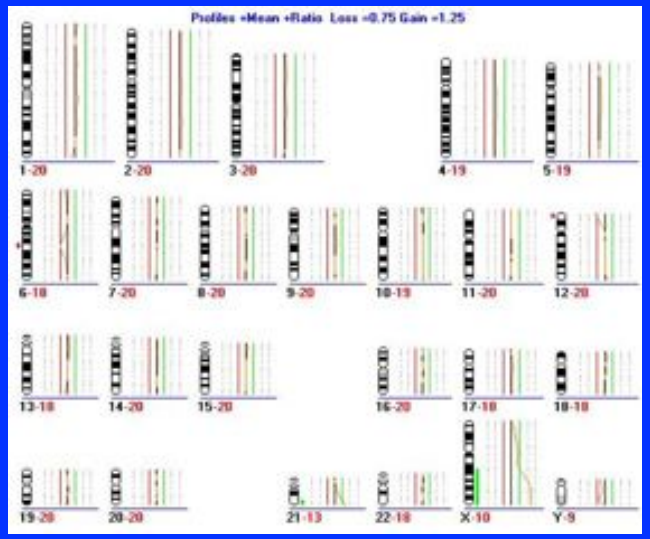


TEL/AML1 fusion x1,
AML1 residual x2



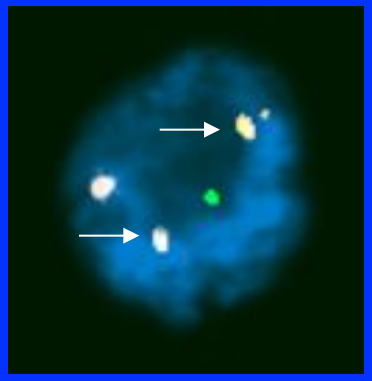
WCP12 (green),
WCP 21 (orange)





Case 6

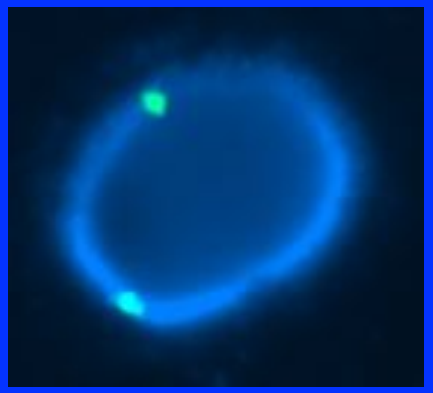
TEL/AML1 fusion x2



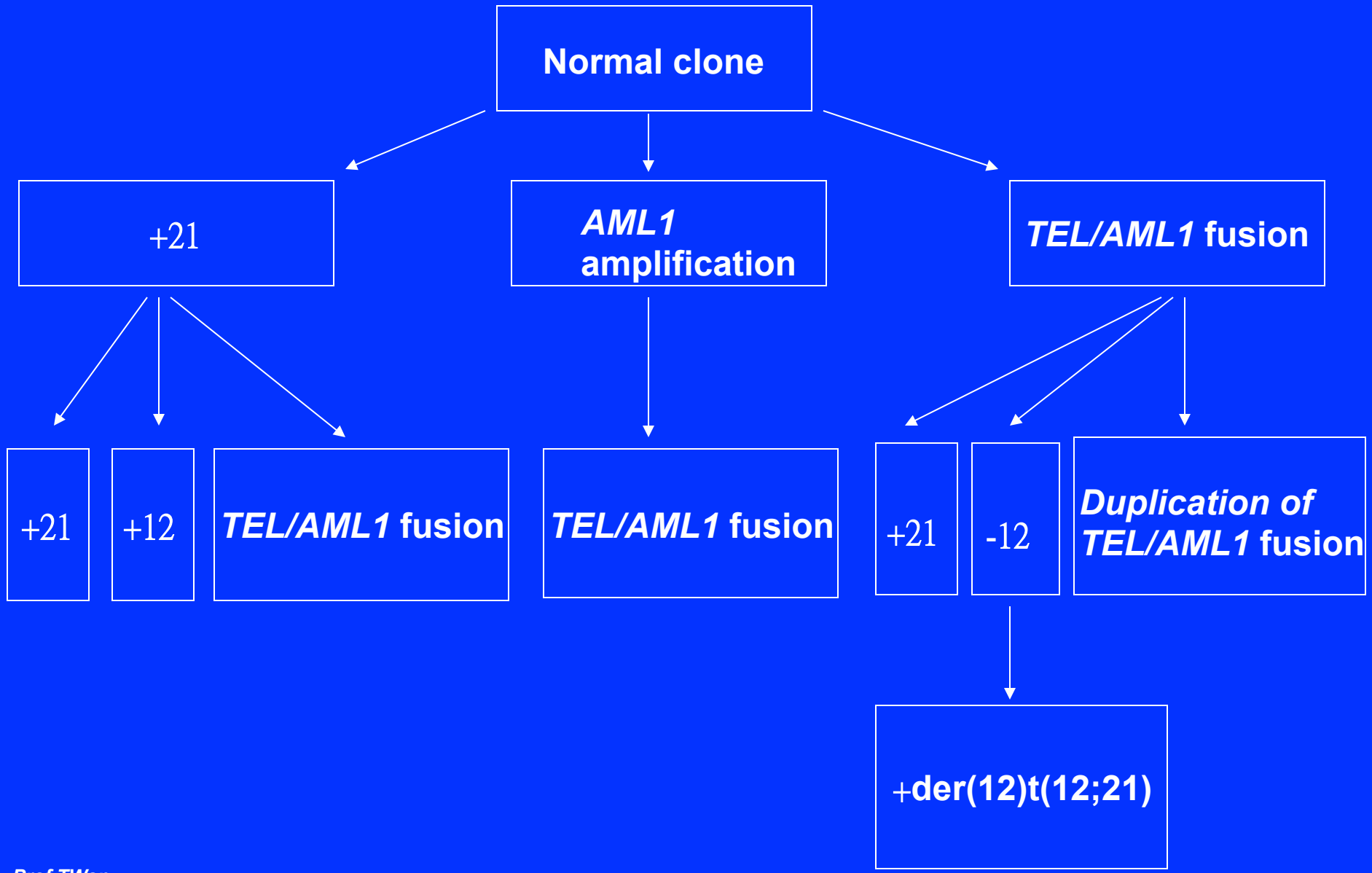
Case 7

TEL/AML1 fusion,
Normal *TEL* deletion

CEP 12

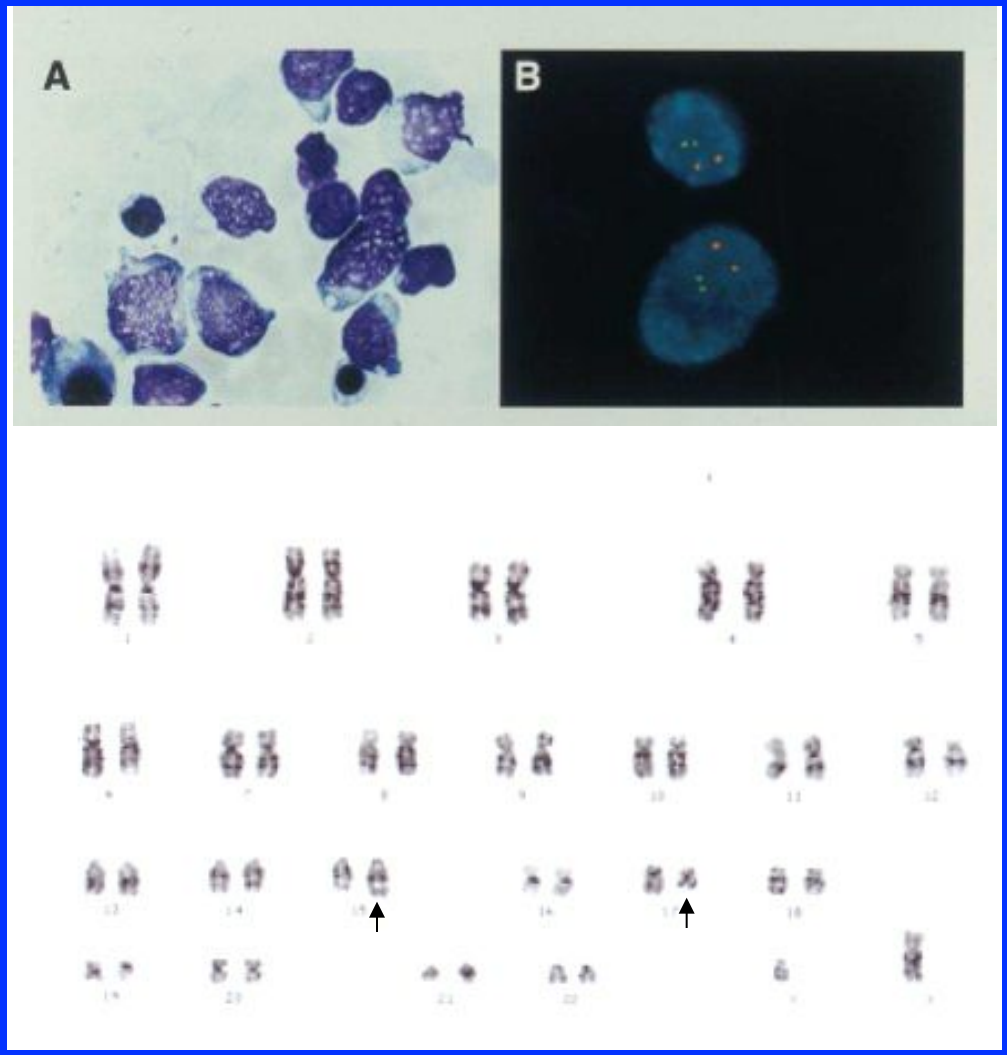


Pathways for cytogenetic evolution in childhood ALL with *TEL/AML1* fusion



FISH vs Conventional cytogenetics

t(15;17) not associate with APL and negative for *PML/RARA* fusion

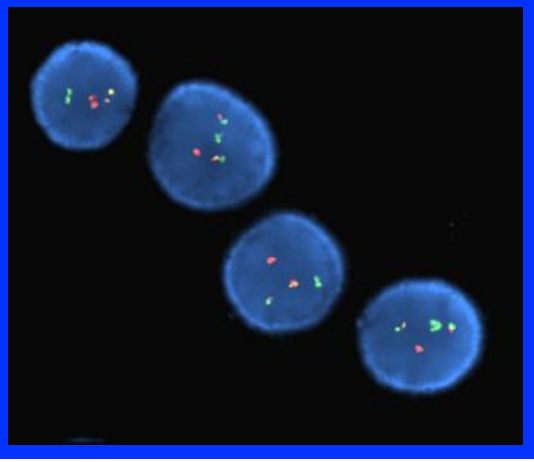


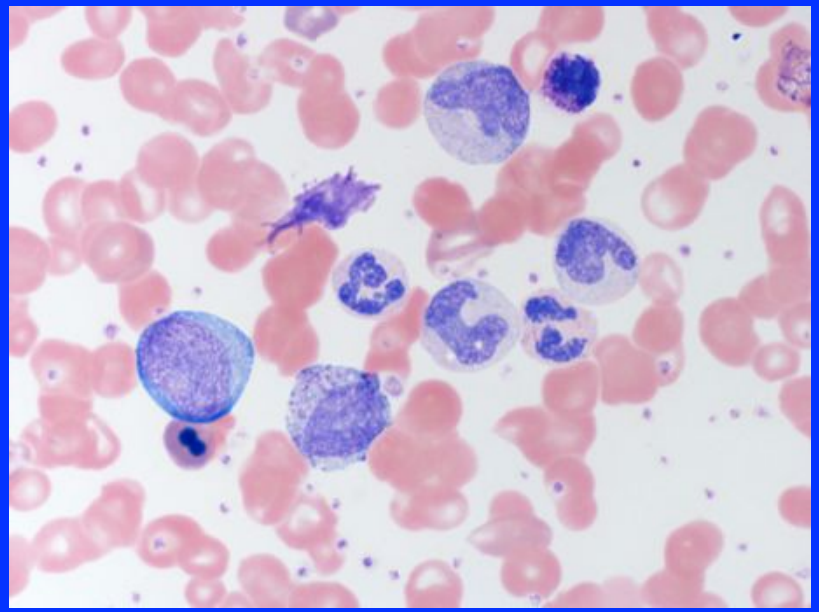
Ma, Wan, *et al*. (2000) Haematologica 85:768-769.

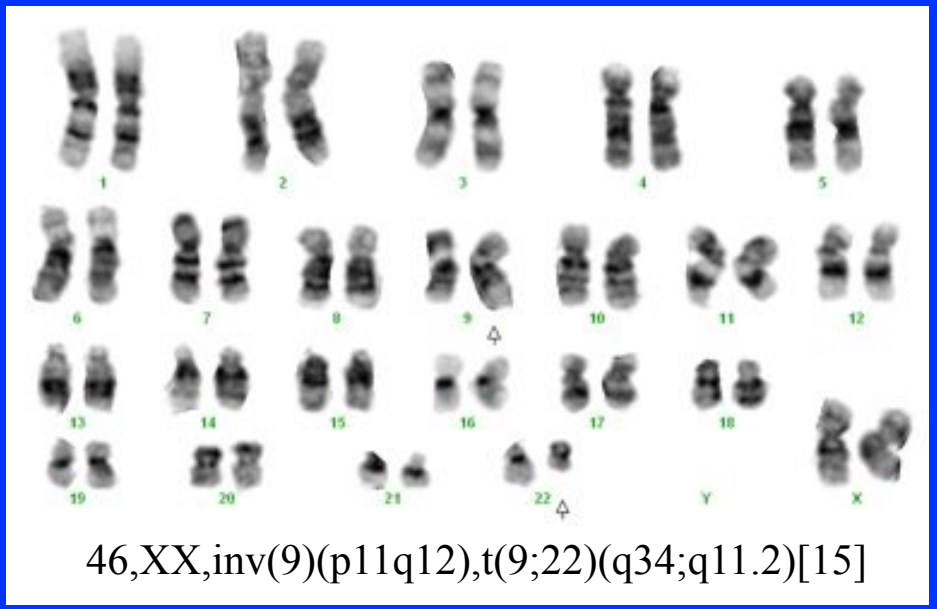
FISH vs molecular technique

Ph +ve CML with BCR-ABL variant transcript

- F/51, BM Feb 2007 showed 33% blasts
- April 2007: Hb 11.1 g/dL,
  WBC 66.1 x 10^9/L and Plt 920 x 10^9/L







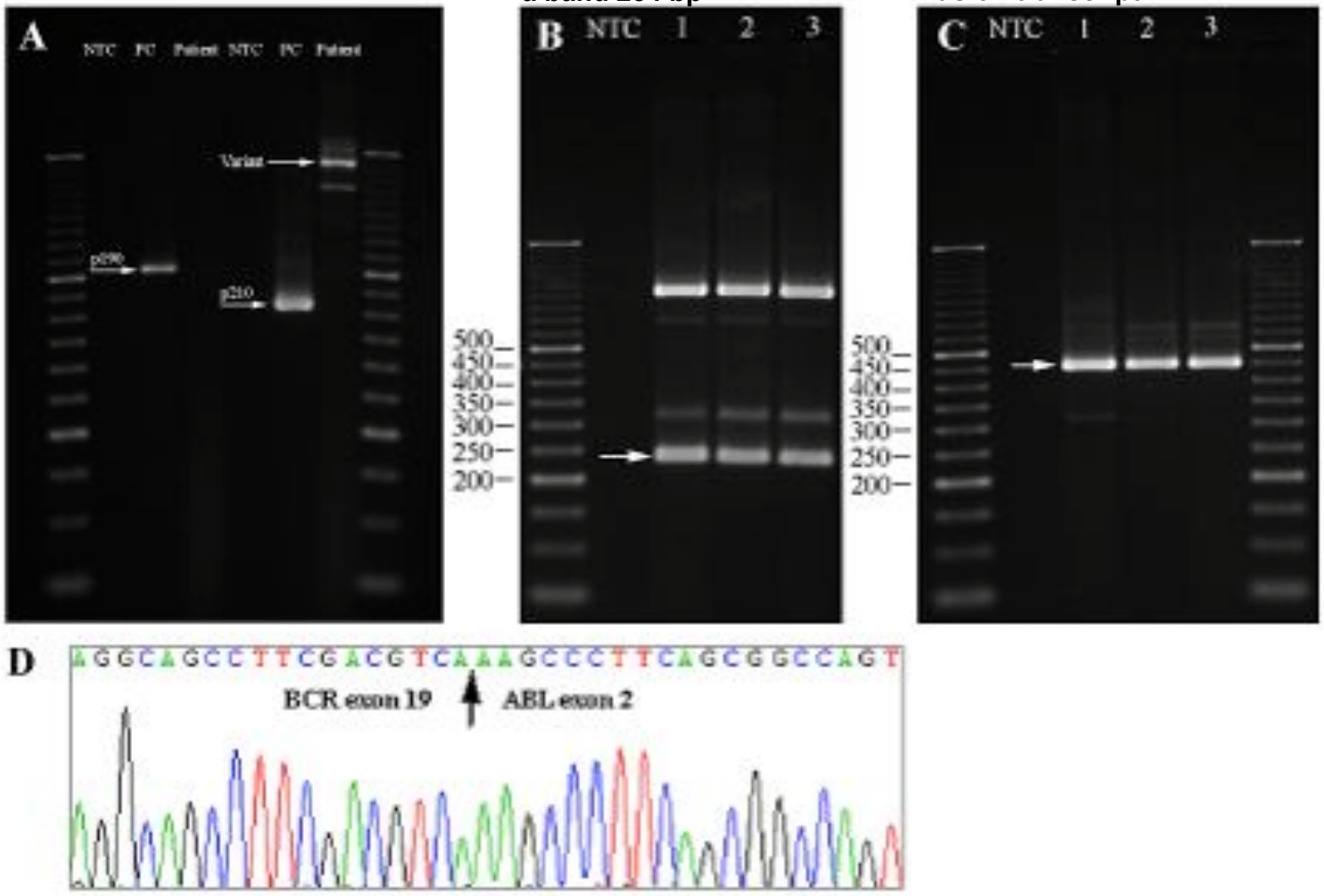
46,XX,inv(9)(p11q12),t(9;22)(q34;q11.2)[15]

Wan et al., Chang Gung Medical Journal, in press.

RT-PCR

Multiplex PCR showing a band 234 bp

RT-PCR targeting the e19a2 fusion transcript



Automated sequencing confirming the e19a2 BCR-ABL fusion transcript

Sample 1: before imatinib; 2: six months after imatinib; 3 12months after imatinib

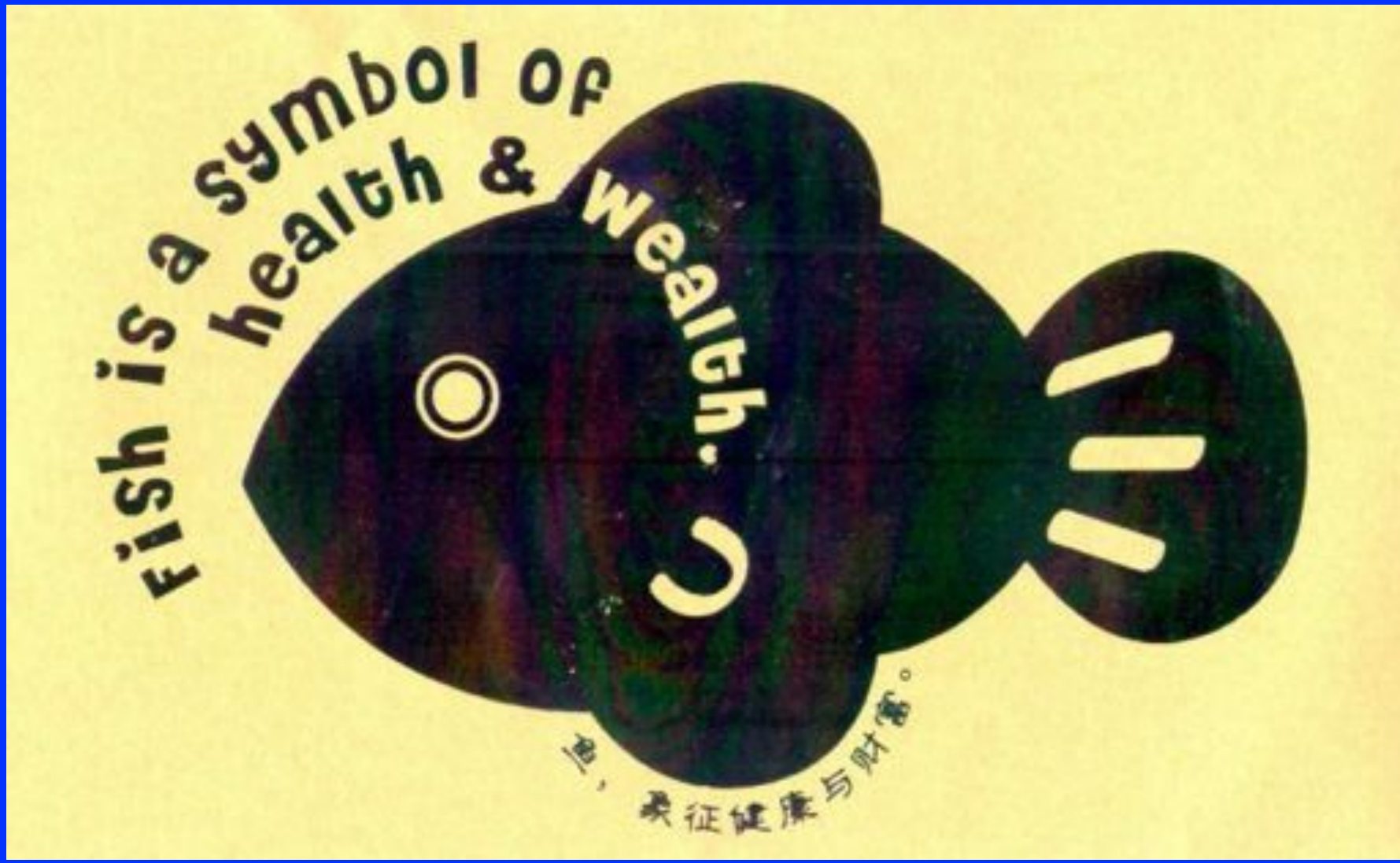
Wan et al., Chang Gung Medical Journal, in press.

Caveats of FISH analysis

- No global view of chromosomal complement
- Requires clinicopathological or prior cytogenetics information
- Issues related to analytical sensitivity and probe specificity
- Susceptibility to artifacts
- Cannot detect minute aberrations (< 20 kb)
- Aneuploidy versus amplification

Any role for FISH in the post-genomic era?

- Manageable by routine diagnostic laboratories
- Answer to specific clinical questions
- Support the practice of personalized medicine
- Practical advantages
  - Numerical abnormality
  - Multiple fusion partners
  - Breakpoint heterogeneity
- Applicable to many specimen types


Fish is a symbol of
health & wealth.
鱼，象征健康与财富。