GENETIC MARKERS IN LYMPHOMA

a practical overview

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- **B and T cell monoclonalities**

  Rearrangement of immunoglobin and TCR genes

  → **may help to establish the malignant nature of a lymphoproliferative lesion**

- **Identification of non-random chromosomal abnormalities**

  t(14;18) or t(11;14) translocations in FL and MCL respectively

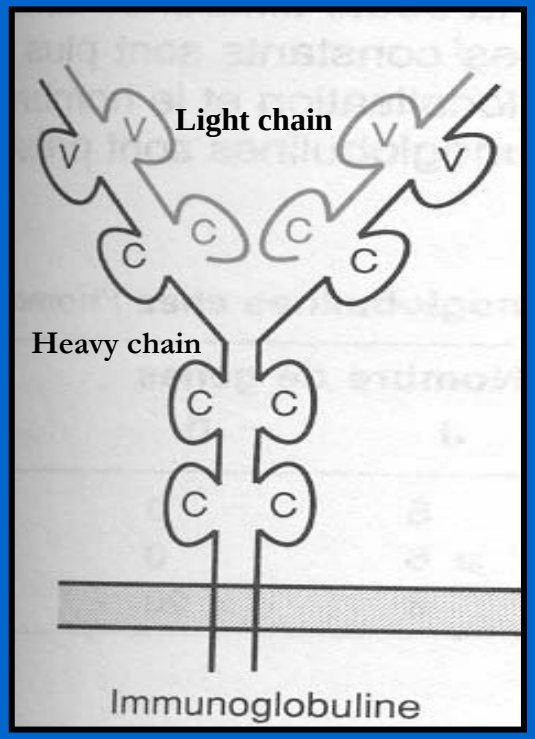
  → **allow lymphoma subtype classification**

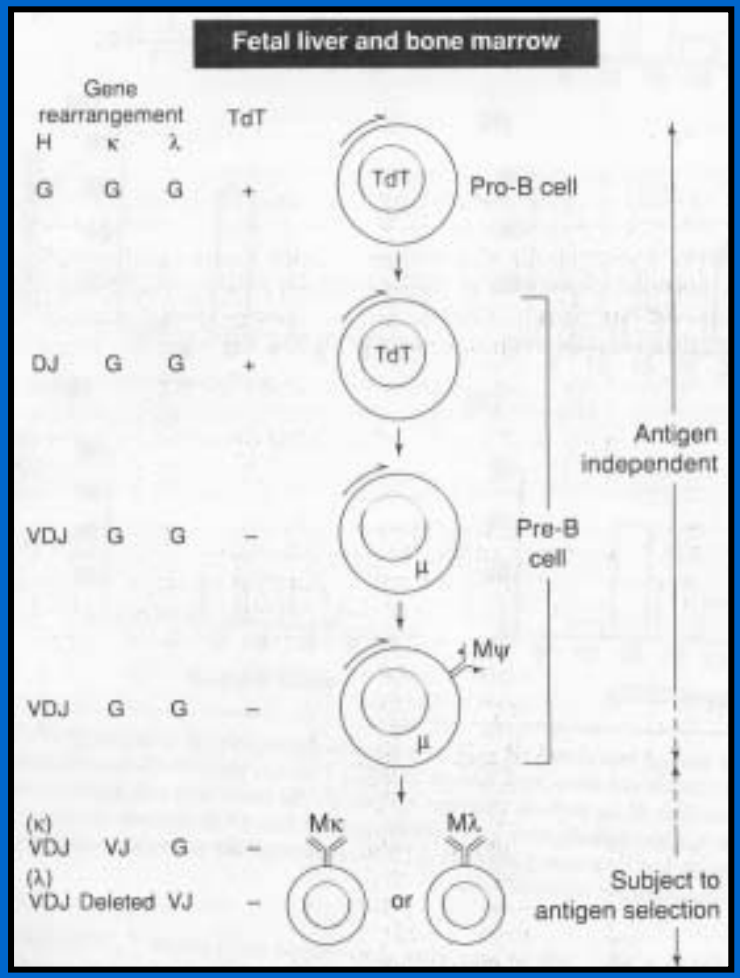
B and T cell monoclonality

what does that mean?

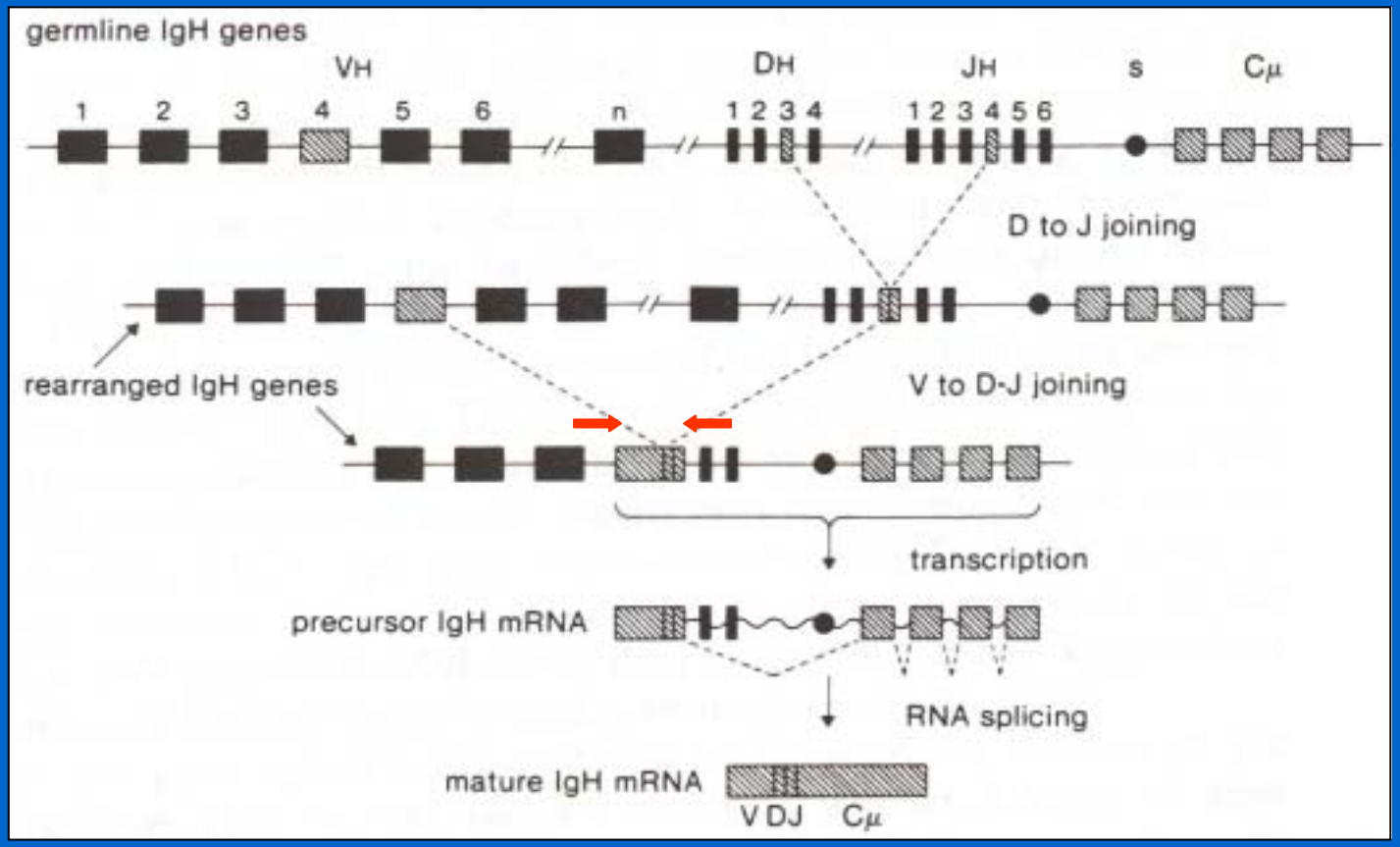
During early lymphoid development, the genes encoding antigen receptor undergo rearrangement

example of the Ig heavy chain locus (IgH)



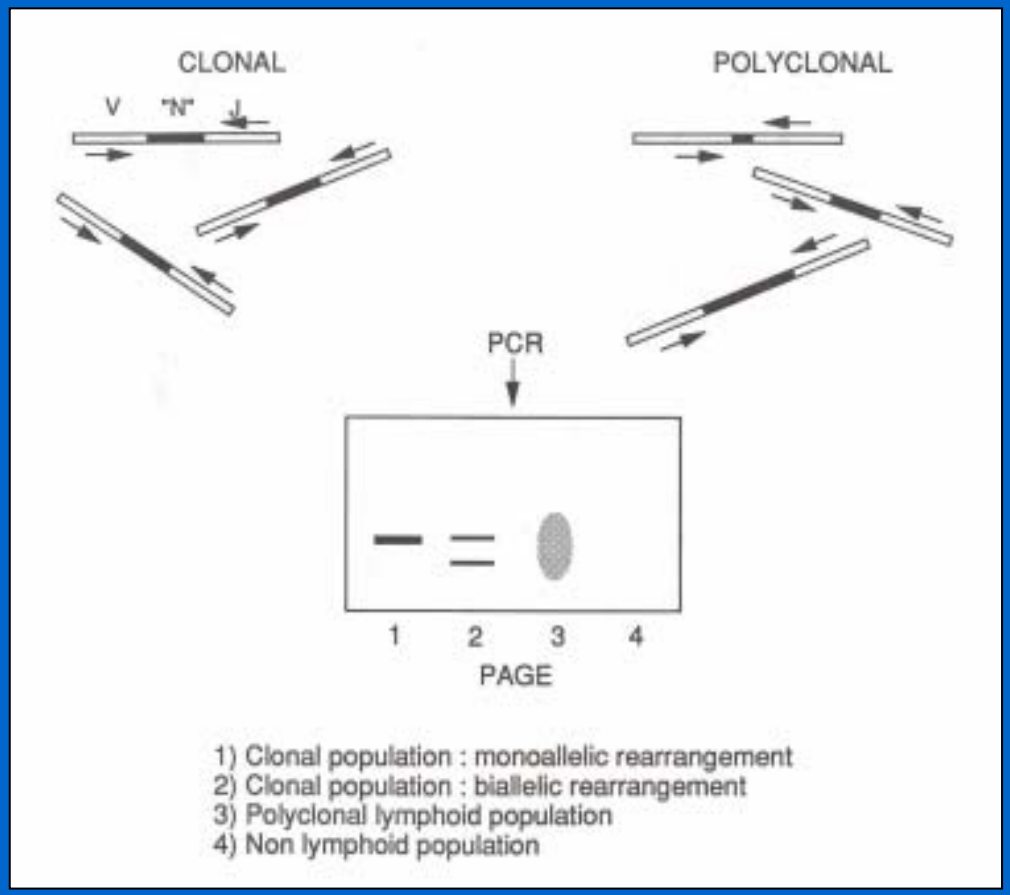


Schematic diagram of IgH gene rearrangements



➡ : indicate the primers location for PCR method

Schematic representation of mono and polyclonal populations detected by PCR.



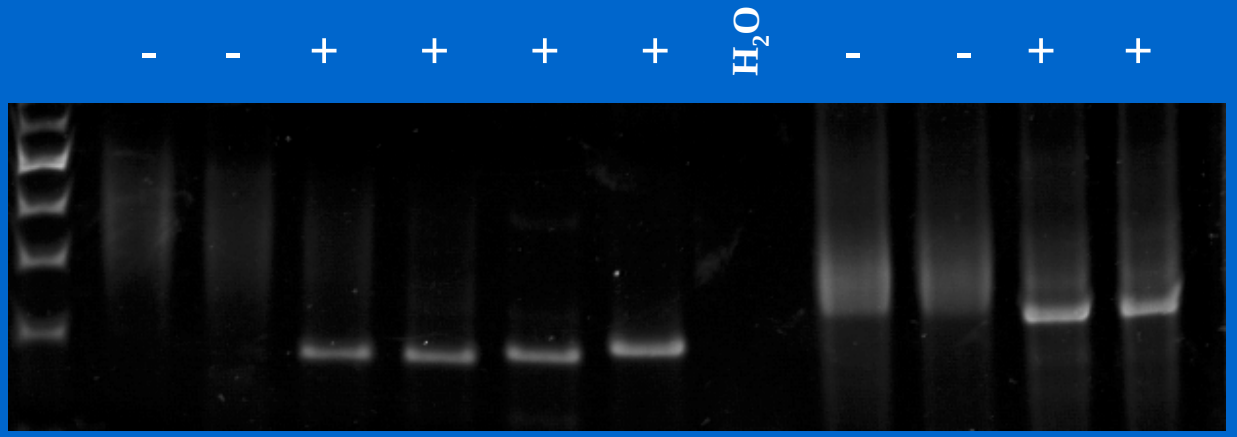
- monoclonal population implies malignant process

- polyclonal population implies benign lymphoid proliferation

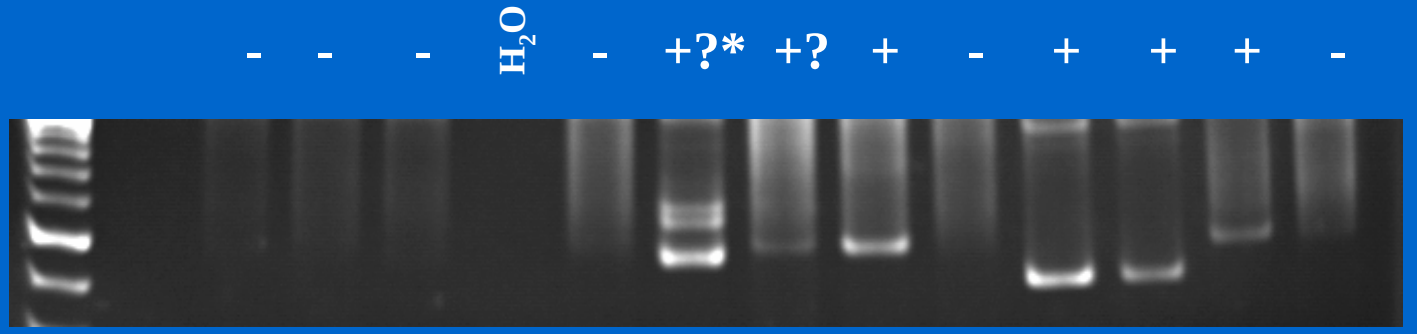
but...
the rule is not absolute

B and T cell monoclonalities - PCR

Illustration on ethidium-bromide-stained gel



B cells



Tcells

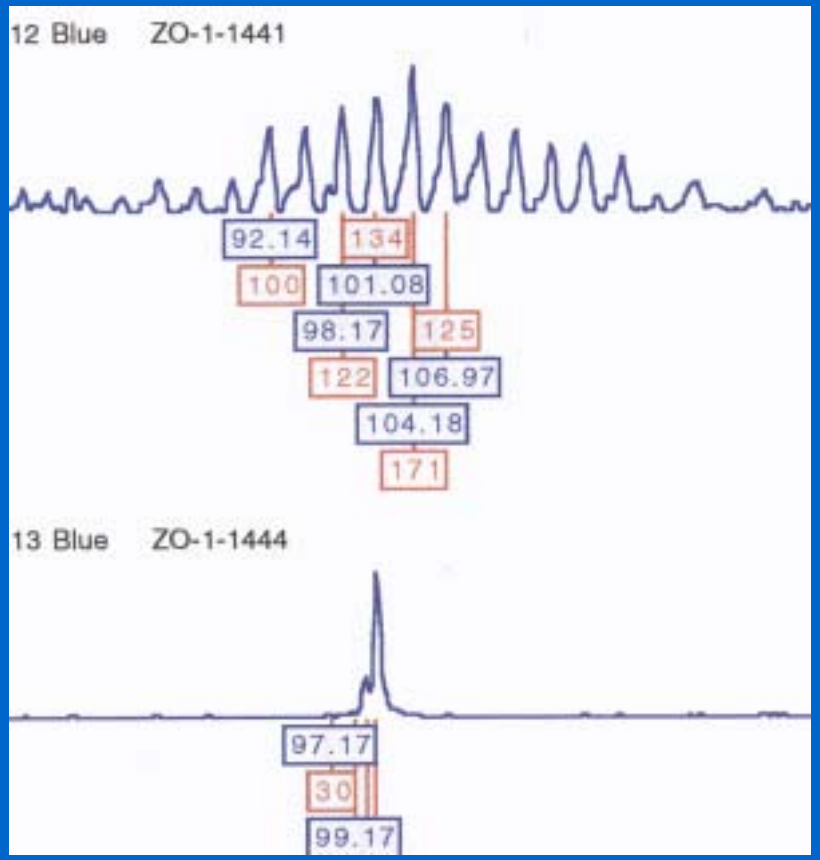
*? oligoclonality

B and T cell monoclonalities - PCR

Illustration on Genescan

polyclonality

monoclonality



PCR

Advantages: (vs Southern Blot)

- simple and faster
- requires much less amounts of pathological material
- greater quantitative sensitivity
- can be applied on DNA paraffin-embedded tissue

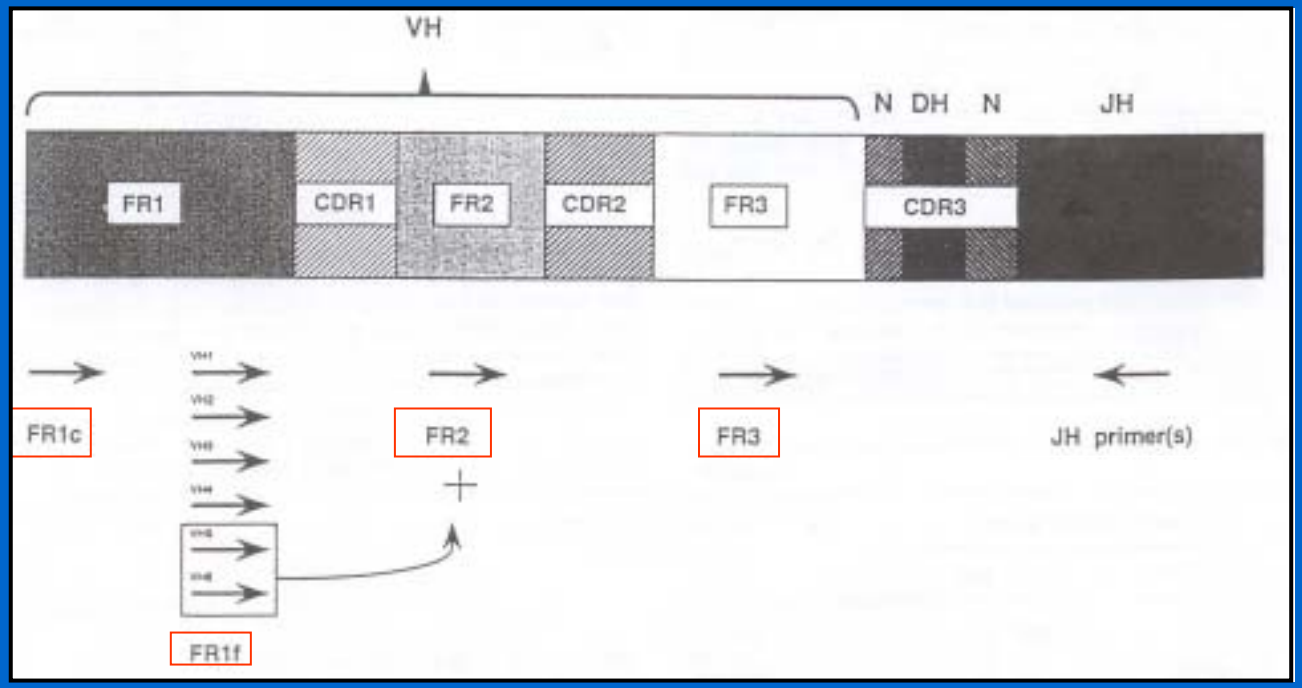
Disadvantages: (vs Southern Blot)

- lower qualitative sensitivity
- need to use several different PCR strategies in order to increase the overall detection rate *

*genomic sequence in a given antigen receptor may vary significantly from one to another and multiple sets of primers may be required

PCR strategies

- Necessity to use several sets of primers in order to increase the overall detection rate (~90 %) of the PCR method: FR3 -JH
  FR1c-JH
  FR1f-JH,...



- This detection rate varies according to the underlying disorders

Detection rates by PCR according to the subtype of B-cell neoplams

- SLL	**~ 100 %**
- MCL	**~ 100 %**
- DLBCL	**~ 60 %**
- FL	**~ 50 %**

PCR - Pitfalls

False negative:

- chromosomal translocations into the IgH locus (in FL or DLCL)
- Somatic hypermutation (in FL and DLCL)
- partial D-J rearrangements (in immature malignancies)
- no VDJ rearrangement produced (in immature malignancies)
- failure of the IgH primers to recognize the VH segment involved

False positive:

- very weak amount of DNA
- reactive lymphoid populations

Rules to known *(1)*

- Genotype does not correspond to phenotype !

Lineage infidelity of Ig and TCR gene rearrangements ("Illegitimate rearrangements"):

- **50-60 % of lymphoblastic B cell malignancies.**
- **20-30% of lymphoblastic T cell malignancies.**
- **~10% of mature B and T cell malignancies.**

Therefore, Ig and TCR gene rearrangements should not be systematically used as markers for B and T cell lineages, respectively.

Rules to known *(2)*

- Monoclonality is not always equivalent to malignancy !

  - Clinically benign lymphoproliferations may consist of clonal cell populations.

  - Although this pitfall is encountered in B cells, it is mainly observed in T cell monoclonality (cf limited combinatorial diversity of TCR-γ and -δ genes)

Rules to known *(3)*

- Some cases of unequivocal B-cells lymphoma do not generate a clonal signal by PCR despite histological and immulogic evidences of malignancy

- Any result must be interpreted in view of other findings and clinical informations

Chromosomal abnormalities

Chromosomal abnormalities

closely associated with particular morphological subtypes of lymphoma

→ diagnostic markers

prognostic/predictive markers

molecular targets for rationale therapies

mainly chromosomal translocations

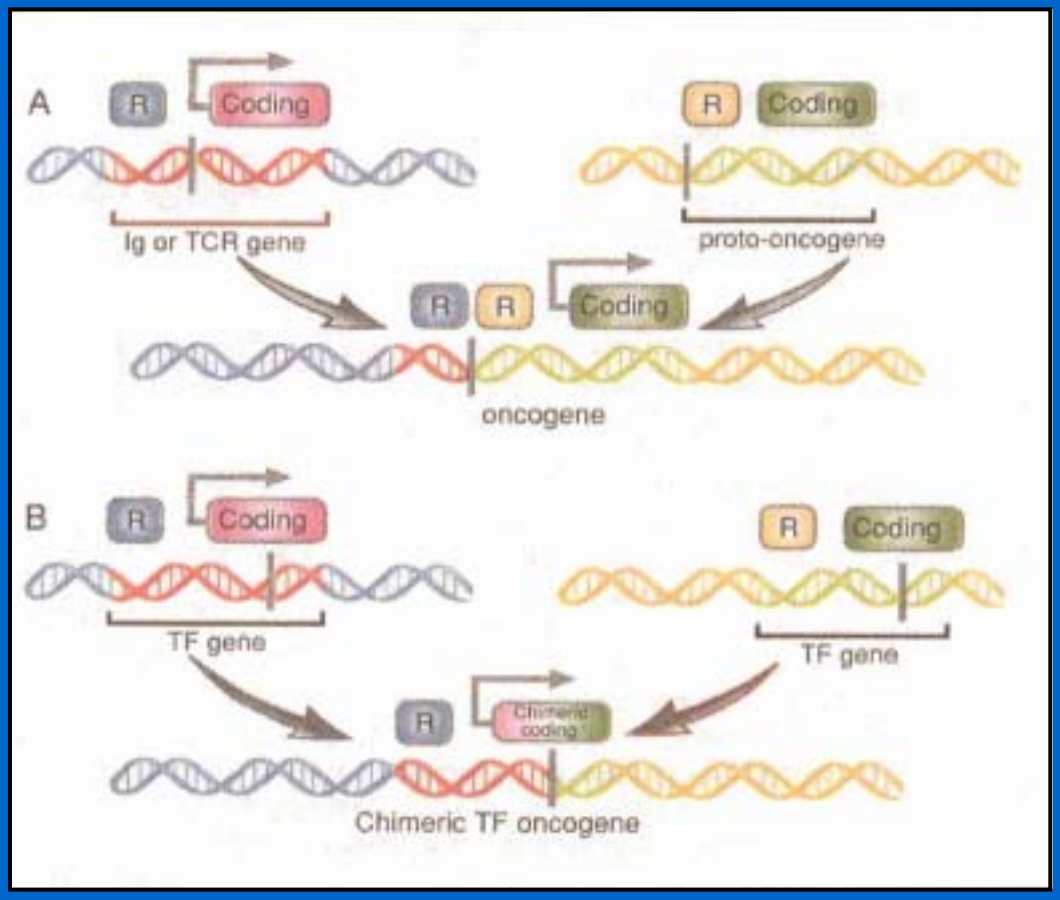
Two distinct types of chromosomal translocations at molecular level

A. Quantitative changes

BCL2-J_H, BCL1-J_H,…

B. Qualitative changes

ALK-NPM, API1-MALT



Recurrent genetic abnormalities in lymphoma

t(14;18) / *BCL2* - J_H	**in follicular lymphoma**
t(11;14) / *Bcl1* - J_H	**in Mantle Zone lymphoma**
t(11;18)/ *API2-MALT1*	**in Marginal Zone lymphoma**
del(7q), +3	
t(3;14) / *BCL6* - J_H	**in Diffuse Large Cell lymphoma**
t(8;14) / *cMYC* - J_H	**in Burkitt lymphoma**
t(2,5) / ALK-NPM	**in Anaplastic Large Cell Lymphoma**

Follicular lymphoma *(1)*

t(14;18)(q32;q21) - *BCL2-IgH* oncogene

⇩

overexpression of the antiapoptotic Bcl2 protein

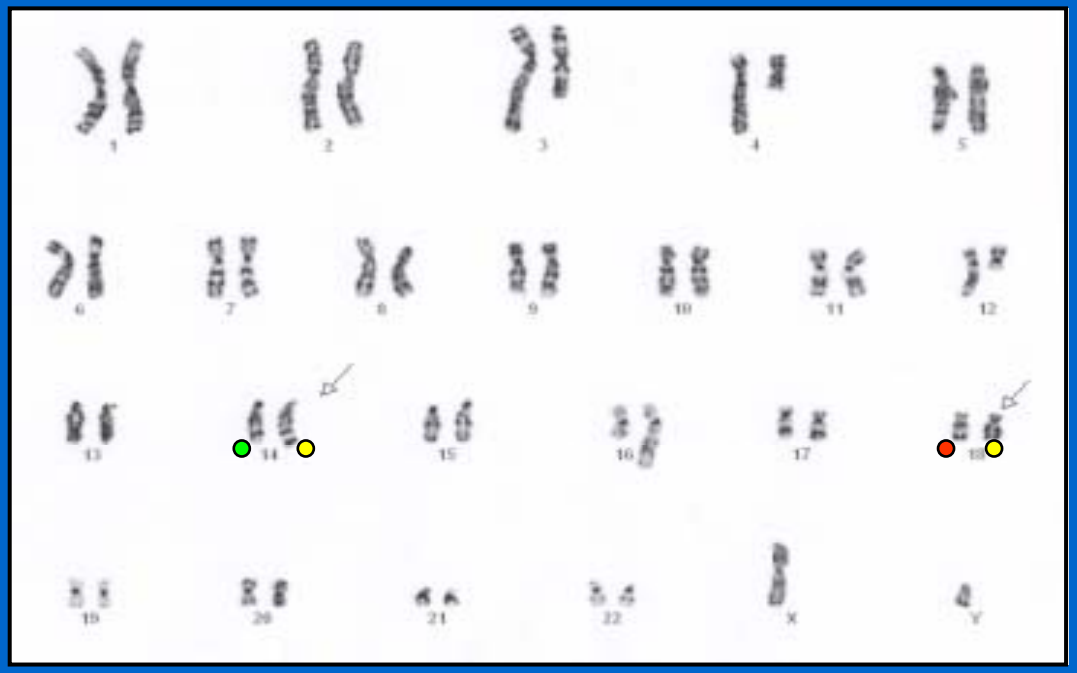
⇩

cell survival favoring increased genomic instability

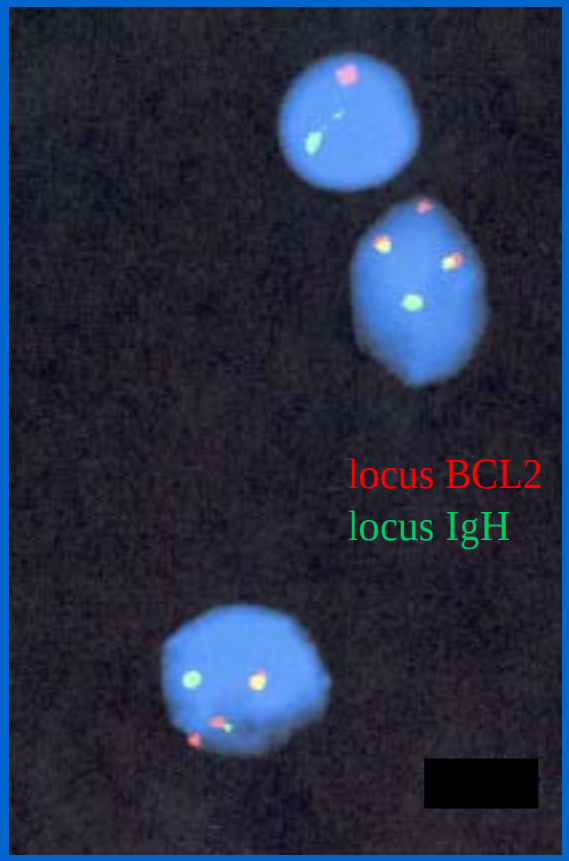
⇩

Follicular lymphoma

Follicular lymphoma *(2)*



t(14;18)(q32;q21) / *BCL2 -IgH*



FISH: « double fusion strategy »

Follicular lymphoma *(3)*

what to know

grade 1

grade 2 → t(14;18) positive in 80-90% of cases

grade 3 → t(14;18) positive in ± 30 % (mainly grade 3a)

t(14;18) negative in ± 70% (mainly grade 3b)

↓

BCL2 overexpression

or

no BCL2 overexpression

3q27 / *BCL6* rearrangement

Follicular lymphoma *(5)*

what to know

Conventional cytogenetic and/or FISH

"golden standard methodologies"

PCR: - four known different breakpoints on *Bcl2* gene

mbr	**in ~ 45 % of cases**
mcr	**in ~ 7 % of cases**
3'UTR	**in ~ 10 % of cases**
icr	**in ~ 10% of cases**

→ several sets of primers required

- some breakpoints are still unknown

Follicular lymphoma *(4)*

what to know

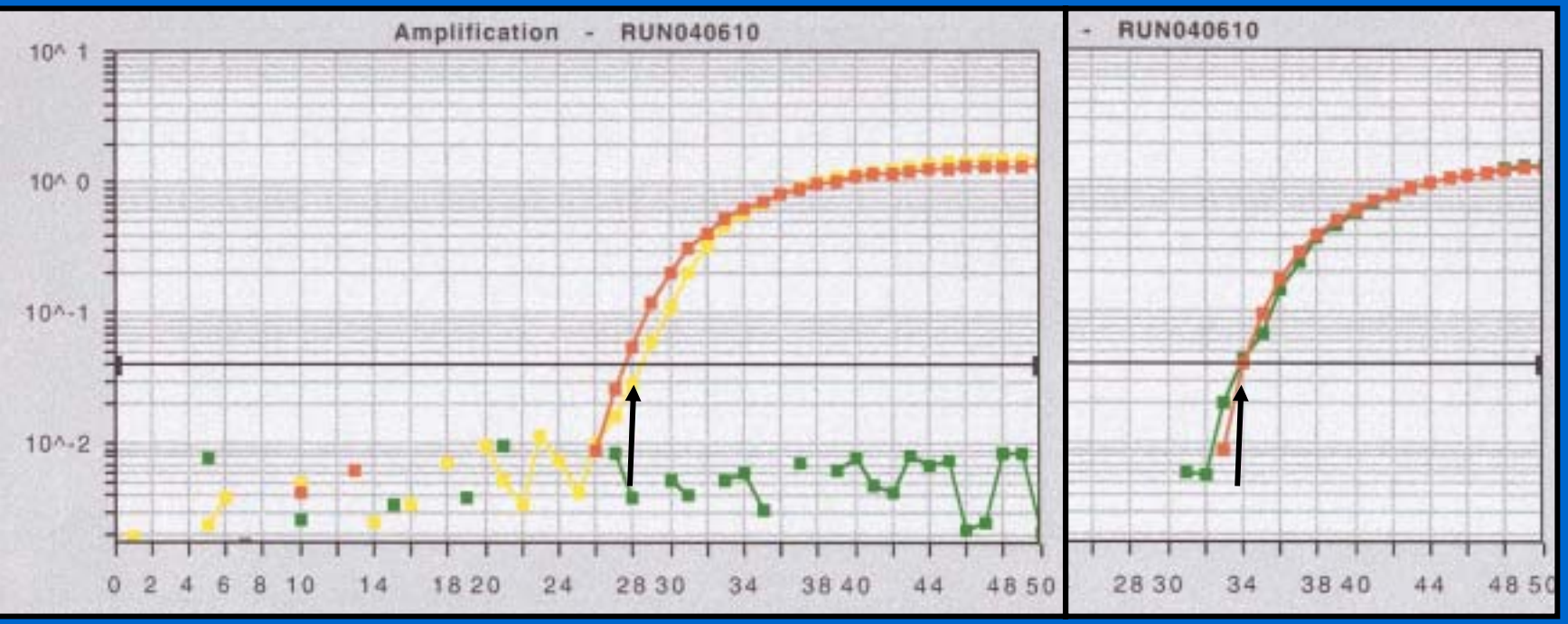
Methods : different levels of qualitative sensitivity

FISH	**> 95%**
Cytogenetics	**~ 80-90%**
PCR BCL2(mbr)-JH	**40-50%**
PCR BCL2(mcr)-JH	**~ 10%**

PCR Bcl2-JH in follicular lymphoma

Illustration

at diagnosis **Follow up**



the persistance of a positive result or a molecular re-emergence after one year of treatment is highly predictive of a clinical relapse.

Follicular lymphoma *(5)*

what to know

- **At diagnosis: CC and/or FISH ***
- **Follow up: Quantitative PCR**

* FISH can be performed on fresh touch print or paraffin-embedded tissue

Mantle Cell lymphoma

t(11;14)(q13;q32) - *BCL1-IgH* oncogene

⇩

overexpression of the Bcl1/cyclin D1 protein

⇩

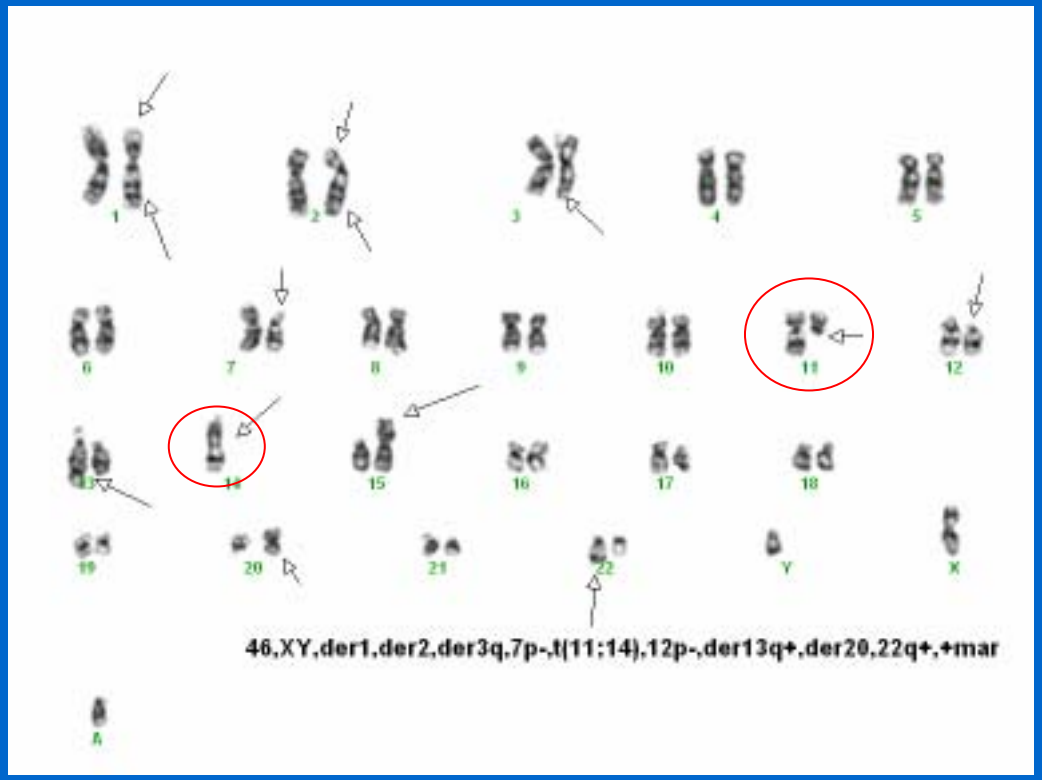
cell cycle activation (G1/S phase)

(+ other genetic alterations involving TSG such as *p16*)

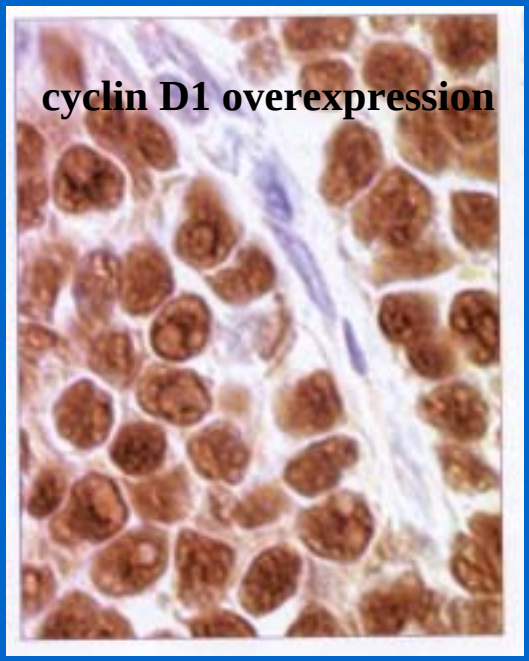
⇩

mantle cell lymphoma

Mantle cell lymphoma *(2)*



t(11;14)(q13;q32) / *BCL1-IgH*



FISH: « double fusion strategy

Mantle cell lymphoma *(3)*

what to know

Conventional cytogenetic and/or FISH

"golden standard methodologies"

PCR: - one major known breakpoints on *Bcl1* gene

MTC in ~ 50 % of cases

- other breakpoints are heterogeneous and difficult to detect

(large target region for possible rearrangement breakpoints)

Mantle cell lymphoma *(3)*

what to know

Methods : different levels of qualitative sensitivity

FISH	**> 95%**
Cytogenetics	**~ 80%**
PCR BCL1(MTC)-JH	**~ 50%**
RT-PCR (CyclinD1 overexpression)	**~ 100% ***

* results difficult to interpret

Marginal cell lymphoma *(1)*

Distribution of chromosomal abnormalities according to the three ≠ subtypes

MZL of MALT type

chromosomal translocations with site-specificity in terms of their incidence

splenic MZL

numerical abnormalities (mainly trisomies 3, 7, 18)

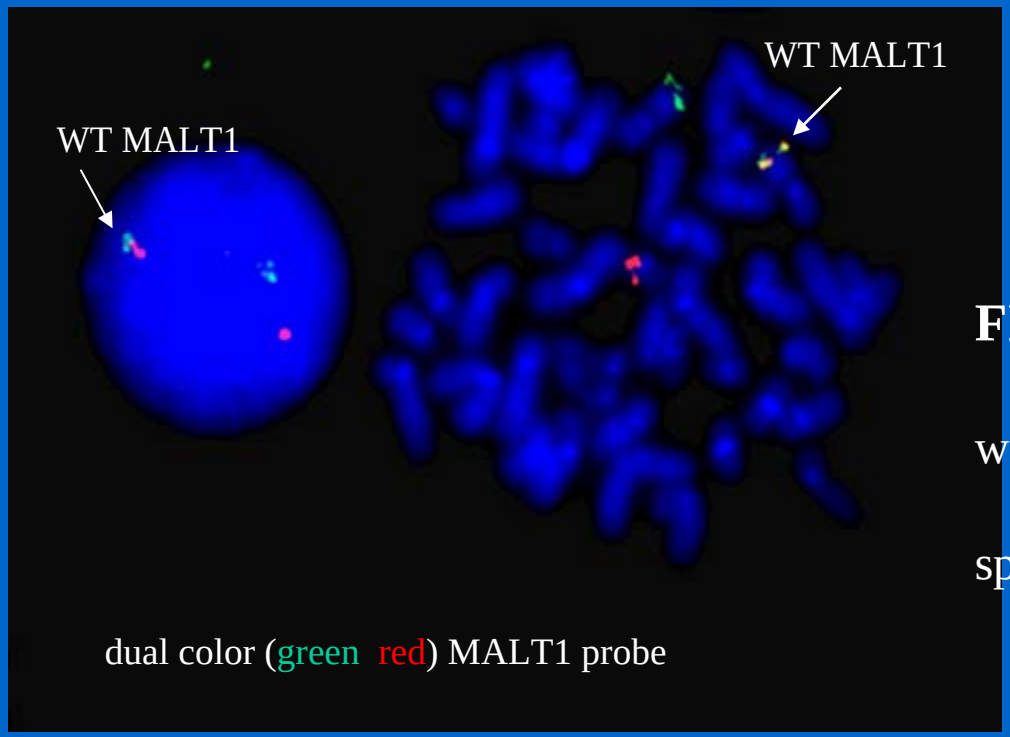
nodal MZL

numerical and structural abnormalities: del(7q),+3

Marginal cell lymphoma (*2*)
MALT type

t(11;18)(q21;q21)	***API2-MALT1***	**15 -40%**	**stomach intestine lung**
t(14;18)(q32;q21)	***MALT1-IgH***	**20%**	**salivary gland ocular adnexa skin, liver, lung**
t(1;14)(p22;q32)	***BCL10-IgH***	***1-2%***	**stomach, lung**
t(3;14)(p14;q32)	***FOXP1-IgH***	***5%***	**thyroid, skin, ocular adnexa**

t(11;18)(p21;q21) / *API2-MALT* in gastric MALT lymphoma



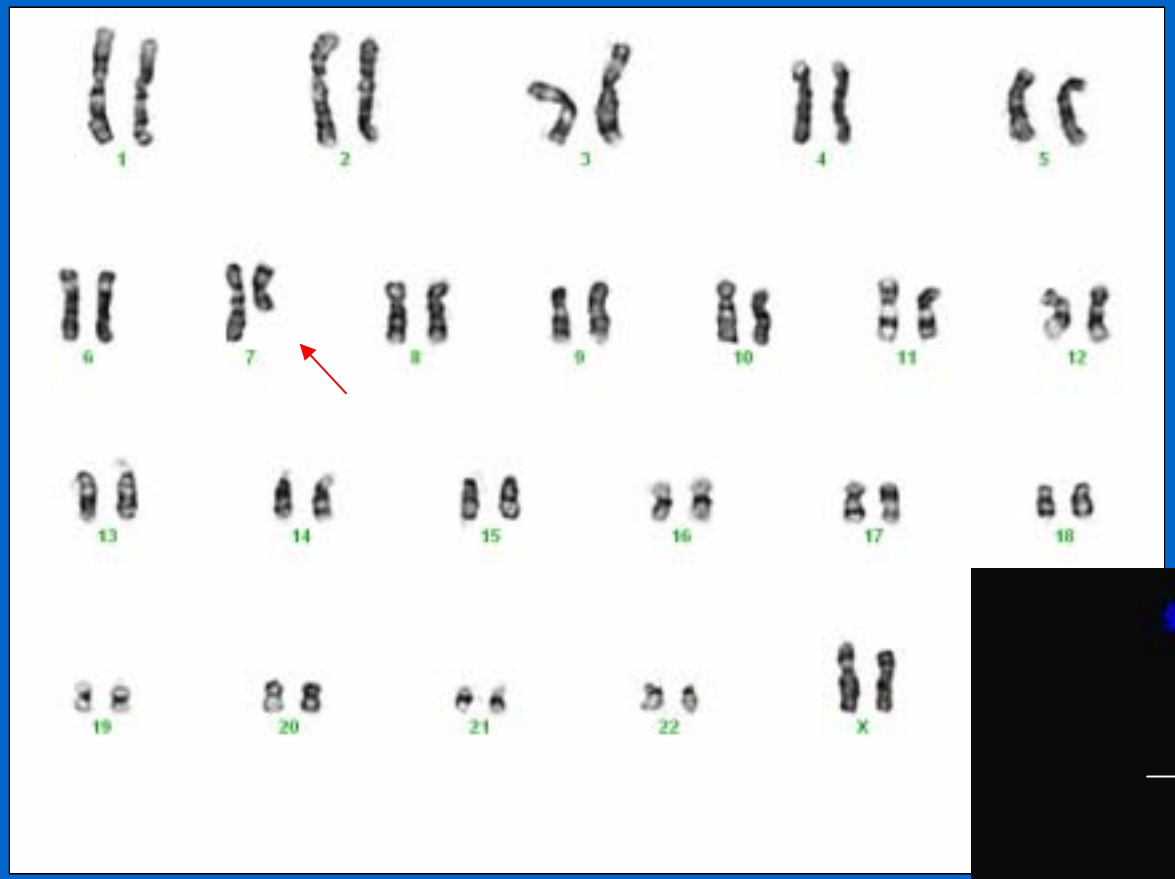
FISH: "break-apart probe strategy

wild type MALT1: 1 yellow spot

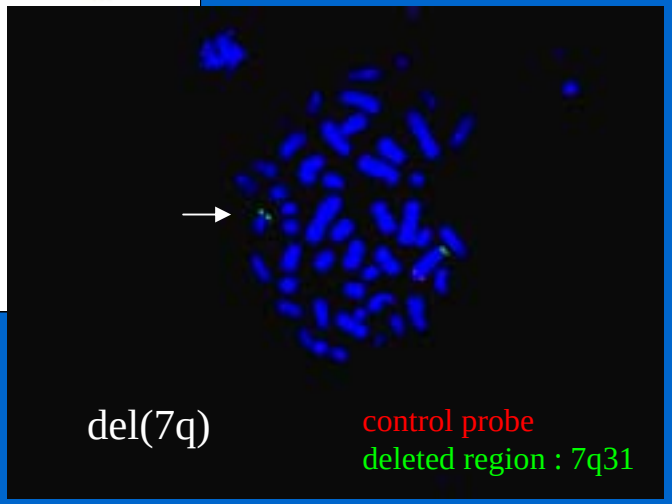
splited MALT1: 1 green and 1 red

gastric MALT with t(11;18) do not respond to *Helicobacter pylori* antibiotic

splenic Marginal cell lymphoma *(3)*



46,XX,del(7)(q22q32)



Diffuse Large B cell lymphoma *(1)*

t(3;14)(q27;q32) — ***BCL6-IgH* oncogene**

t(3q27;v) — ***BCL6-non IgH* oncogene**

in 30-40% of DLBCL

⇩

BCL6* oncogene overexpression

⇩

cell survival and proliferation

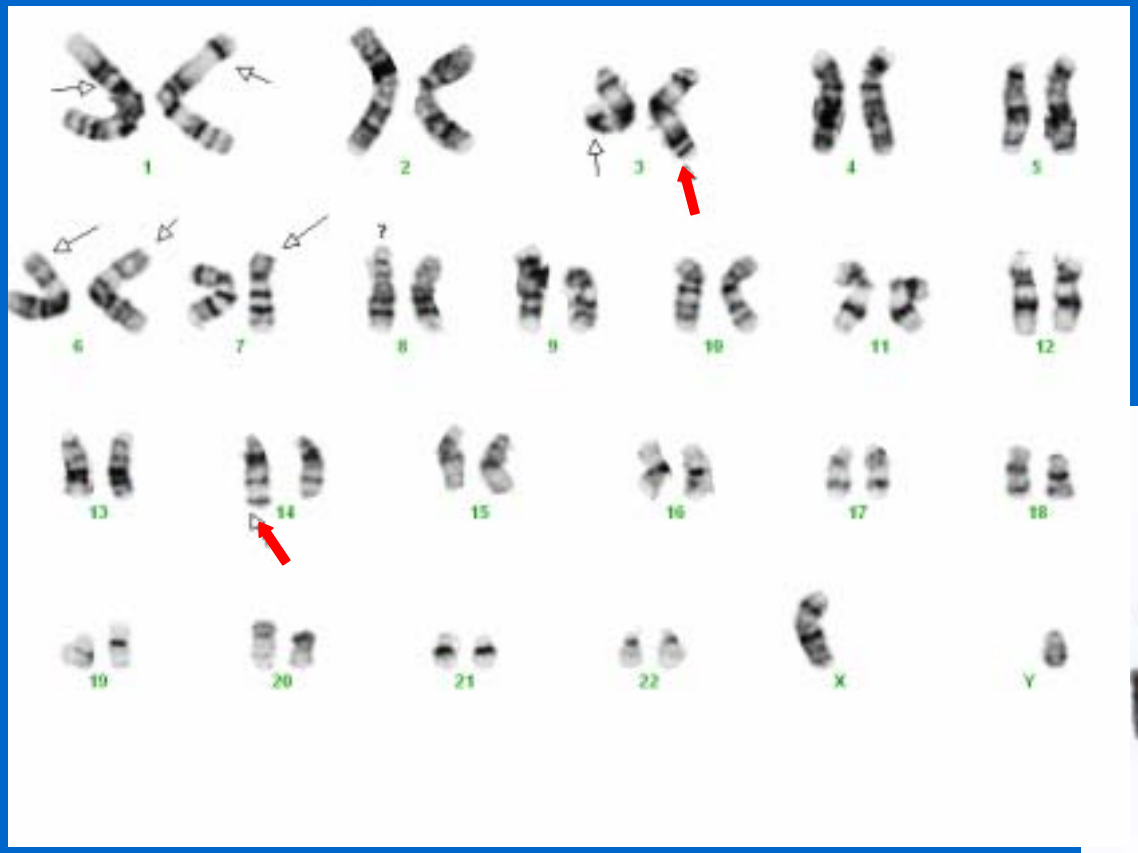
⇩

DLBCL

* high *BCL6* overexpression ⟶ better outcome

Diffuse Large B cell lymphoma *(2)*

Illustration



BCL6 probe

t(3;14)(q27;q32) ***BCL6-IgH***

FISH: “break-apart probe strategy”