Applications of Fluorescence in situ hybridization (FISH) in hematology

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Mosul medical collage

What is FISH ?

It is process of painting the whole chromosome or only part of the chromosomes (protein) with florescence molecule to identify chromosomal abnormality and presence of a region of DNA or RNA within the chromosome.

History:

- In 1969, Gall and Pardue introduced *in situ* hybridization (ISH) to localize nucleic acids in individual cells.
- Pinkel (1986), described the Immunofluorescence technique .
- Afterwards, so many techniques derived from FISH have been developed.

Other techniques:

- Q-FISH.
- Fiber FISH.
- Multi colour FISH(M-FISH or SKY).
- Flow-FISH.
- Comparative genomic hybridization(CGH).
- SNP(single nucleotide polymorphism array).

Targets:

- Metaphase chromosomes.
- Interphase cells.
- Tissue sections---from tissue biopsy slides.
- Cells in culture.

What does FISH need?

- Sample.
- Probe.
- Fluorescence microscope.

Samples:

- Blood.
- Bone marrow(aspirate , biopsy).

•Probe:

It is a specific DNA fragment, usually 1 to 100 kb length, complementary to the chromosome site that we are interested in e.g of probe:

- ❖ Single colour
- ❖ Dual colour _ dual fusion
- ❖ Dual colour _ single fusion

Probe Labeling:

- Direct labeling : the probe directly labeled with fluorochromes such as Spectral Green and Spectral Orange(One-step hybridization).
- Indirect labeling : needs antibodies to complete FISH procedure(Haptens-Biotin-dUTP, digoxigenin-dUTP).

Fluorescence microscope:

Light source:

High-pressure mercury vapor lamps, tungsten-halogen lamps or xenon lamps.

Filters:

1. Exciting filter: to let a certain wave length of light passes so that can excite the given fluorochrome carried on sample.
2. Barrier filter: to allow the visible light passes so that the fluorescence can be seen by eyes or the image can be captured.

   Note: there should be no auto-fluorescence in any part of light path except for samples

Procedure:

- Denaturation of chromosome.
- Denaturation of probe.
- Hybridization: It is the formation of duplex between 2 complementary sequences.
- Florescence staining.
- Examination of the slide or store in dark.

The principles of fluorescence in situ hybridization:

(a) The basic elements are a DNA probe and a target sequence.

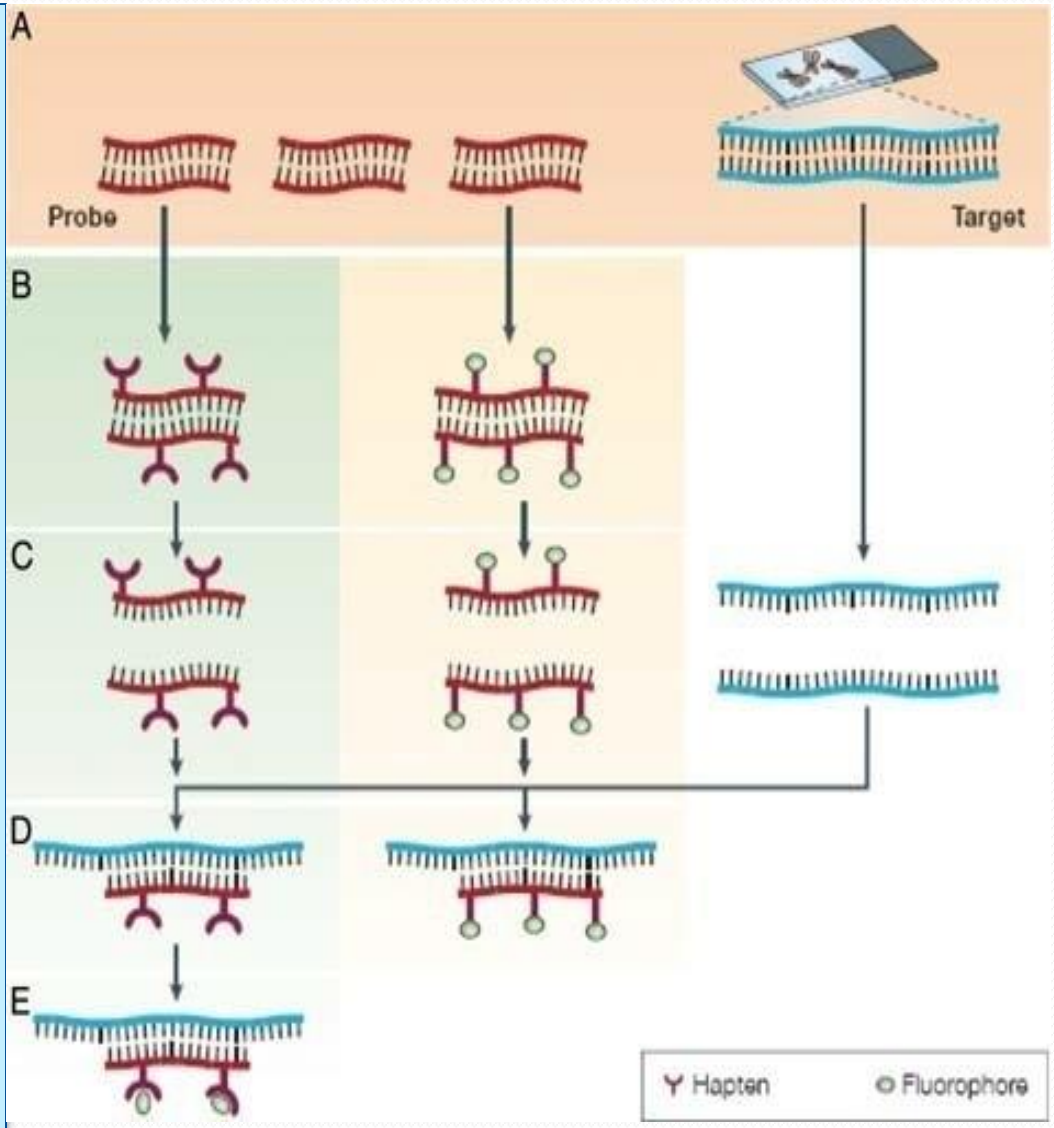
(B) Before hybridization,

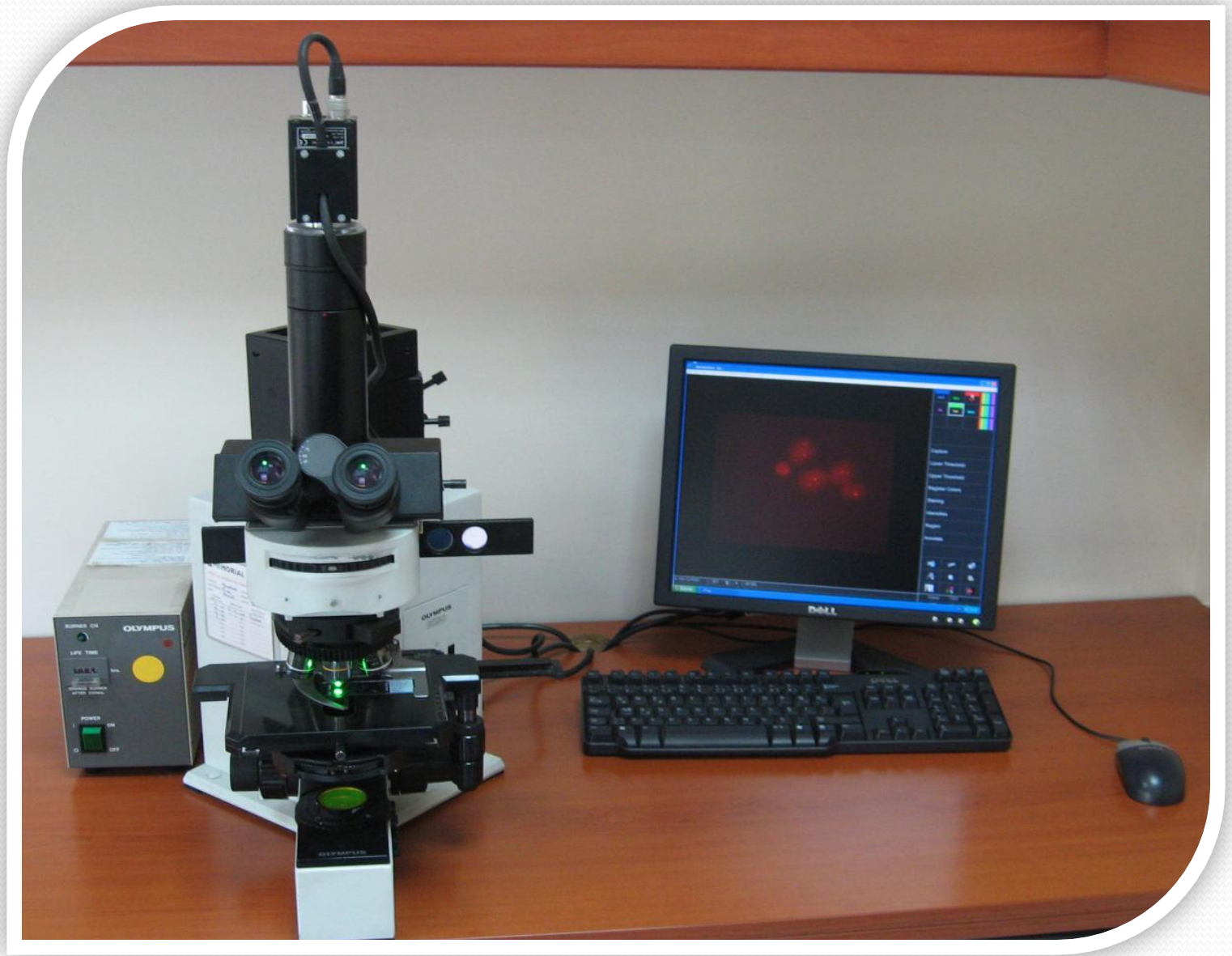
the DNA probe is labelled indirectly with a hapten (left panel) or directly labelled via the incorporation of a fluorophore (right panel).

(C) The labelled probe and the target DNA are denatured to yield single-stranded DNA.

(D) They are then combined, which allows the annealing of complementary DNA sequences.

(E) If the probe has been labelled indirectly, an extra step is required for visualization of the non-fluorescent hapten that uses an enzymatic or immunological detection system. Finally, the signals are evaluated by fluorescence microscopy




OLYMPUS
DELL

A good FISH method should have:

- An extremely high specificity(extremely low background).
- A good sensitivity(good hybridization efficiency).
- Unambiguous recognition of the hybridization signal.

FISH limitation:

- Probe design requires knowledge of specific chromosomal abnormality to be studied.
- Cut off signals may be different among laboratory.
- Processing error, imperfect hybridization, non specific binding, photo bleaching, inter observer variability and false positive and negative.

Advantages:

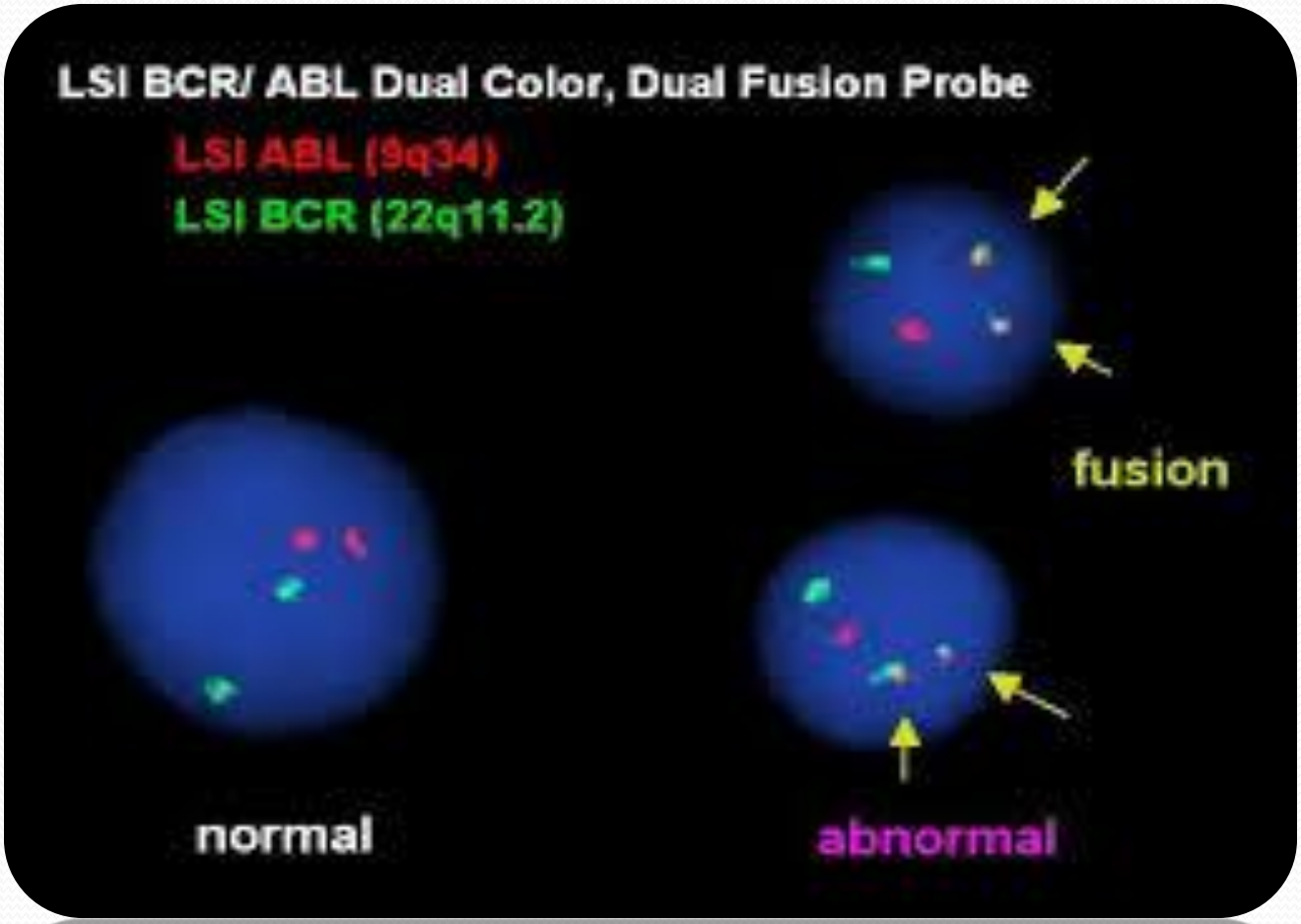
- It is useful in establishing the percentage of neoplastic cells at the time of diagnosis and after therapy.
- FISH studies are used to investigate the origin and progression of hematologic malignancies and to establish which hematopoietic compartments are involved in neoplastic processes.
- Quick and correct results save time and money by preventing unnecessary additional diagnostics and suboptimal treatment approaches.
- less labor-intensive method for confirming the presence of a DNA segment within an entire genome than other conventional methods

Advantages:

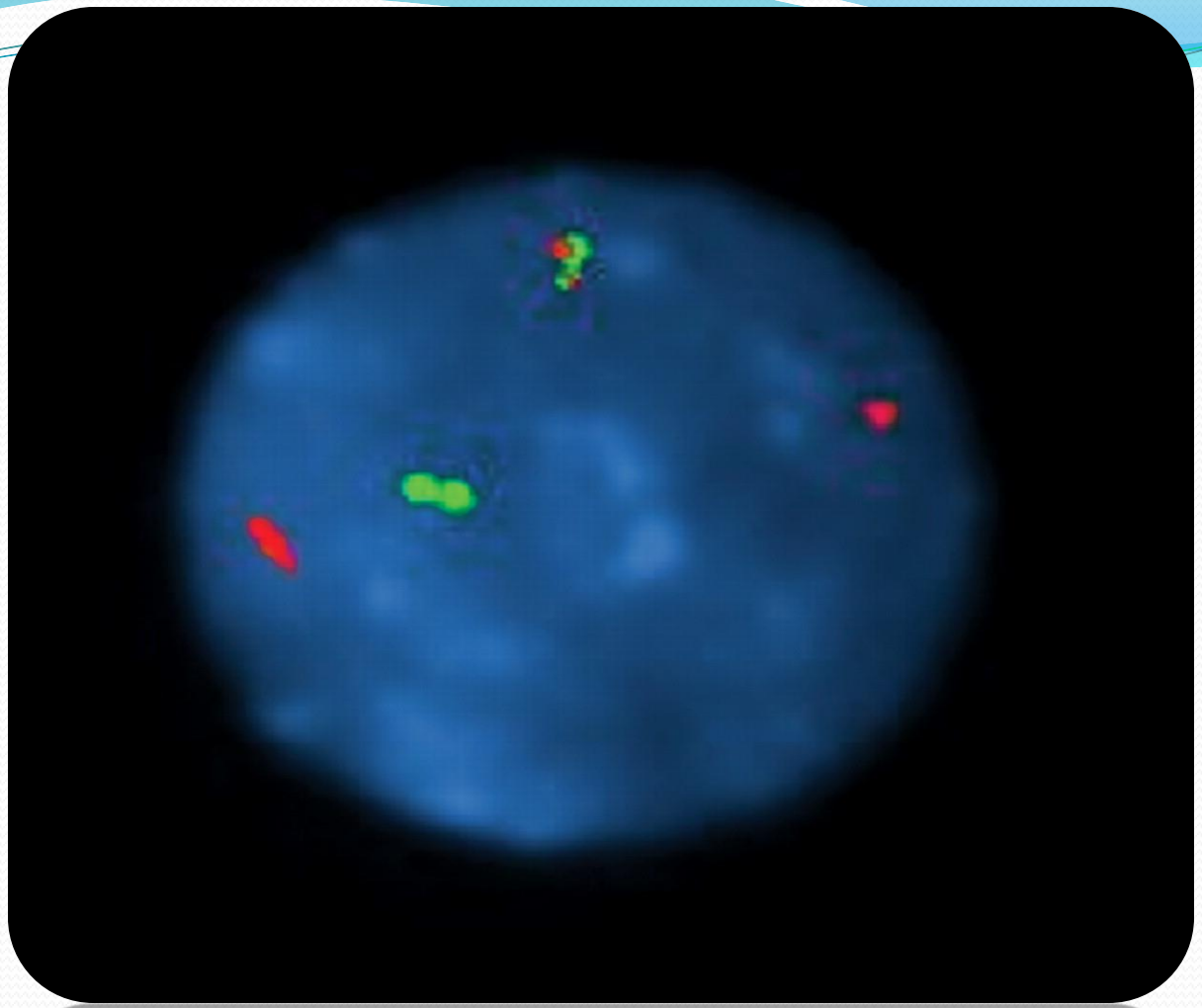
- Fast 48-72 hr.
- Reliable and unbiased result.
- Architecture of specimen remain intact.
- Old specimen can be used up to (7 years).
- It can detect numerous abnormalities(gain, losses of whole chromosome or deletion/duplication) superior to PCR.
- Used in diagnostic evaluation specially like cryptic abnormality that not evident by conventional karryotyping.

Application of FISH in haematology:

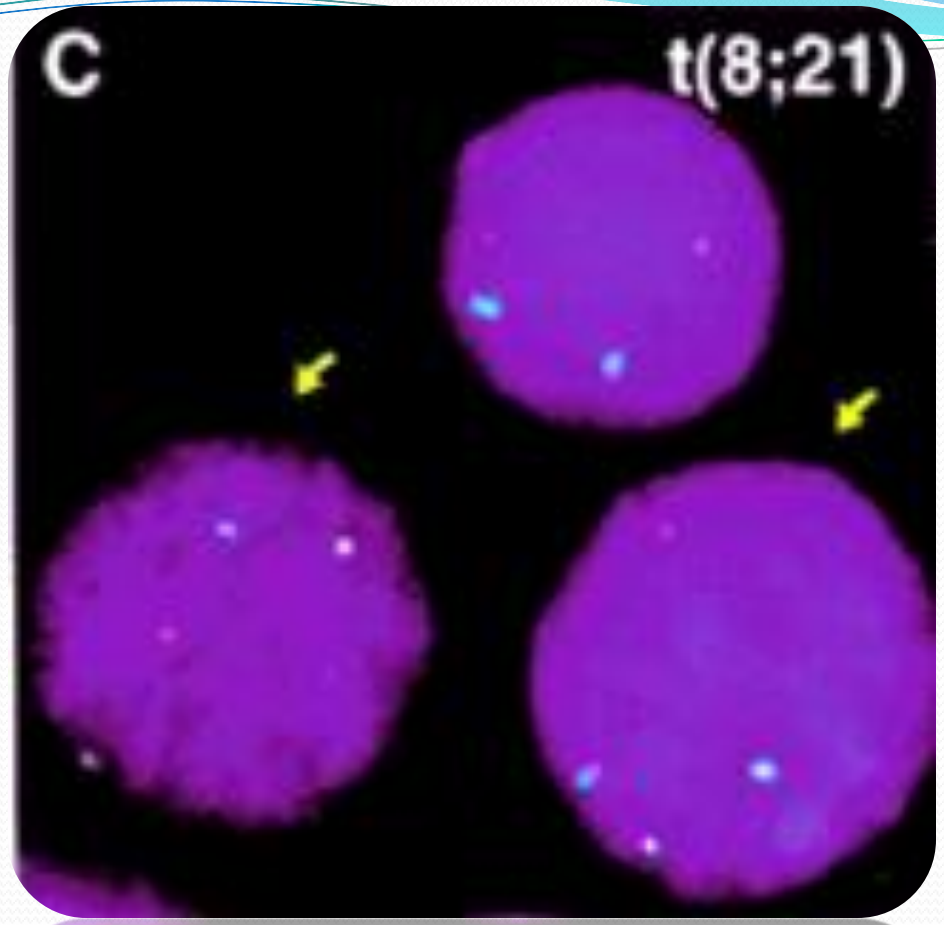
- Acute Lymphoblastic Leukemia t(4;11).
- Acute Myeloid Leukemia(PML/RARA t(15;17), AML/ETO t(8;21)
- Chronic Myeloid Leukemia(ABL/BCR t(9;22) .
- Chronic Lymphocytic Leukemia(del13q14).
- Myelodysplastic Syndromes.
- Plasma cell myeloma(deletion of 13q 14 or monosomy 13).
- Non Hodgkin Lymphoma(t(11;14) in mantle cell lymphoma).
- CLL/small lymphocytic lymphoma.
- Mismatched BM transplantation diseases relapse.



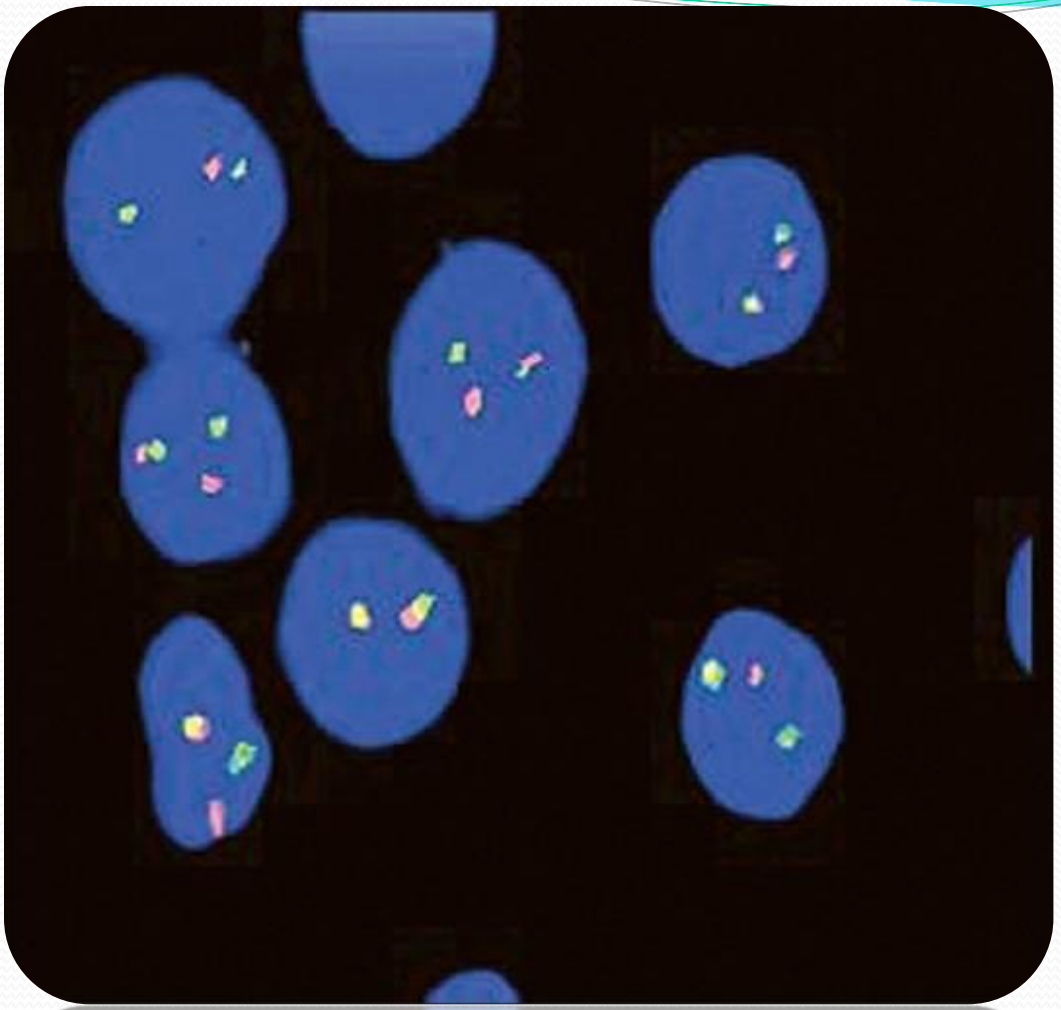
chronic myelogenous leukemia A *BCR-ABL1* amplification seen on interphase fluorescence in situ hybridization (FISH; dual-color, dual-fusion probes for *BCR,* green, and *ABL1,red*).



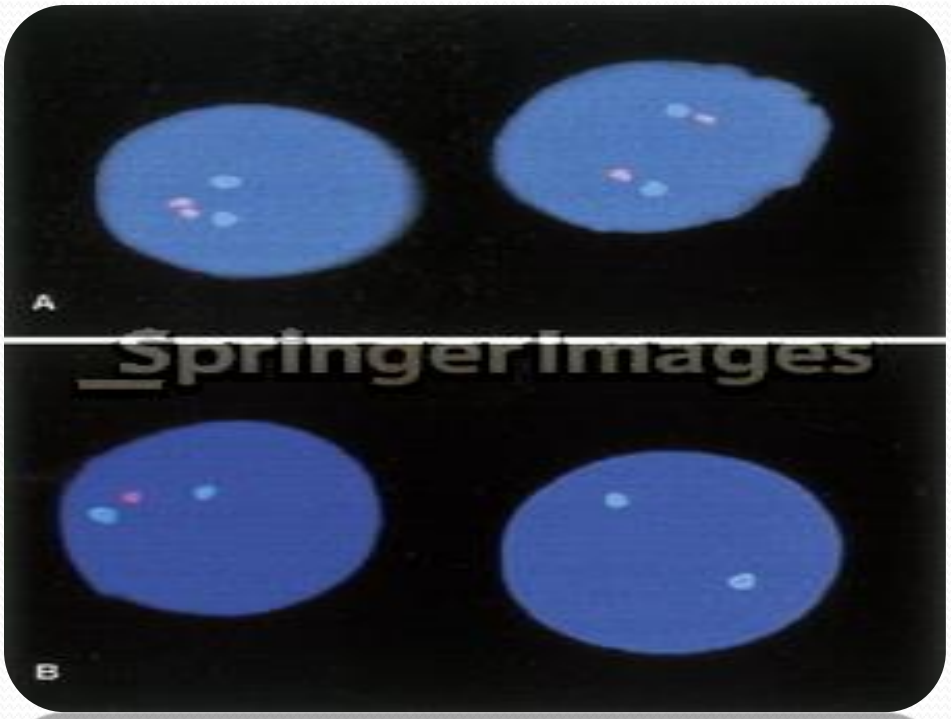
Acute Promyelocytic Leukemia FISH of an interphase nucleus with a *PML* probe (red fluorophore) chromosome 15 and an *RARα* probe (green fluorophore) chromosome 17demonstrating normal-intensity green and red signals, a fusion signal, and a red signal of diminished intensity.



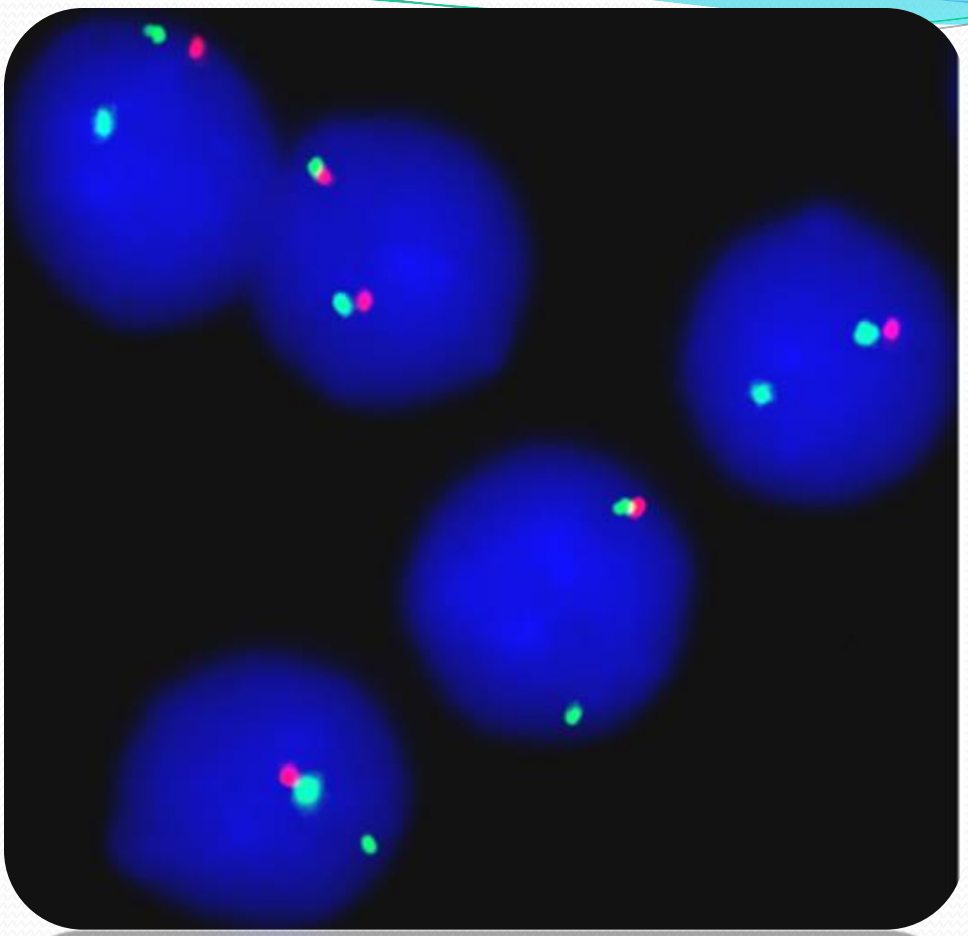
c) Dual-fused approach: AML1/ETO D-FISH probe (Oncor) labeled with Rhodal green and Texas red for detection of t(8;21)(q22;q22).



Mantle cell lymphoma t(11;14) yellow fusion signal.



Interphase fluorescence in situ hybridization analysis in MM patients, using specific probes for chromosome 17 centromere (*green dots*) and *P53* locus (*orange dots*). *A* Two hybridization signals for each probe in normal interphase nuclei. *B* Abnormal nuclei exhibiting a monoallelic (*left*) and biallelic (*right*) deletions of *P53* locus



Deletion of chromosome 13q14.2 occurs in multiple myeloma, non-Hodgkins lymphoma, chronic lymphocytic leukemia, myeloproliferative disorders, myelodysplastic syndrome, and acute nonlymphocytic leukemia.


Thank
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