The use of FISH techniques in the diagnosis of Haematological Malignancies

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Introduction

Association of specific, non-random chromosome aberrations with defined sub-types of haematological malignancy is accepted.

Aberrations can be either numerical or structural.

- Trisomy 12 and monosomy 13
- Translocations, inversions, deletions

Identification of specific aberrations can provide both diagnostic and prognostic information.

- t(15;17) is a specific diagnostic test for APML
- Deletion *p53* is a marker of poor prognosis

O'Connor & Barrans, HMDS (2005)

Detection of chromosome aberrations

Conventional karyotyping – Global

Requires cell culture

- good for acute leukaemias
- but a problem with more differentiated leukaemias

Labour intensive with low throughput

Expensive

PCR based – specific

- Requires DNA or RNA
- High throughput possible

FISH – specific

- Direct FISH interphase cells
- High throughput possible

Detection of specific genetic abnormality

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Immunoglobulin translocations associated with B cell lymphoma

Disease type	Ig translocation	Frequency	Gene deregulated
Burkitt	t(8;14)(q24;q32)	100%	*C-MYC*
DLBCL	t(3;14)(q27;q32)	5-10%	*BCL-6*
	t(10;14)(q24;q32)	<1%	*NFKB2*
FL	t(14;18)(q32;q21)	80%	*BCL-2*
MZL	t(1;14)(p22;q32)	<5%	*BCL-10*
	t(9;14)(p13;q32)	50%	*PAX5*
MCL	t(11;14)(q13;q32)	95%	*BCL-1*
CLL	t(14;19)(q32;q13)	<1%	*BCL-3*
Myeloma	t(4;14)(p16;q32)	15%	*FGFR3*
	t(11;14)(q13;q32)	25%	*MYEOV*

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Interphase FISH tests carried out as routine by HMDS (Spring 2004)
B-Lymphoproliferative Diseases

Assay	Cytogenetic abnormality	Gene	Control probe	Main association
Translocation	t(8;14)(q24;q32)	*C-MYC;IgH*	α8	Burkitt, DLBCL
Translocation	t(11;14)(q13;q32)	*BCL-1;IgH*	α11	Mantle cell lymphoma, myeloma
Translocation	t(14;18)(q32;q21)	*BCL-2;IgH*	α18	Follicular lymphoma, DLBCL
Translocation	t(4;14)(p16;q32)	*FGFR3;IgH*		Myeloma
Breakapart	3q27	*BCL-6*	α3	DLBCL
Breakapart	18q21	*MALT-1*	α18	Extra-nodal MZL
Deletion	11q23	*ATM*	α11	B-CLL, MCL
Deletion	13q14	unknown	13q34	B-CLL, MCL
Deletion	17p13	*p53*	α17	All types
Trisomy	α12			B-CLL

O'Connor & Barrans, HMDS (2005)

HMDS FISH strategy

- FISH requested at screening (see screening/reporting SOP)
- If the total B cell total is **<5%** the sample is unsuitable
    - especially for chromosome **deletion** analysis

- Samples are divided into 3 groups*
  - **CD5 positive ~ B-CLL or MCL**
    - alpha satellite 12 (trisomy 12), and assess for deletions of 13q14 (? *LEU5*)), 11q23 (?*ATM, Bob-1, Bam32*) and 17p13 (p53).
    - Cases that are CD5 + CD23 – (?MCL) require t(11;14) (*BCL-1*/*IgH*)

  - **CD5 negative ~ FL, MZL, Burkitts or DLBCL**
    - require t(14;18) (*BCL-2* / *IgH*) and assess for deletion of 17p13 (*p53*)
    - Extranodal marginal zone lymphoma needs MALT-1
    - If the final diagnosis is DLBCL do t(8;14) (*C-MYC*/ *IgH*) and 3q27 (*BCL-6*)
      - If Burkitts Lymphoma is suspected t(8;14) (*C-MYC* and *IgH*) is essential and may be required urgently.

  - **Myeloma or Plasma cell disorders**
    - deletions of 13q14 or monosomy 13, t(11;14) (*BCL-1*/*IgH*), t(4;14) (FGFR3/IgH

*Most cases will not have a complete diagnosis at this stage but provisional FISH panels can be selected based on the immunophenotyping results

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Routine FISH panels in HMDS

3 FISH panels

- CLL / Mantle Cell Lymphoma ~ CD5+
  - Alpha satellite 12, 13q14, 11q23 (*ATM*),17p13 (*P53)*
    - t(11;14) (for CD5+ CD23- cases)
- Marginal Zone Lymphoma / Follicular Lymphoma DLBCL / Burkitt lymphoma ~ CD5-
  - t(14;18) & 17p13 (*P53)*
  - MALT-1, 6q21, 7q31
  - 3q27, t(8;14), t(14;18) & 17p13 (*P53)*
- Plasma cell disorders
  - 13q14/13q34, IgH, t(11;14), t(4;14)

O'Connor & Barrans, HMDS (2005)

Guide to tutorial

- Part 1
  - Techniques
  - Probe details
  - Sample preparation
- Part 2
  - Disease specific
  - Prognostic value of cytogenetic abnormalities

Part 1

Techniques

FISH based techniques

- ‘standard FISH’ - specific
- FICTION - specific
- Fiber FISH - specific

- CGH * - unbalanced global DNA
  - CGH is reverse FISH and ‘odd-one-out’ compared to other FISH protocols ~ tumour DNA becomes the probe

- M-FISH - global, metaphases
- SKY - global, metaphases

Examples of these FISH techniques follow

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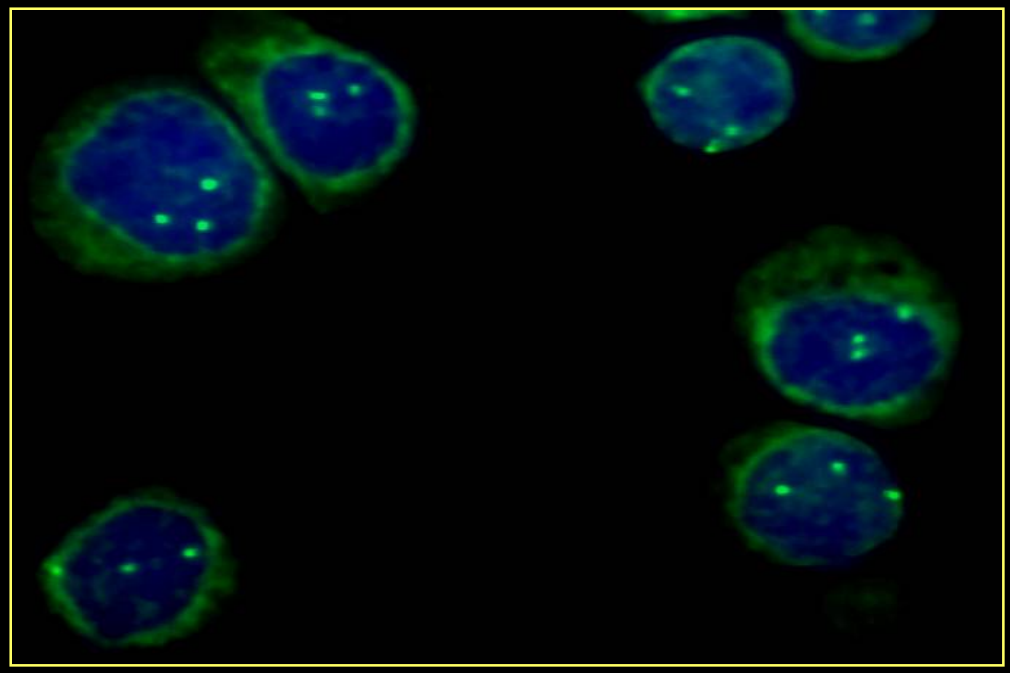
‘Standard’ FISH

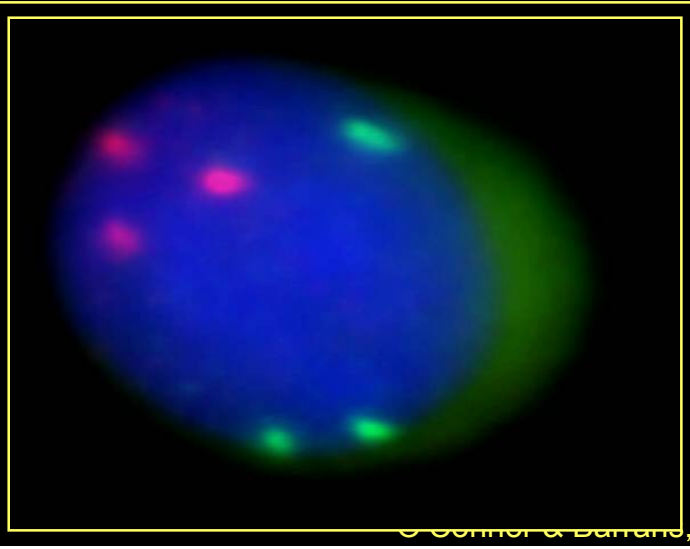
AML-M5a with Trisomy 8

- Bone marrow smear
- Alpha sat 8 probe FITC



Myeloma ~ single plasma cell

- Trisomy 3 (Rhodamine)
- Trisomy 11 (FITC)

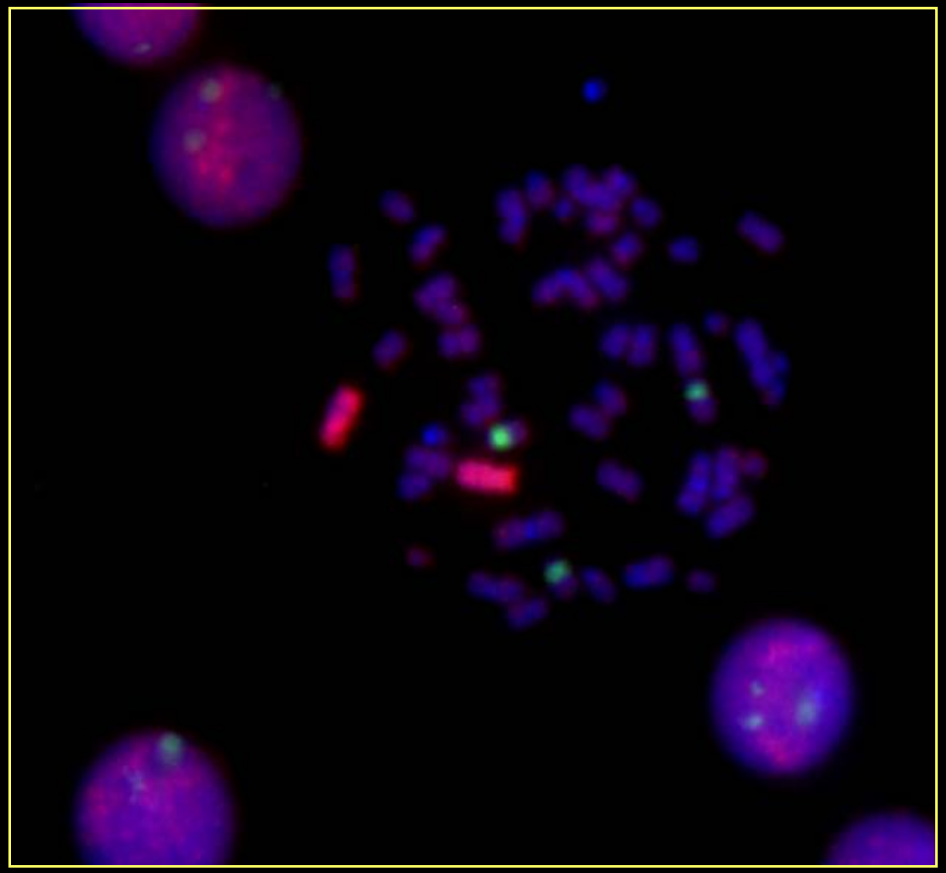


‘Standard’FISH - Paints

Paints are cocktails of probes that are specific for whole chromosomes.

Metaphase FISH usually.

Image shown is a metaphase from atypical B-CLL with trisomy 12 and normal Chromosome 4.



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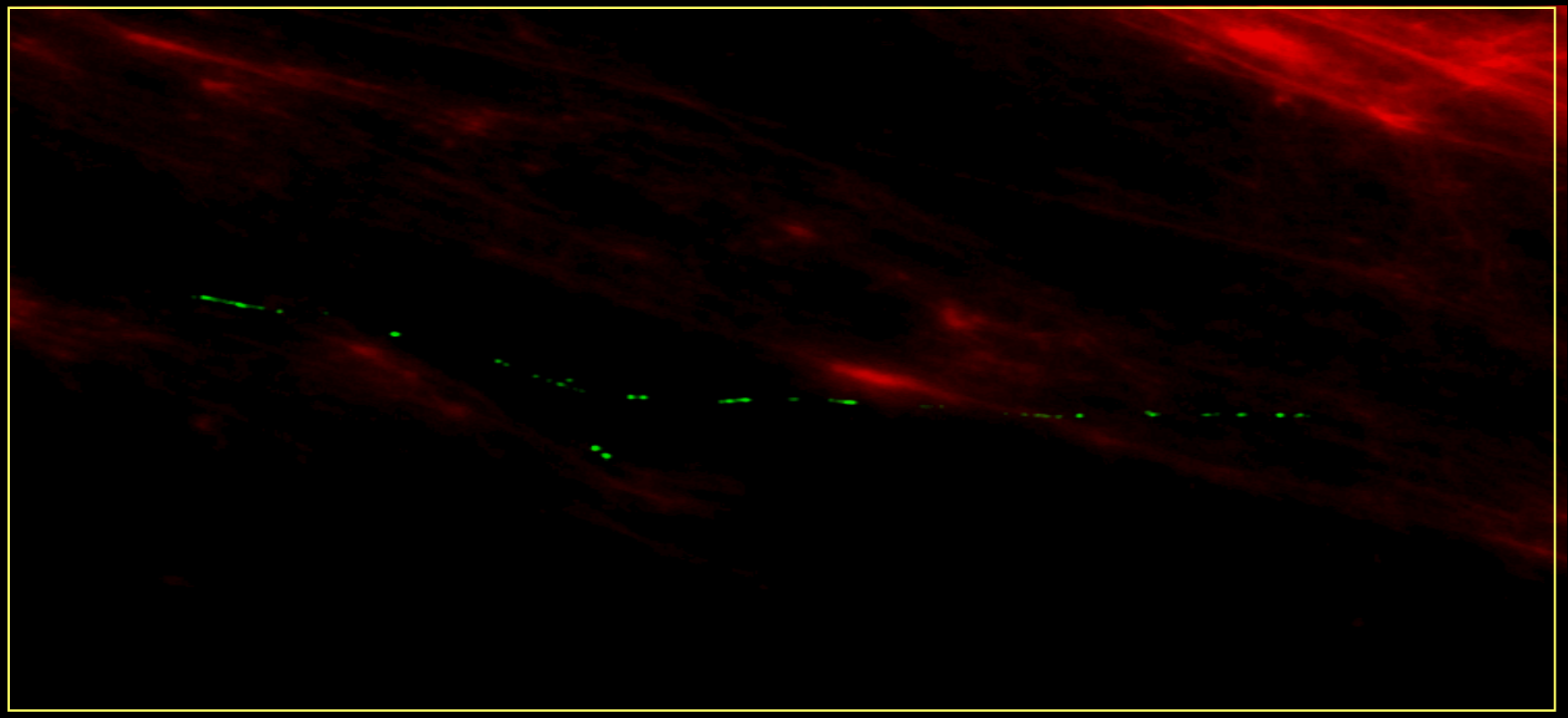
Fiber FISH



Research method for high resolution gene mapping

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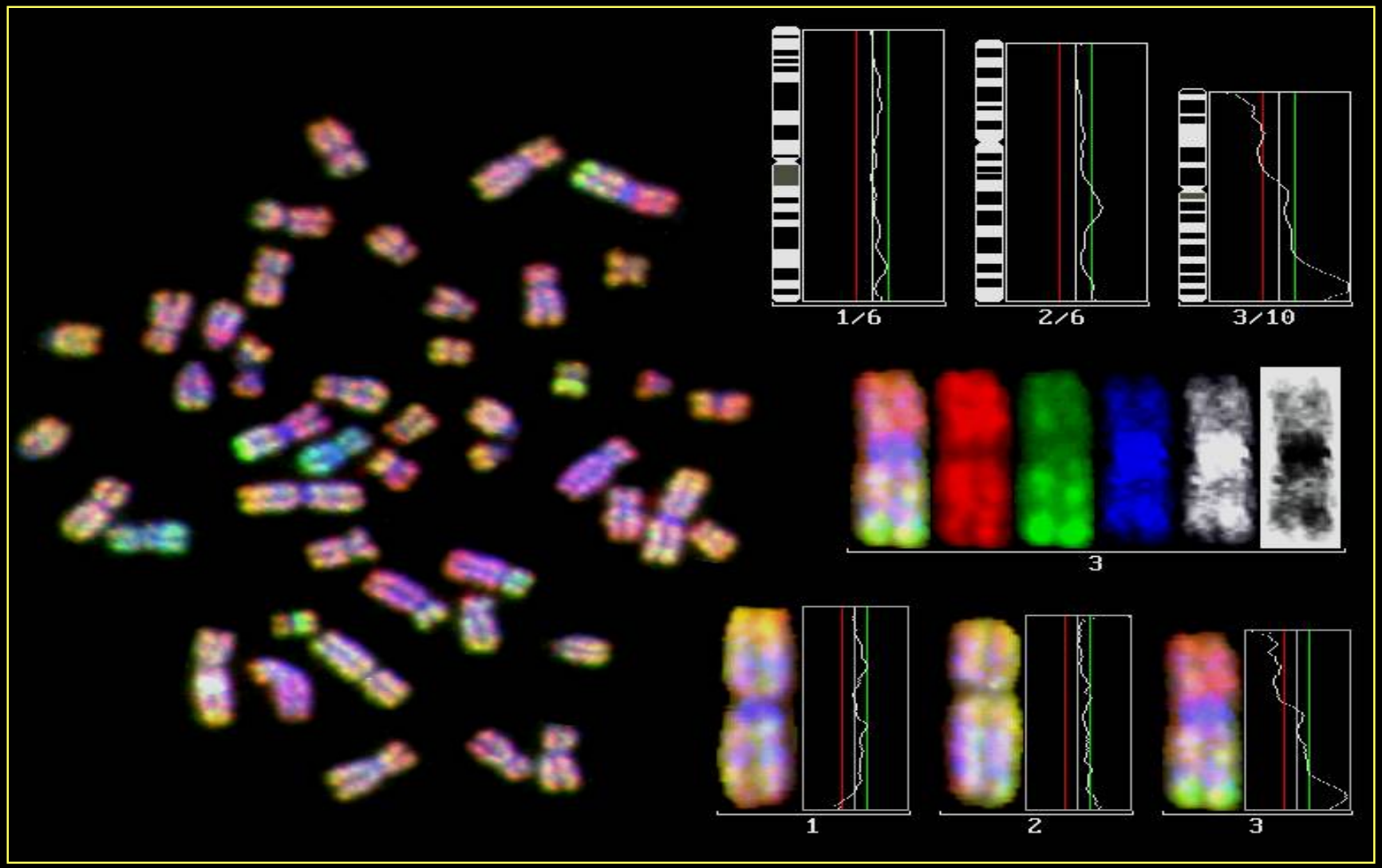
Comparative Genomic Hybridisation



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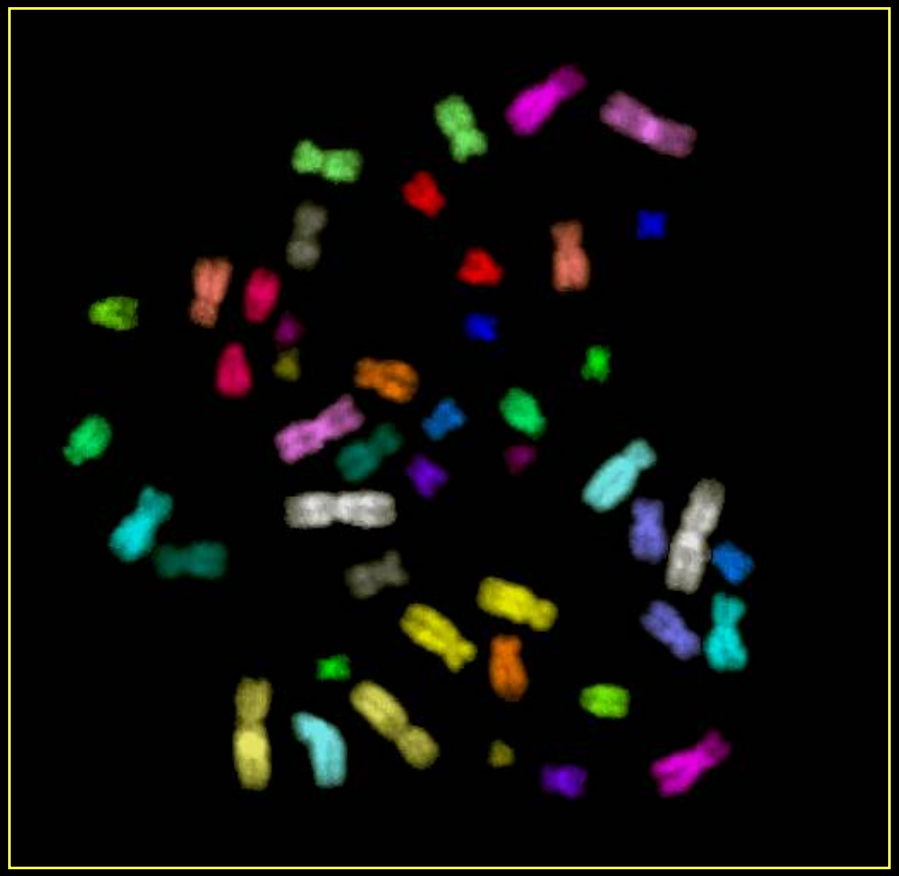
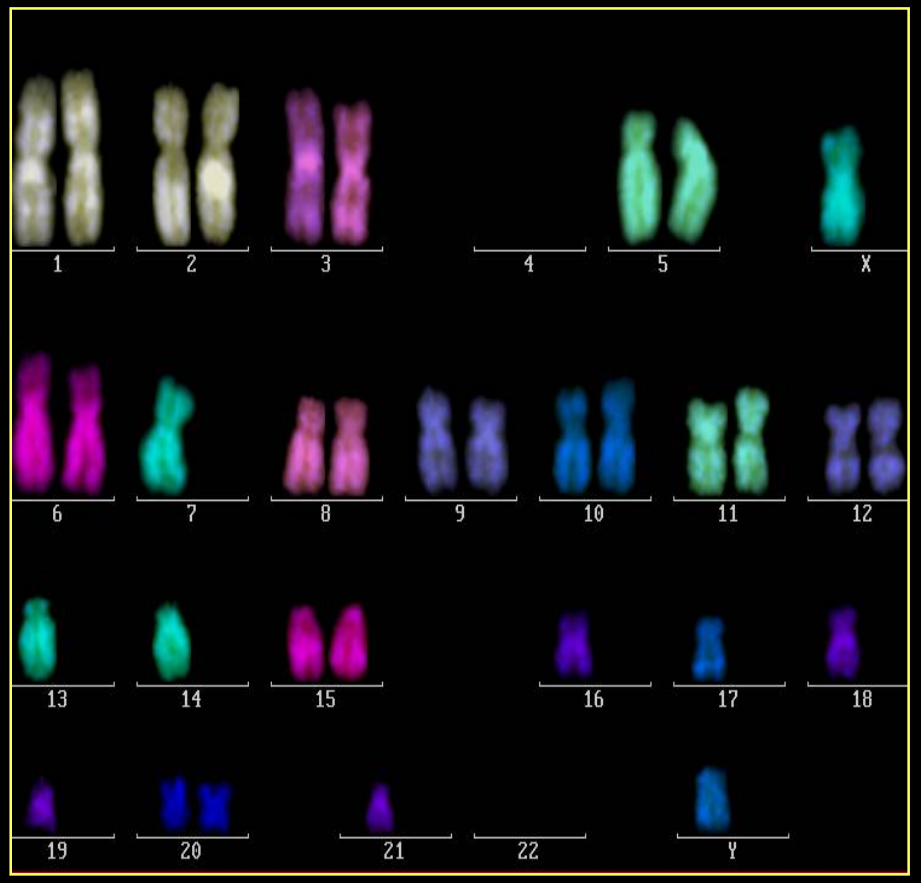
Multiplex-FISH (M-FISH)





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M-FISH colour combinations

Chromosome	DEAC	FITC	SpOrange	Texas Red	Cy5	False colour
1					Cy5	
2	DEAC					
3				Texas Red		
4		FITC				
5			SpOrange			
6		FITC			Cy5	
7	DEAC				Cy5	
8				Texas Red	Cy5	
9			SpOrange		Cy5	
10	DEAC	FITC				
11		FITC		Texas Red		
12		FITC	SpOrange			
13	DEAC			Texas Red		
14	DEAC		SpOrange			
15			SpOrange	Texas Red		
16	DEAC	FITC			Cy5	
17		FITC		Texas Red	Cy5	
18		FITC	SpOrange		Cy5	
19	DEAC			Texas Red	Cy5	
20	DEAC		SpOrange		Cy5	
21			SpOrange	Texas Red	Cy5	
22	DEAC	FITC		Texas Red		
X	DEAC	FITC	SpOrange			
Y	DEAC		SpOrange	Texas Red		

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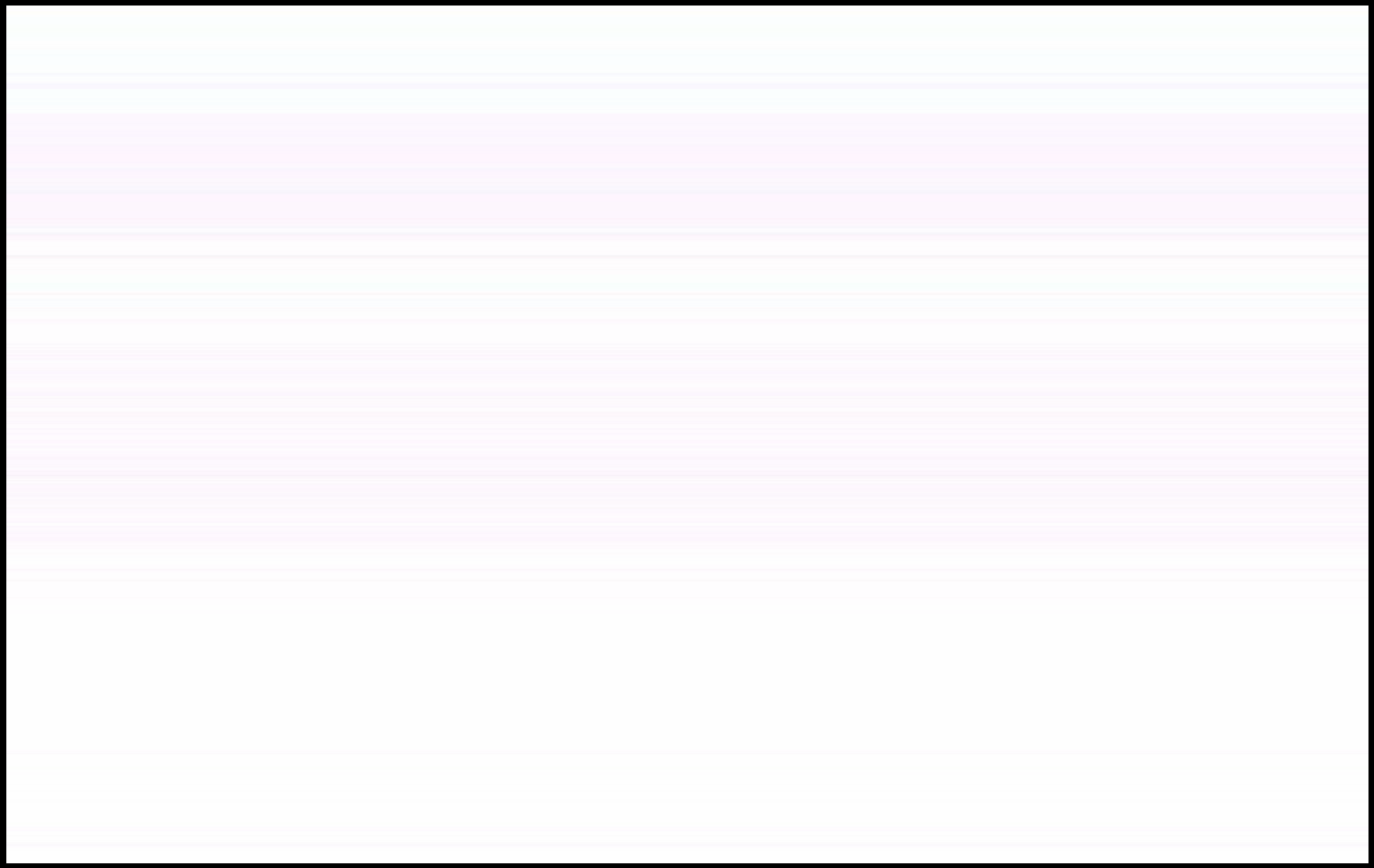
Spectral Karyotyping (SKY)



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Detail of SKY image

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Which technique to use – FISH or PCR?

- Depends on hardware and software availability;
  - microscope and filter configuration
  - camera and workstation
- Local availability of alternative techniques
  - karyotyping & RT-PCR
- Sample type and degree of urgency
  - very few molecular cytogenetic tests need to be done urgently (APML use PML stain)

O'Connor & Barrans, HMDS (2005)

FISH Probes

O'Connor & Barrans, HMDS (2005)

FISH probes

- Commercial
- good for numerical (centromeric, telomeric and paints)
- probe availability increasing all the time
- high cost*

- Home Produced
- needs local expertise & suitable facilities**
- probe production can be unpredictable
- potential low long term costs and ‘unlimited supply’

*Cost coming down with increasing availability / competition

**New EEC legal requirements will be enforced from 2005, difficult to comply with standards for in-house probes

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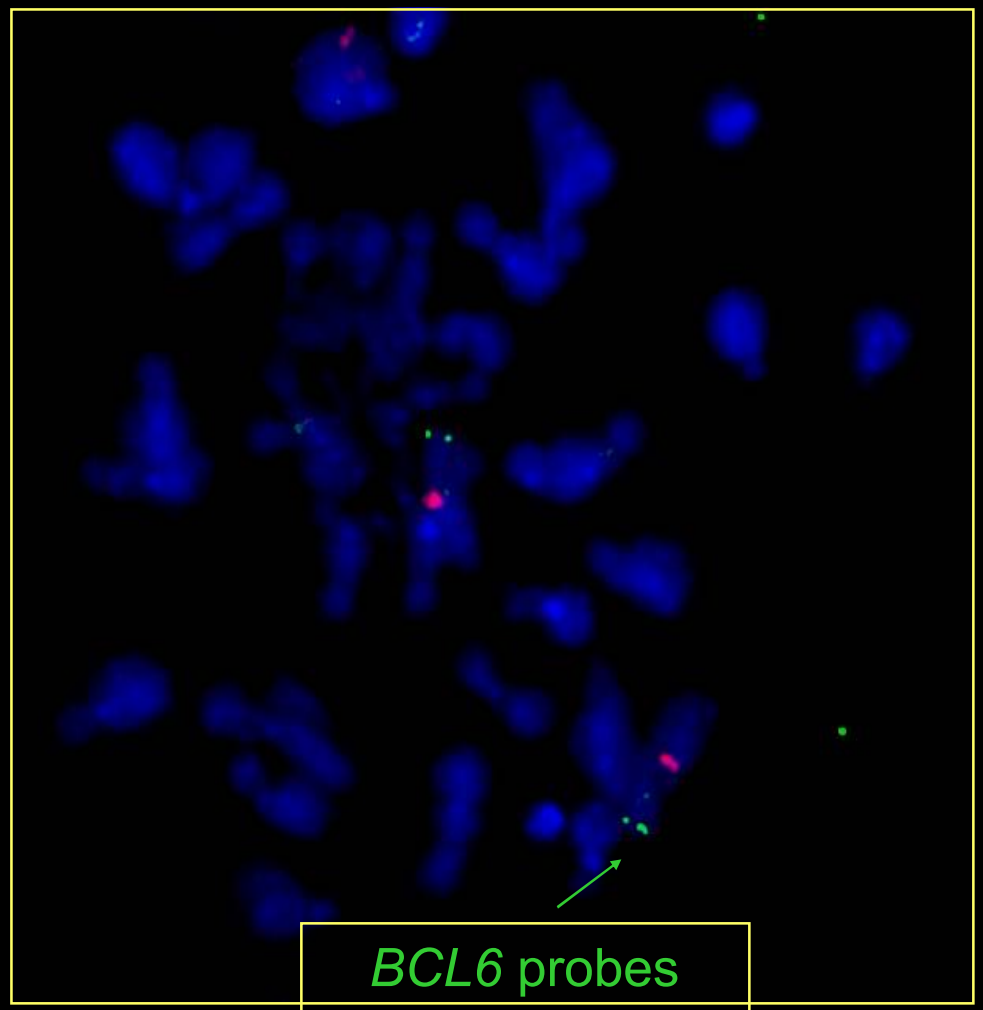
Validation of home produced probes

- Mapped to normal metaphase spreads
  - exact FISH conditions determined (each probe is unique this can take days or months)
  - applied to normal interphase cells
  - applied to known abnormal cases
- Home-grown PAC probe (Prof Dalla-Favera)

  BCL6 labelled green

  alpha sat 3 labelled red



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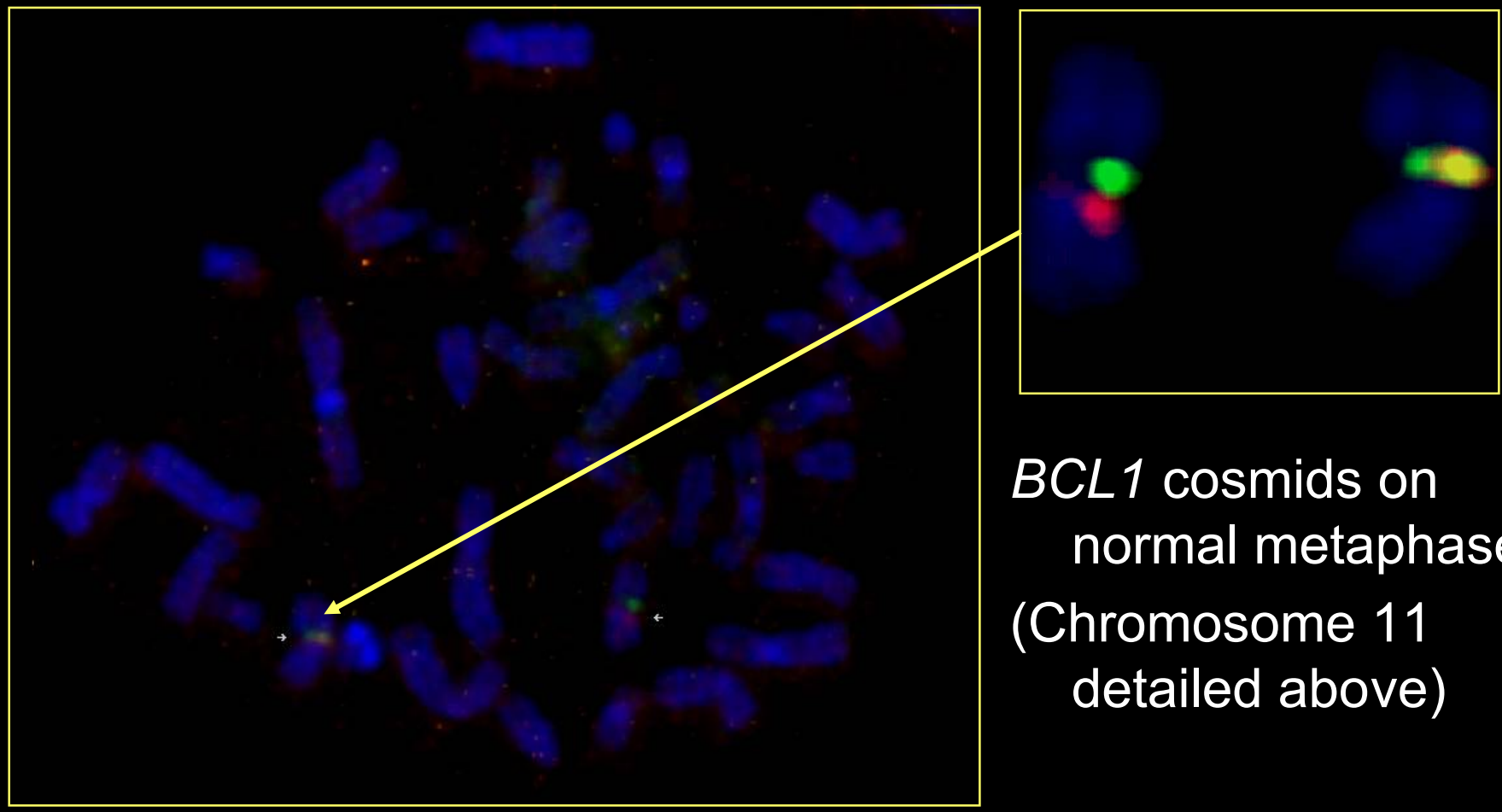
Validation of locus specific probes 11q13 (*BCL1*)



BCL1 cosmids on normal metaphase

(Chromosome 11 detailed above)

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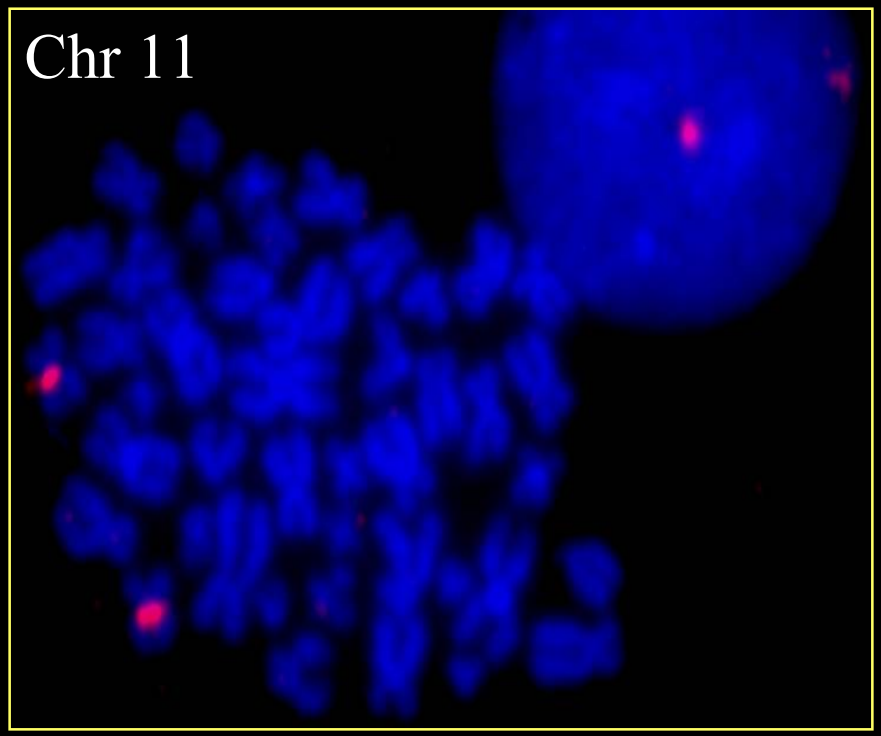
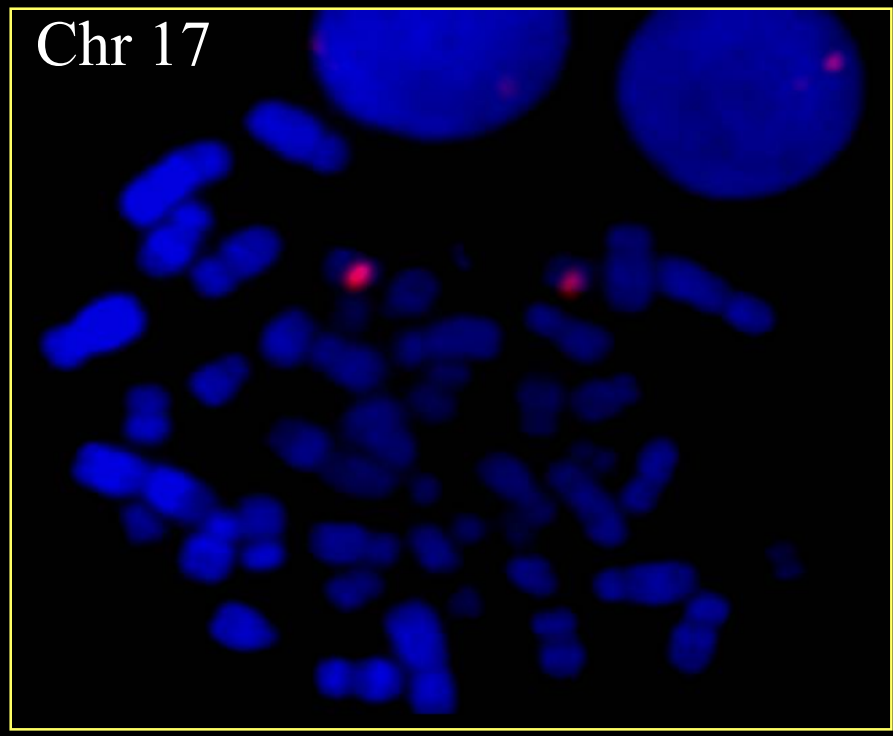
Validation of alpha sat probes





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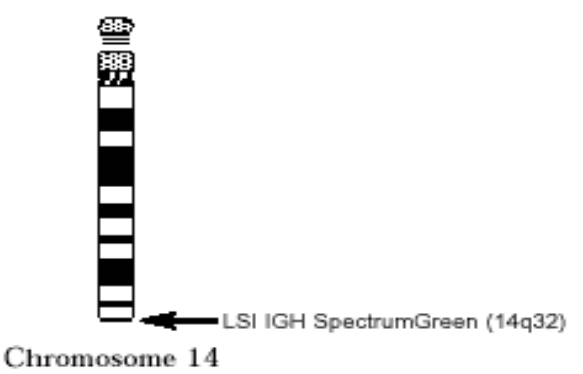
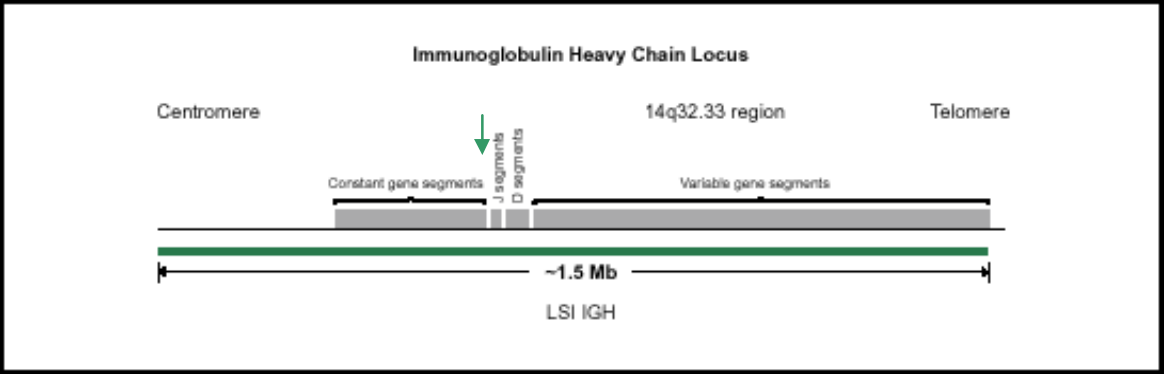
Commerical Probes are validated by Company

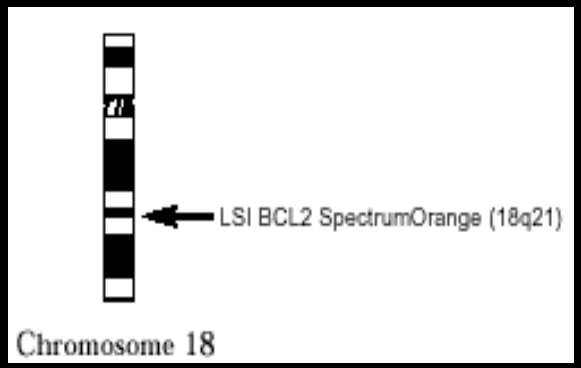
Probe Map Of The Vysis LSI *IgH/BCL2* Dual Colour Probe

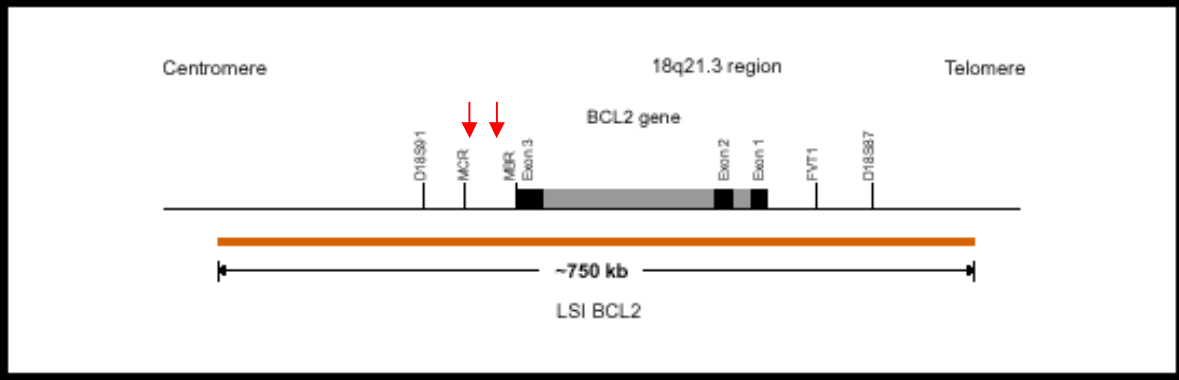
(Cat No: 32-191018)





The *IgH* probe spans approximately 1.5Mb and contains sequences homologous to essentially the entire *IgH* locus, as well as sequences extending about 300kb beyond the 3' end of the *IgH* locus.
The green line indicates the span of the IgH probe, and the arrow indicates the main breakpoint region.





The *BCL2* probe covers an approximate 750kb region, including the entire *BCL2* gene with additional sequences extending approximately 250kb both distal and proximal to the gene.
The span of the *BCL2* probe is indicated by the orange line, the arrows indicate the breakpoint regions.

Diagrams reproduced from the Vysis Website (http://www.vysis.com)

O'Connor & Barrans, HMDS (2005)

FISH labelling strategies

O'Connor & Barrans, HMDS (2005)

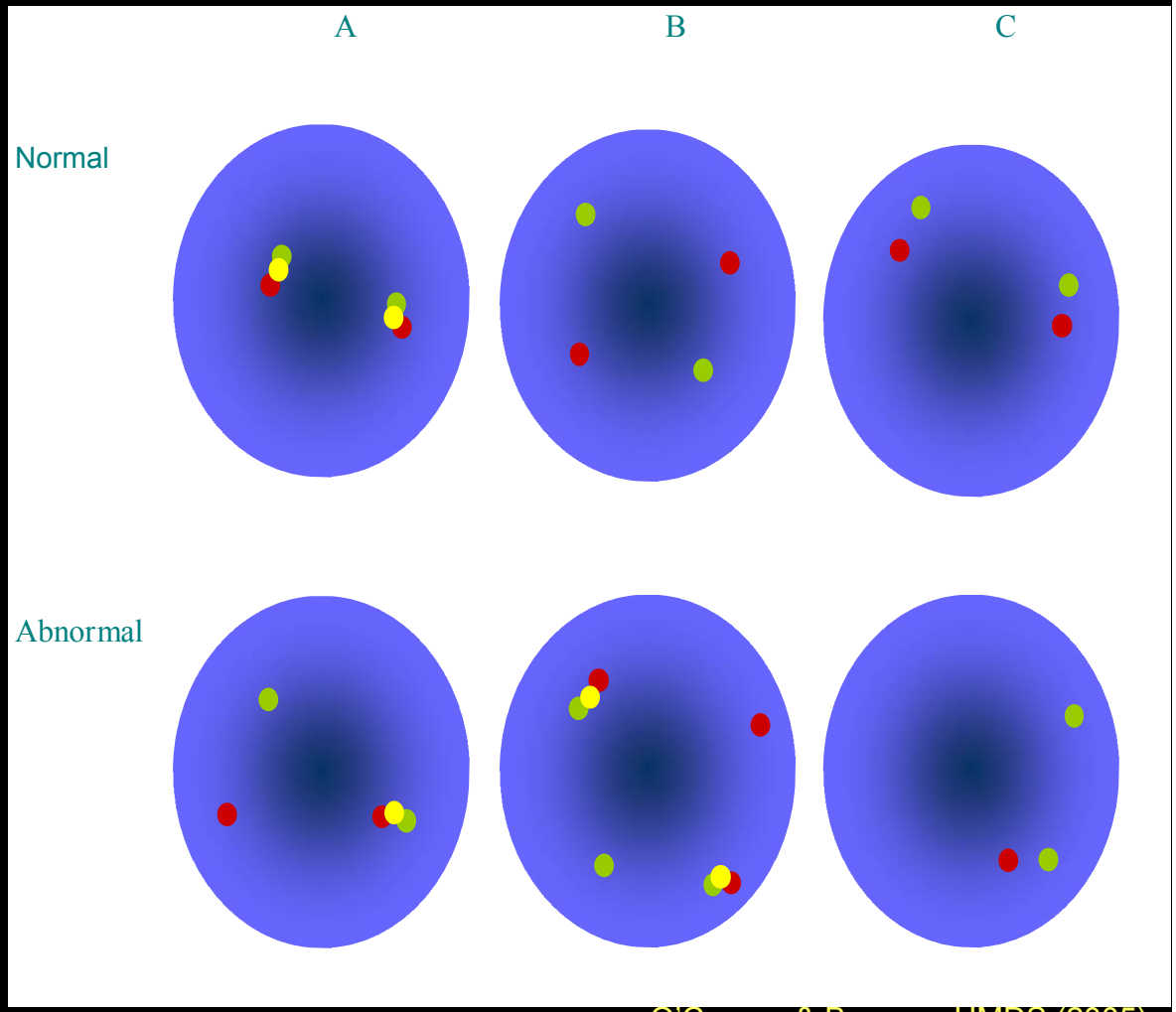
Schematic diagram showing the spot patterns detected with the different 2-colour FISH strategies developed

A. Break-apart protocol.

B. Dual Fusion protocol

Most sensitive strategy for translocations when partner chromosome is known.

C. Deletion assay. Includes control probe



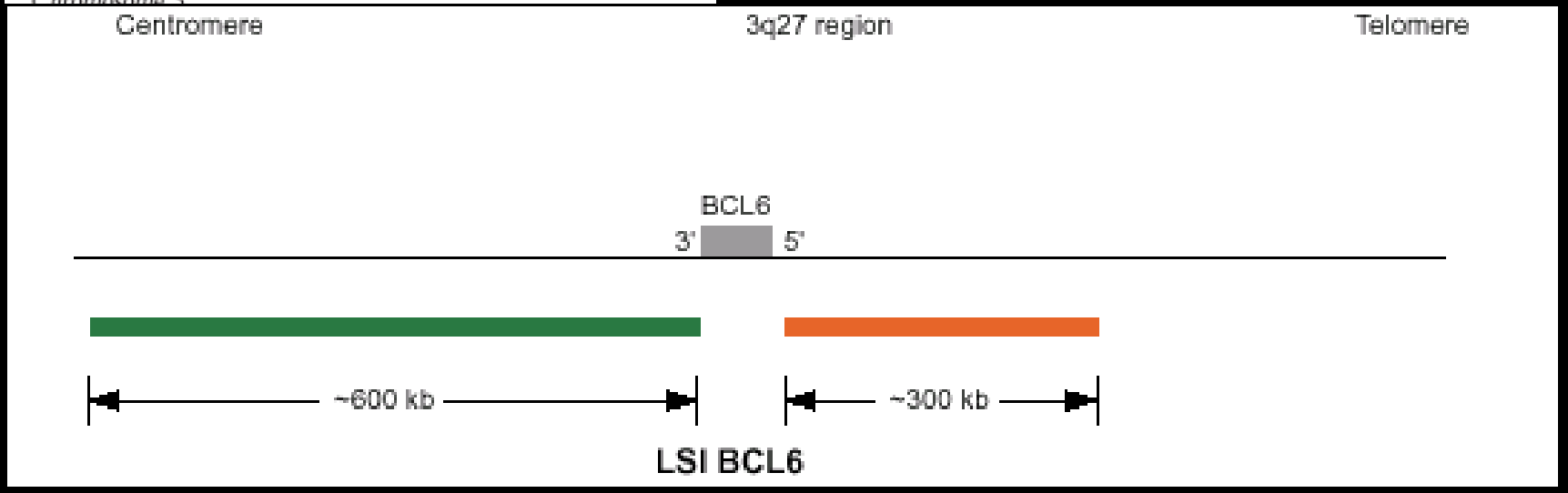
O'Connor & Barrans, HMDS (2005)

‘Break-apart’ FISH assay

LSI® *BCL6* Dual Colour, Break Apart Rearrangement Probe

(Cat No: 32-191016)

DNA Probe Description
The LSI BCL6 Dual Color, Break Apart Rearrangement Probe is a mixture of an approximately 300 kb labelled SpectrumOrange 5' LSI BCL6 probe and a 600 kb labeled SpectrumGreen 3' LSI BCL6 probe. These two probes are separated by a 42 kb gap that contains the entire BCL6 gene, including the BCL6 breakpoint region.



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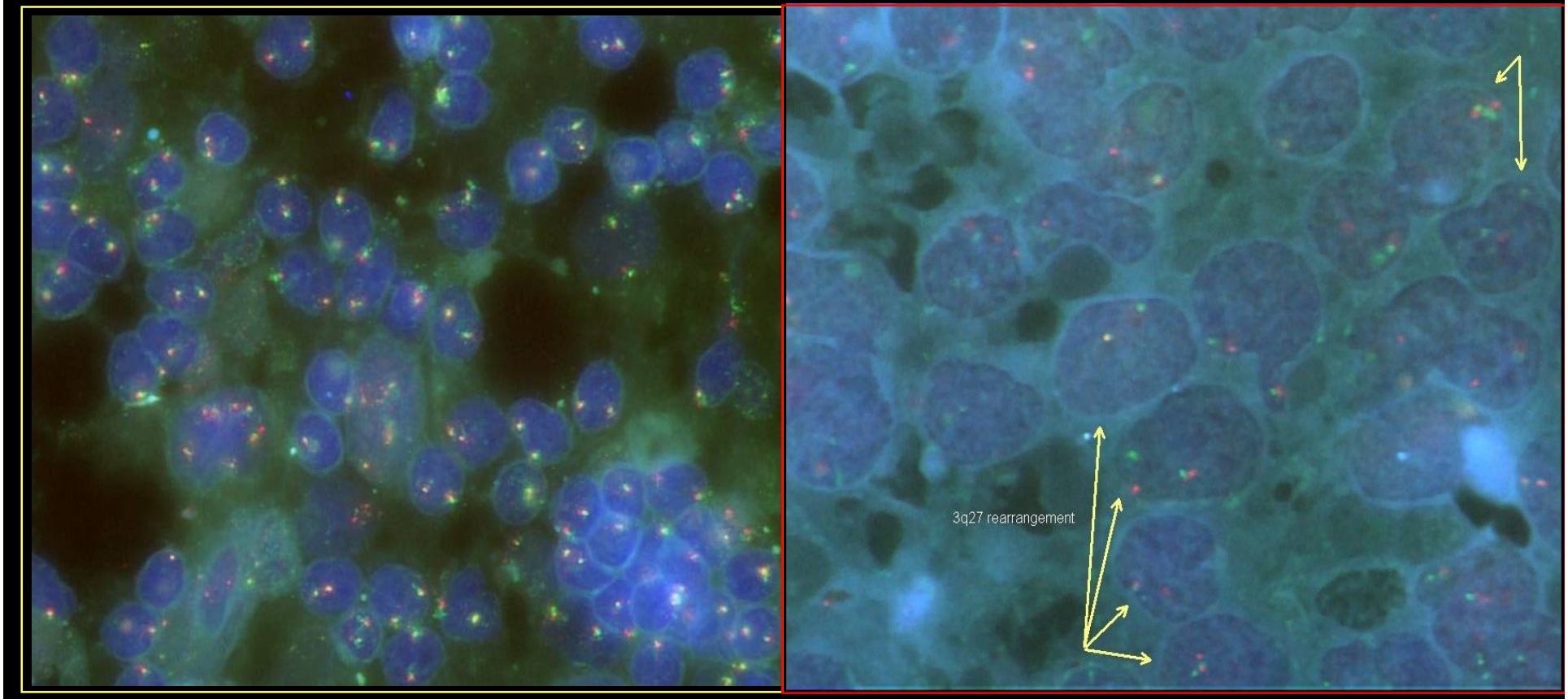
‘Break-apart’ FISH assay



O’Connor & Barrans, HMDS (2005)

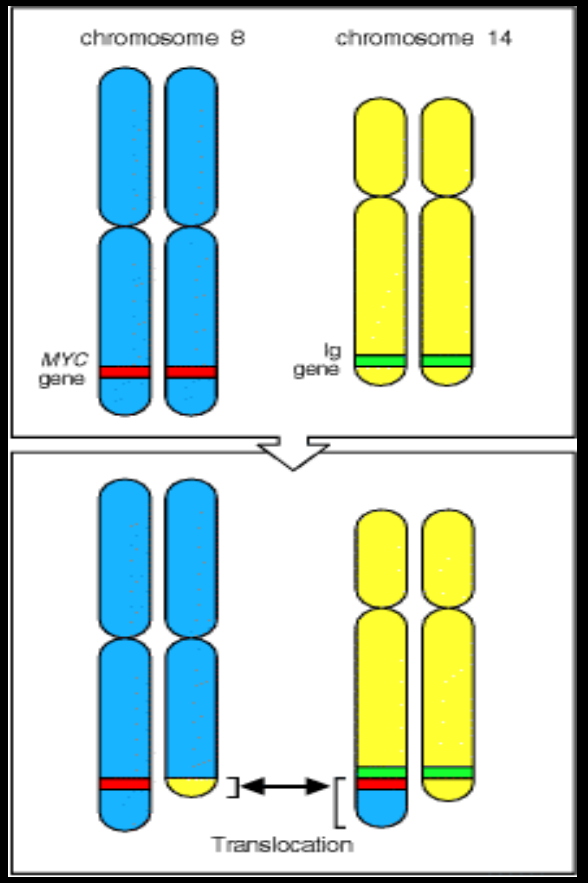
Dual Fusion Translocation Assay

Diagram showing an example of a balanced chromosome translocation

(t(8;14) Burkitts lymphoma)

chromosome 8 and 14 (top) and the result of a balanced reciprocal translocation between 8 and 14 (bottom), which rearranges *IgH* and *C-MYC*.

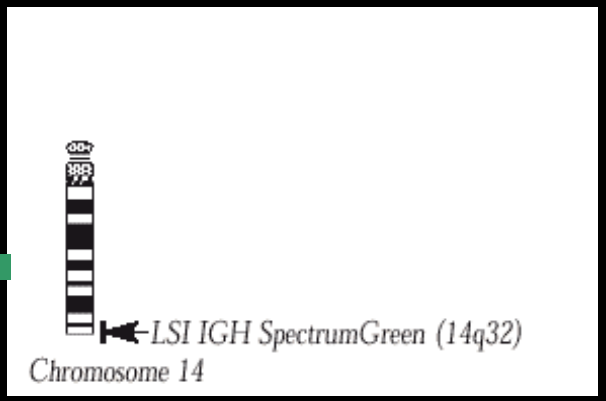
(Diagram from 'ImmunoBiology'. Charles A Janeway, published by Garland Science).

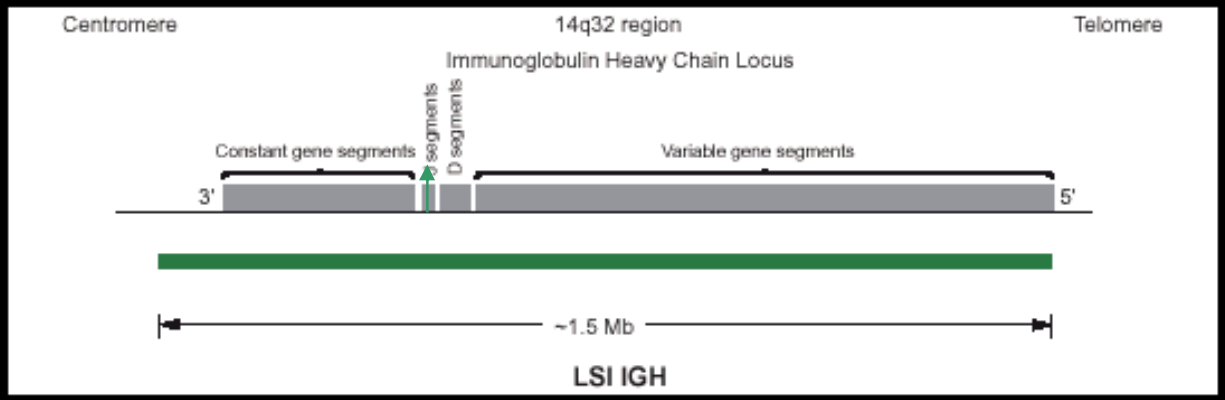


O'Connor & Barrans, HMDS (2005)

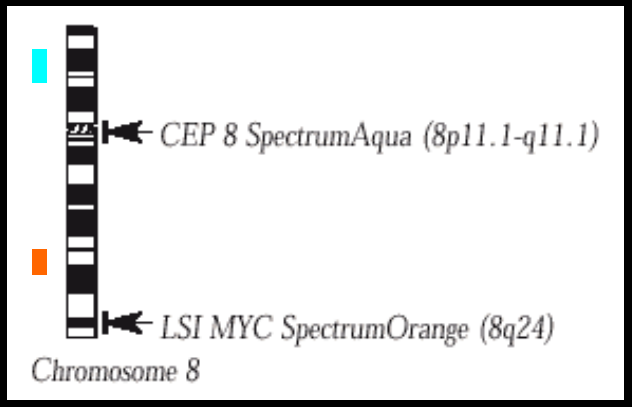
Dual Fusion FISH assay

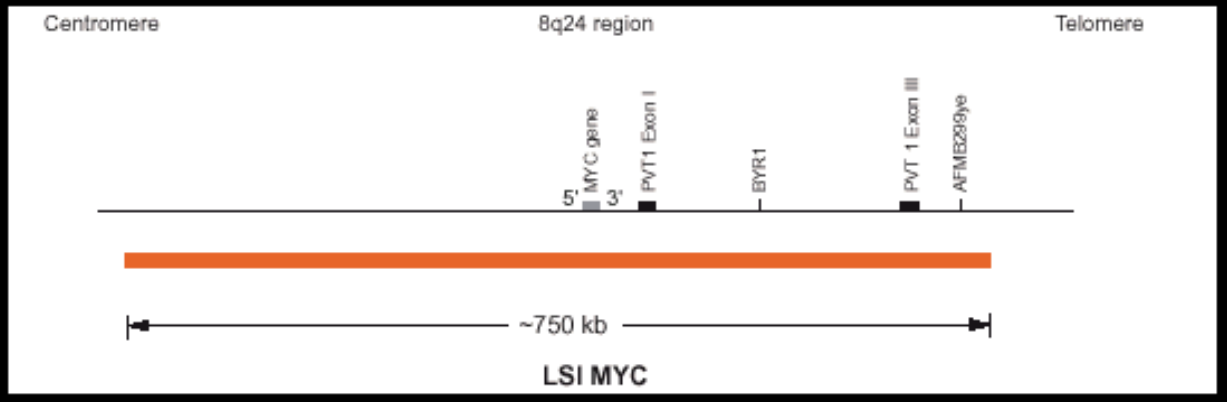
Probe Map Of The Vysis LSI® IGH/MYC, CEP® 8 Tri-color, Dual Fusion Translocation Probe (Cat No: 32-191020)





The *IgH* probe spans approximately 1.5Mb and contains sequences homologous to essentially the entire *IgH* locus, as well as sequences extending about 300kb beyond the 3' end of the *IgH* locus.
The green line indicates the span of the IgH probe, and the arrow indicates the main breakpoint region.





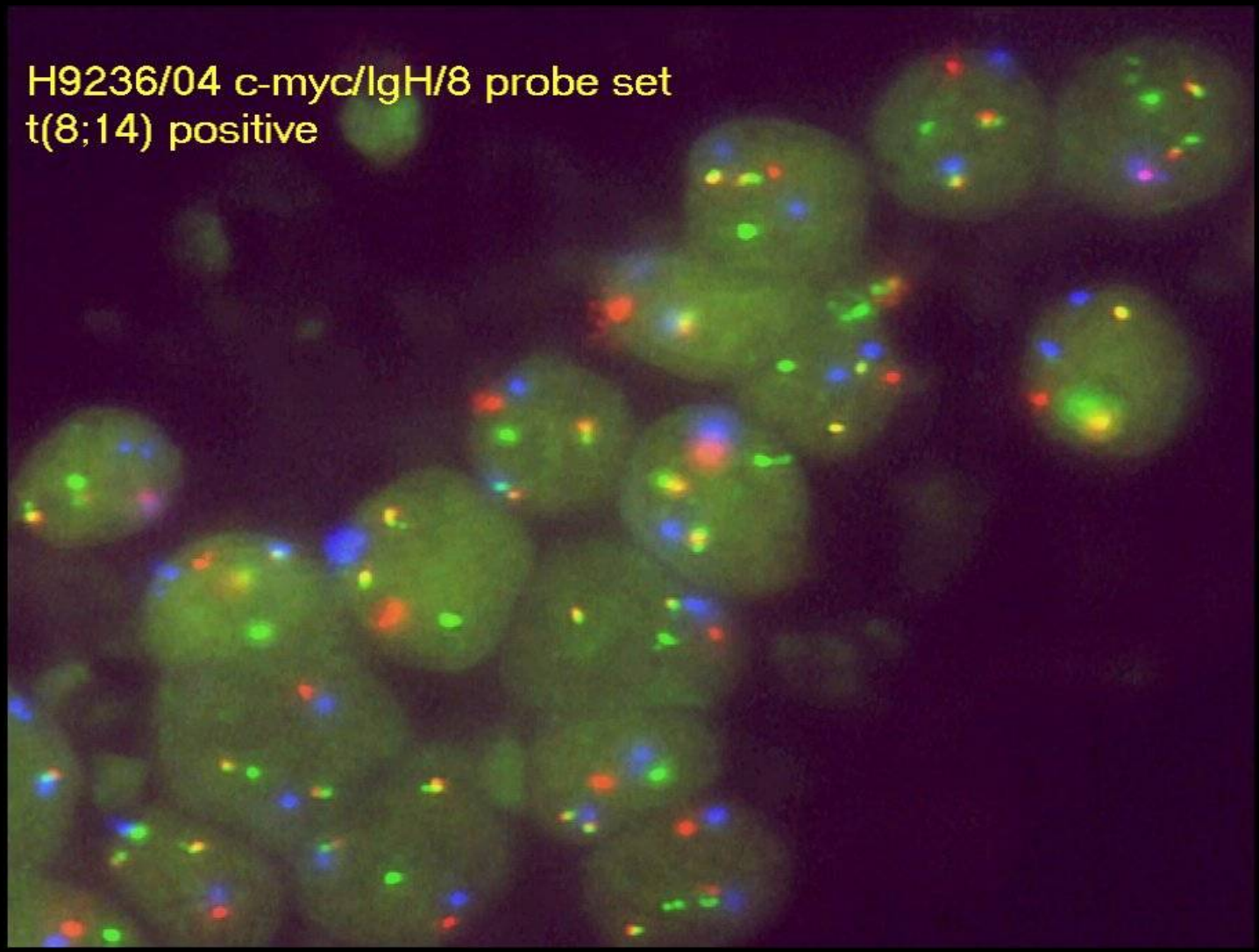
The *cMYC* probe covers an approximate 750kb region, including the entire *cMYC* gene with additional sequences extending both distal and proximal to the gene.
The span of the *cMYC* probe is indicated by the orange line

Diagrams reproduced from the Vysis Website (http://www.vysis.com)

O'Connor & Barrans, HMDS (2005)

Dual Fusion Translocation Assay

Representative Image



O'Connor & Barrans, HMDS (2005)

Deletion Assay
Schematic diagram

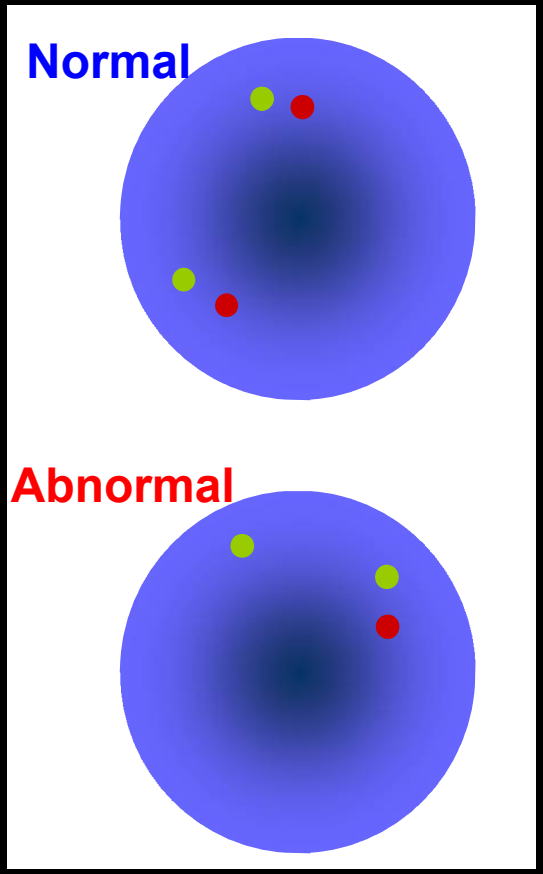
For deletion analysis it is important to select a probe from **within** the minimally deleted region.

The smallest size probe used in practice is a single cosmid (approx. 40KB (to detect a locus specific target).

Interpretation can be difficult due to the small probe size and the sensitivity of deletion assays is poor compared to translocation detection.

A control probe from a non-deleted segment of the same chromosome is essential.

- critical probe RED
- control probe GREEN

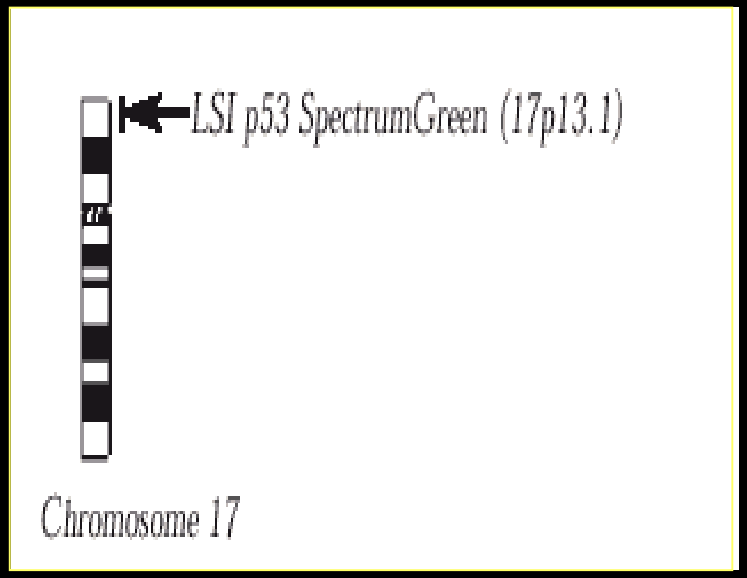


O'Connor & Barrans, HMDS (2005)

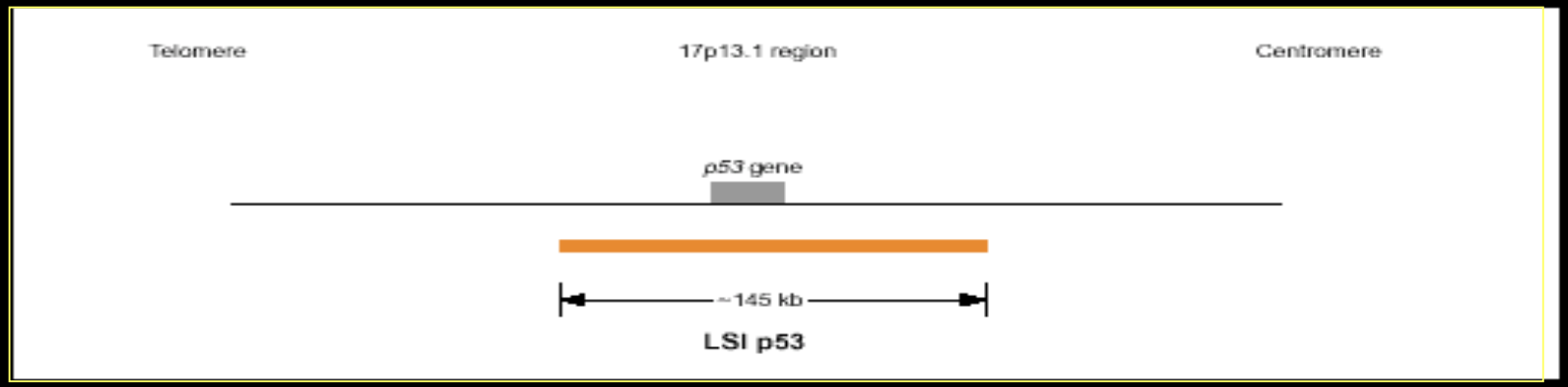
Deletion Assay

Vysis LSI® *P53* (17p13.1) Single Color Probe

(Cat. No. 32-190008)



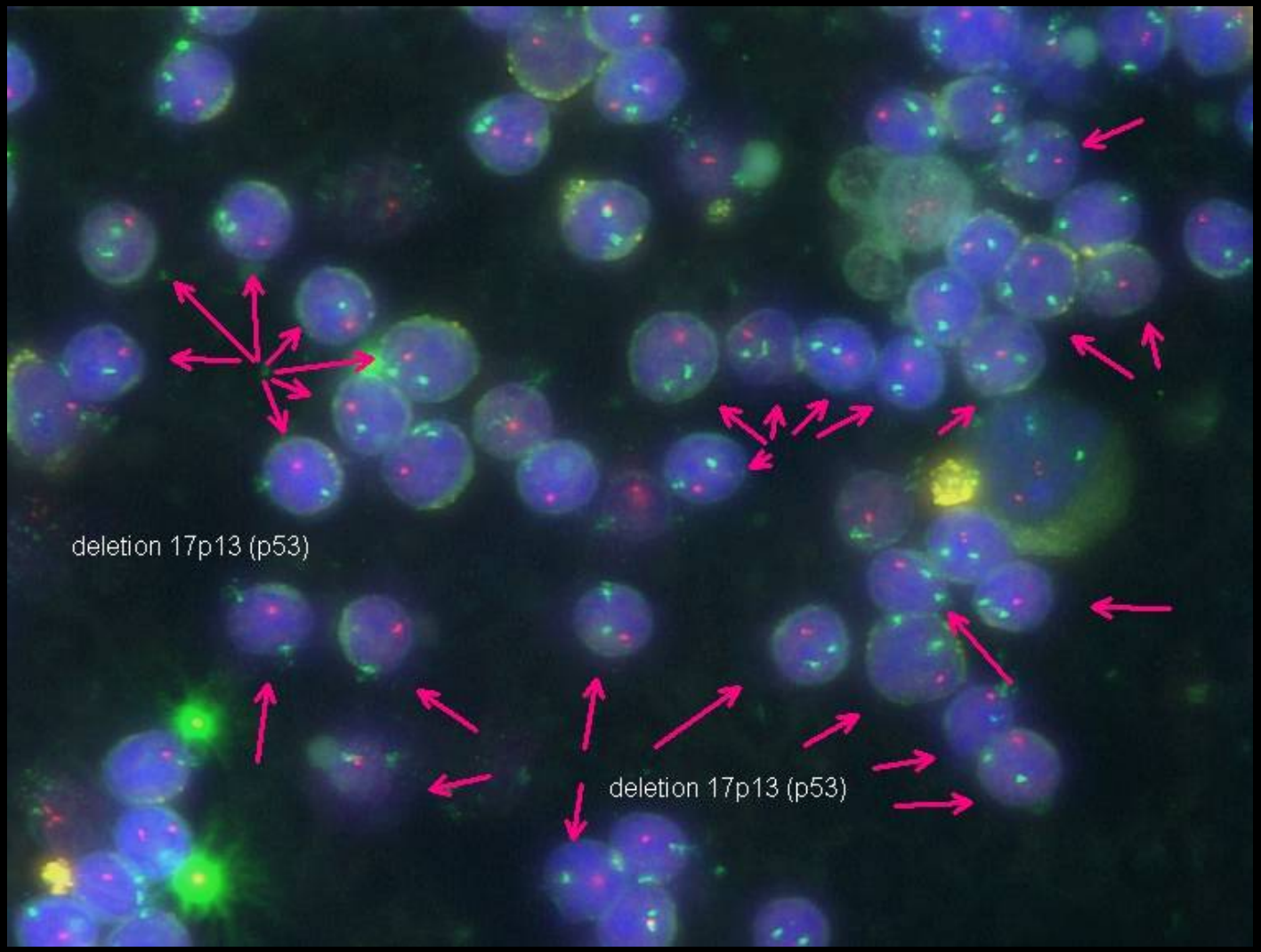
- Probe set will detect deletion of 17p13 region which spans the *P53* gene.
- Mutations of *P53* cannot be detected by this method.



Diagrams reproduced from the Vysis Website (http://www.vysis.com)

Deletion Assay

Representative Image



O'Connor & Barrans, HMDS (2005)

Sample Preparation

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Sample preparation for FISH

- Keep it as simple as possible !

- Fresh material is better
  - Peripheral blood and bone marrow smears
  - Cytospins of fluid samples
  - Tissue biopsy samples
    - Dab preparations of fresh unfixed material
    - Dab preparations of frozen unfixed material

- Fixed tissue
  - Extracted nuclei and thin sections (more to follow)

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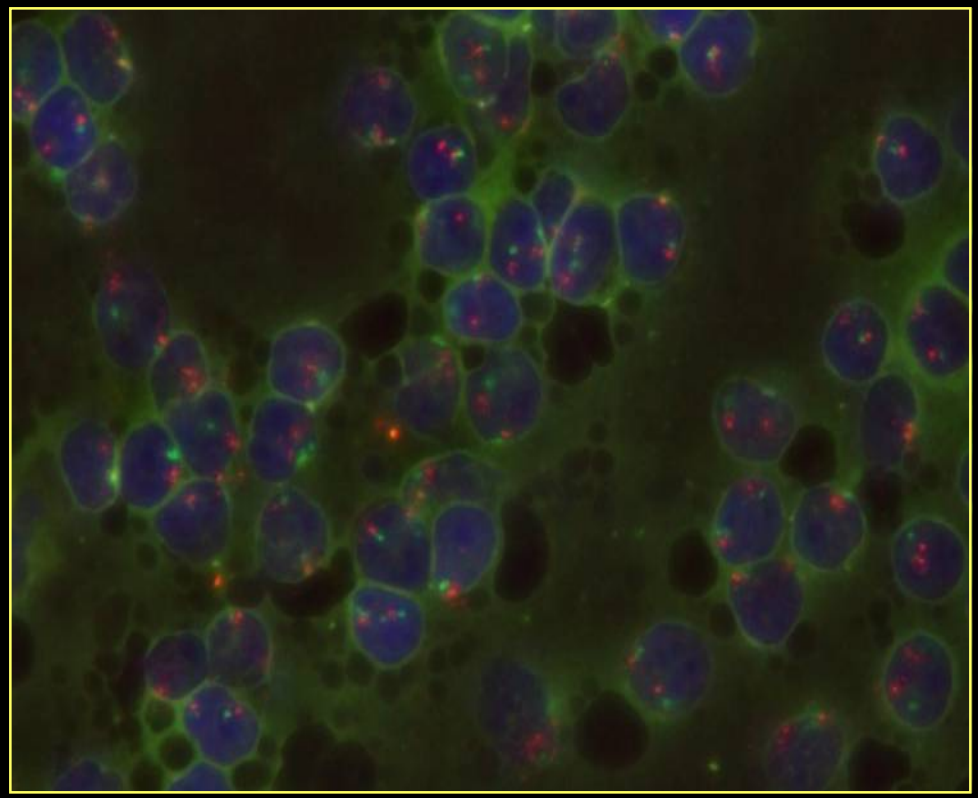
Tissue biopsy DAB preparation

- rapid
- very easy to prepare
- can use the same FISH protocol as PB & BMA
- compare to morphology
- high background
- requires a fresh biopsy



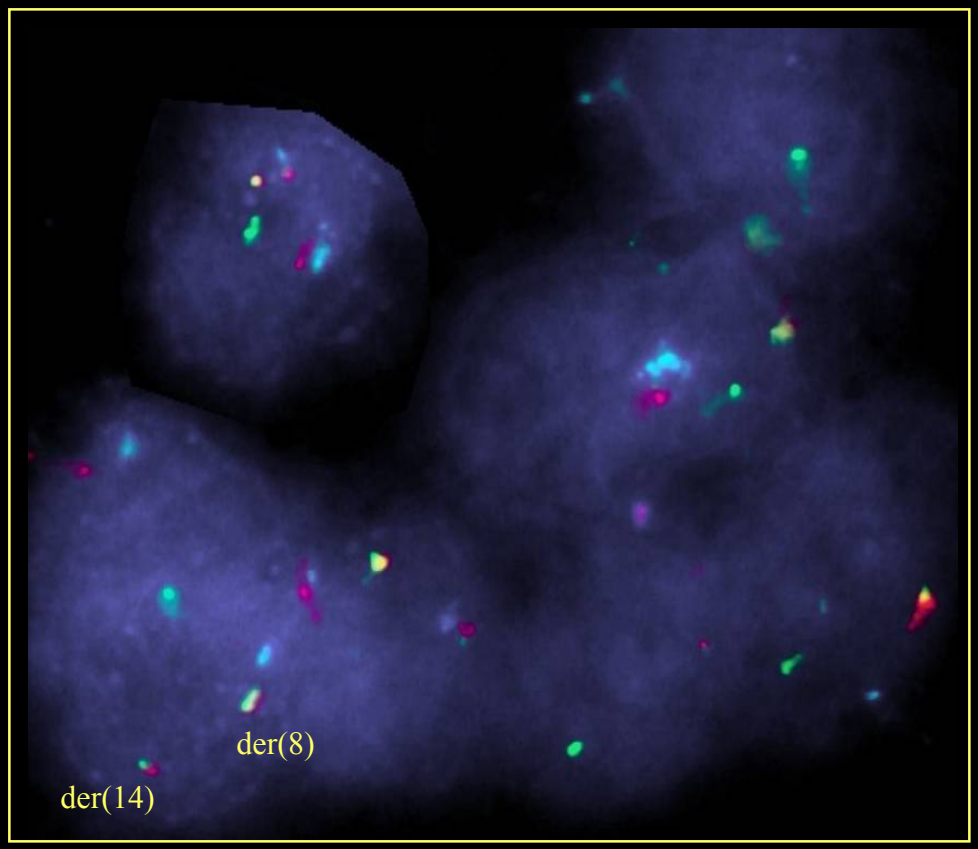
Reactive lymph node DAB
normal FISH

BCL2 red, *IgH* green

O'Connor & Barrans, HMDS (2005)

Tissue biopsy Frozen DAB preparation

- DABs made from frozen biopsy material
- Identical FISH method
- Some reduction of background staining



- *cMYC*/*IgH*/CEP 8 Aqua probe set
- Abdominal mass biopsy – 2x frozen
- Dab preparation
- Double fusions seen
- t(8;14) positive

O'Connor & Barrans, HMDS (2005)

Formalin Fixed Paraffin Embedded Tissue biopsies

- access the huge archive of stored tissue
- lack of suitable fresh material from many centres

- difficult to standardise
- many more variables compared to PB & BMA samples
  fixation, processing, age, degree of fibrosis etc
- **only used as last resort in diagnostic setting!!**

O'Connor & Barrans, HMDS (2005)

Formalin Fixed Paraffin Embedded Tissue biopsies

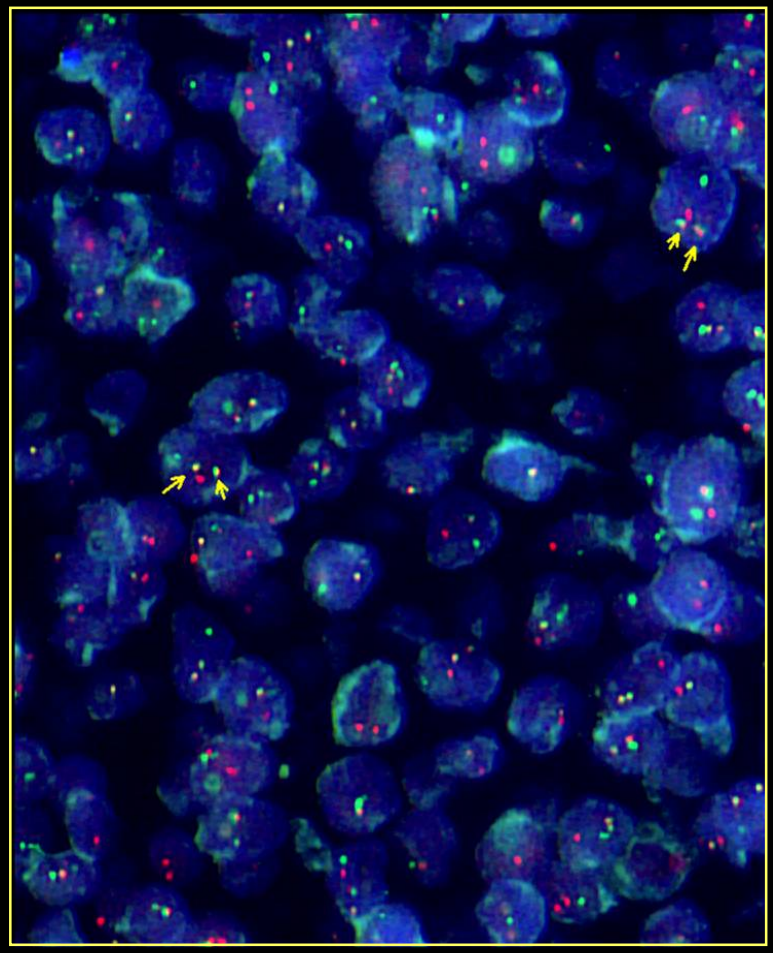
4μm sections or extracted nuclei?

Advantages of thin sections

- Tissue architecture is retained - important in the morphological diagnosis of lymphoma
- Maintain cell membrane and cytoplasm – possibility of FICTION

Disadvantages of thin sections

- Loss or 'slicing through' of signals
- Difficult to standardise
- Difficult to interpret



t(11;14) positive case – thin section

O'Connor & Barrans, HMDS (2005)

Formalin Fixed Paraffin Embedded Tissue biopsies

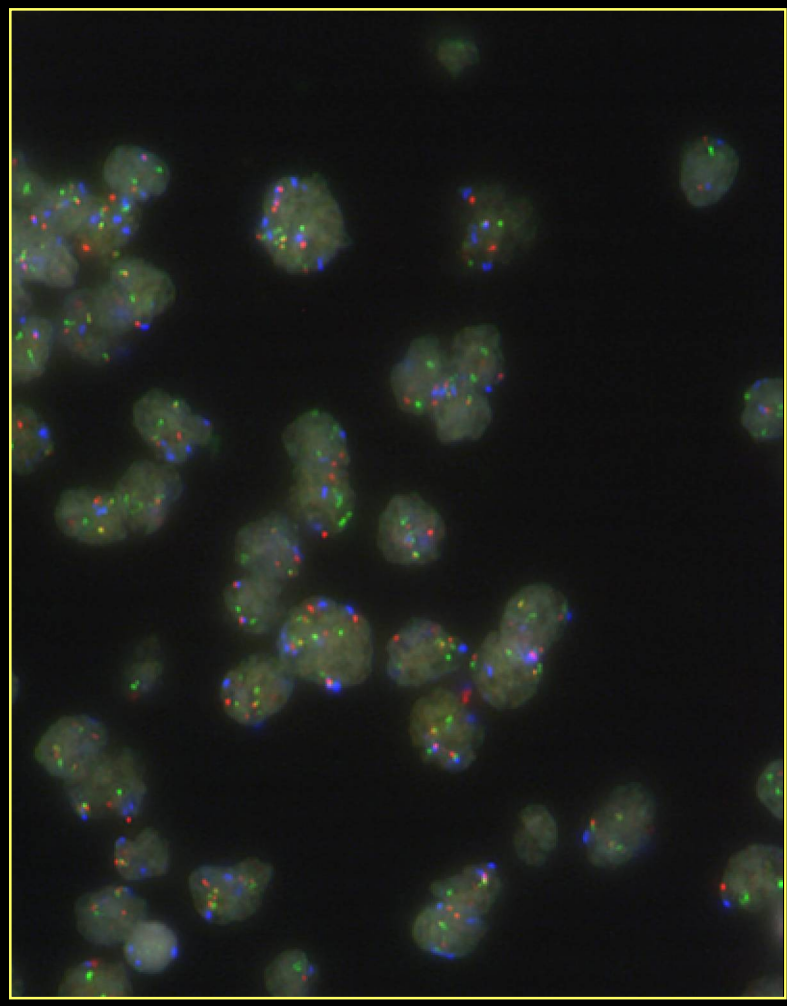
4μm sections or nuclei extracted from thick sections?

Advantages of extracted nuclei

- The complete nucleus is analysed
- Results easier to interpret
- Can adjust pre-denaturation to allow for differences in digestion efficiency

Disadvantages of extracted nuclei

- Loss of the cell membrane and cytoplasm
- Loss of tissue architecture
- The technique is highly complicated - more variables to consider and evaluate.



t(1418) positive case – extracted nuclei

O'Connor & Barrans, HMDS (2005)

FISH on whole nuclei extracted from paraffin-embedded tissue

Principle

- digest sections sufficiently to release whole nuclei into suspension
- allows penetration of the probe, without completely digesting the nucleus

Method

- 35μm thick paraffin sections
- digest in Protease XXIV (Sigma P8038) in Tris buffer containing NP-40 at 37°C
- Resuspend digested nuclei in MAA,
- 'drop' nuclear suspension onto APES-coated microscope slides
- Requires an additional 90°C pre-denaturation step
- Following pre-Denaturation, use standard FISH technique

O'Connor & Barrans, HMDS (2005)

FISH on thin paraffin sections

Principle

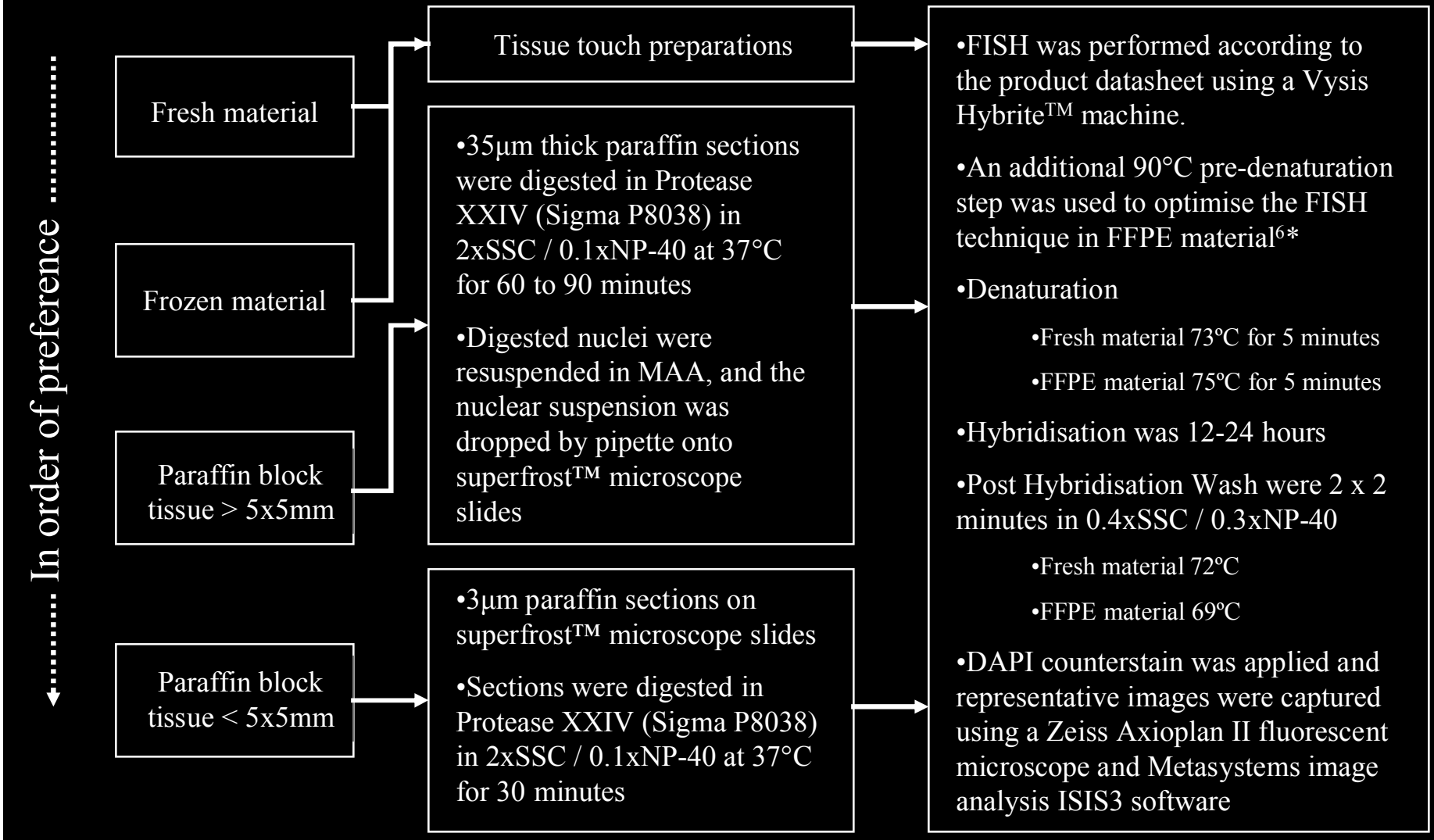
- FISH is performed on routine histological sections (3-4μm)
- Rapid method, can be fitted in with routine FISH on fresh samples

Method

- Take sections to water, ensuring thorough removal of xylene and alcohol
- treat in 2xSSC at 37°C
- digest in Protease XXIV (Sigma P8038) in 2xSSC containing NP-40 at 37°C
- 90°C pre-denaturation step
- Following pre-denaturation, use standard FISH technique

O'Connor & Barrans, HMDS (2005)

Summary of Interphase FISH Method



*Concordance of results obtained between fresh tissue and FFPE tissue is reported

O'Connor & Barrans, HMDS (2005)

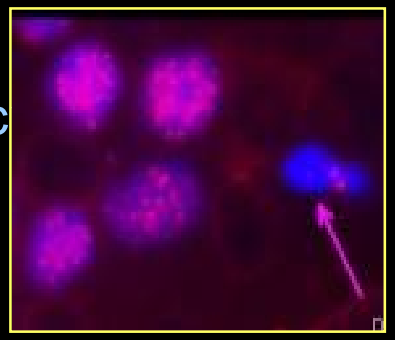
Part 2

Clinical applications of FISH

Myeloid Leukaemias

Cytogenetics of myeloid leukaemias

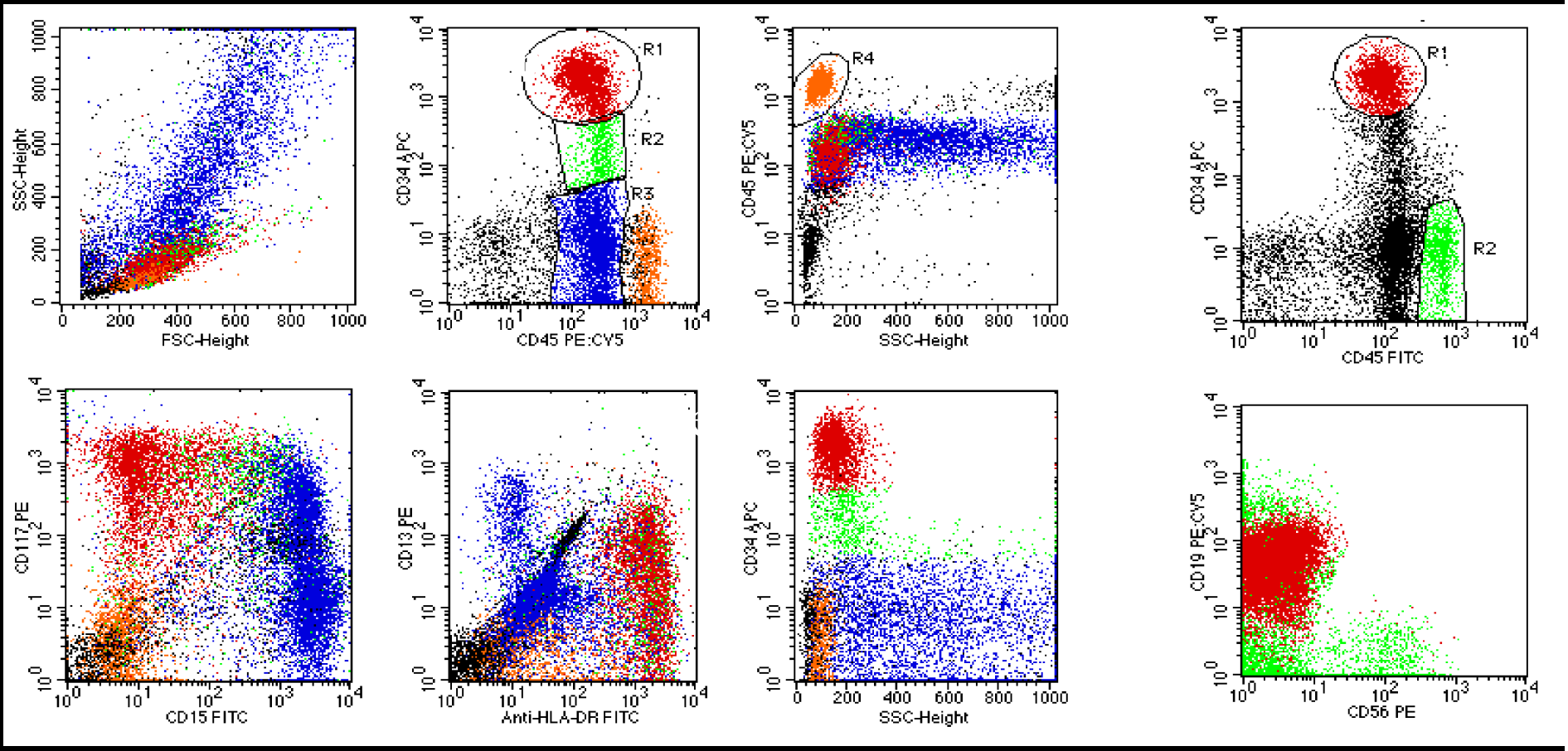
- AML and MDS are associated with specific cytogenetic aberrations
  - Balanced translocations
    - t(15;17) APML approx 10% frequenc
    - t(8;21) AML <5%
    - Inv 16 AML <5%
  - Deletions
    - Loss of 5q and/or 7q (25% overall incidence) seen in tAML, MDS and AML ~ poor prognosis
  - Amplifications
    - Trisomy 8 very frequently seen in monoblastic leukaemia (90%), but also seen all sub-types of myeloid leukaemia (15-20%).
- These are generally diseases of progenitor cells which have proliferative capacity and grow well in culture
  - conventional cytogenetics detects abnormalities ~ 5O%
  - RT-PCR can be used to detect translocations



PML protein ~ rapid test for t(15;17)

O'Connor & Barrans, HMDS (2005)

AML with t(8;21)

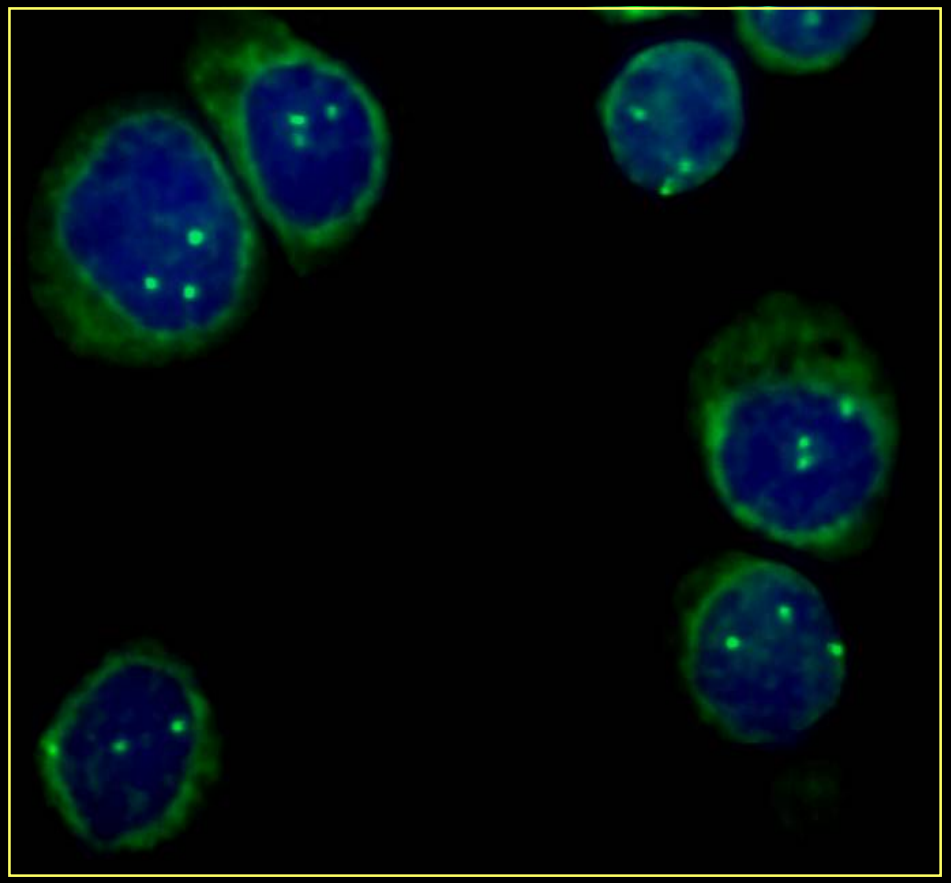


Plots (a, b & c) show standard analysis regions. Plots (d & e) show CD117/CD15 & CD13/HLADR expression by these components. The most immature blast cells (R1) are CD117+CD15-CD13+HLADR+. Maturing blasts (R2) CD117+CD15+ and mature myeloid cells (R3) CD15+CD117+/-HLADR-.
Plots (g & h) show CD19 expression by blast cells, though at levels weaker than on normal B cells.

O'Connor & Barrans, HMDS (2005)

Trisomy 8 in AML

- Common in AML-M5 but also seen in other types of AML.
- Overall 10% +8 occur as an isolated abnormality
- Often seen as part of complex abnormality
- Not specifically associated with prognosis.

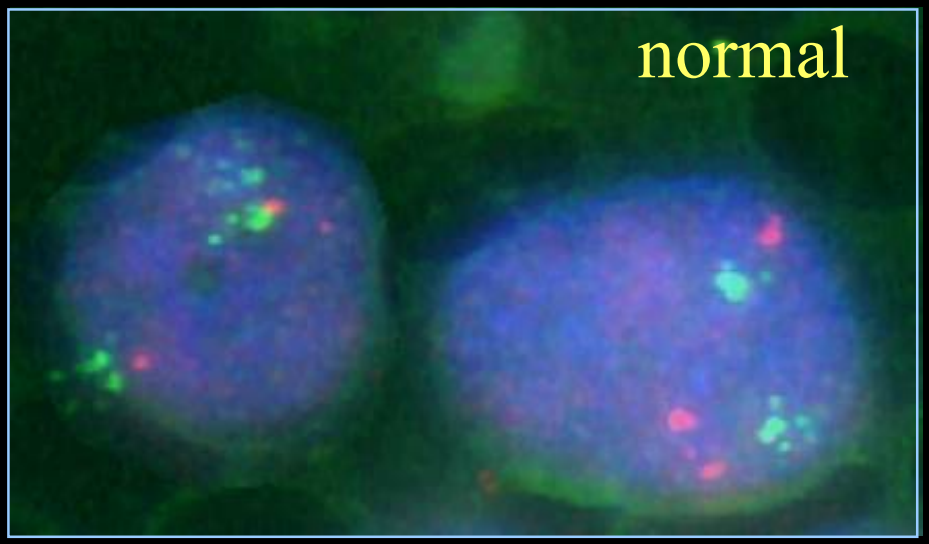


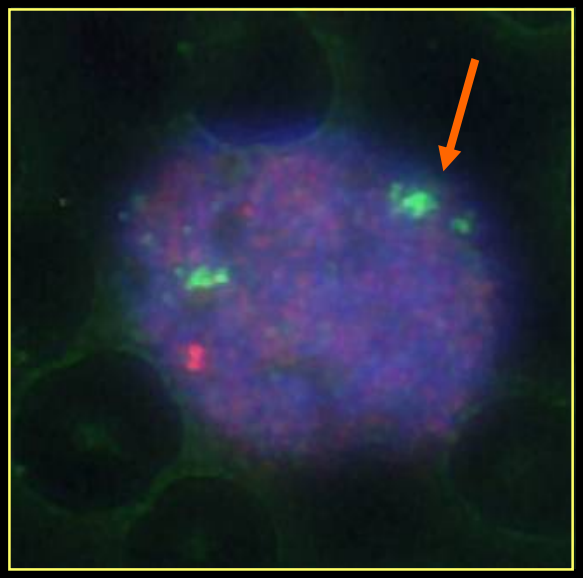
O'Connor & Barrans, HMDS (2005)

Deletion of 5q31 in AML/MDS

- Deletions of the *EGR1* gene at 5q31 are one of the commonest aberrations seen in myeloid malignancy.
- Interphase FISH is a rapid and cost effective way to screen large numbers of cells.
- LSI EGR1 (5q31)/D5S721 probe set

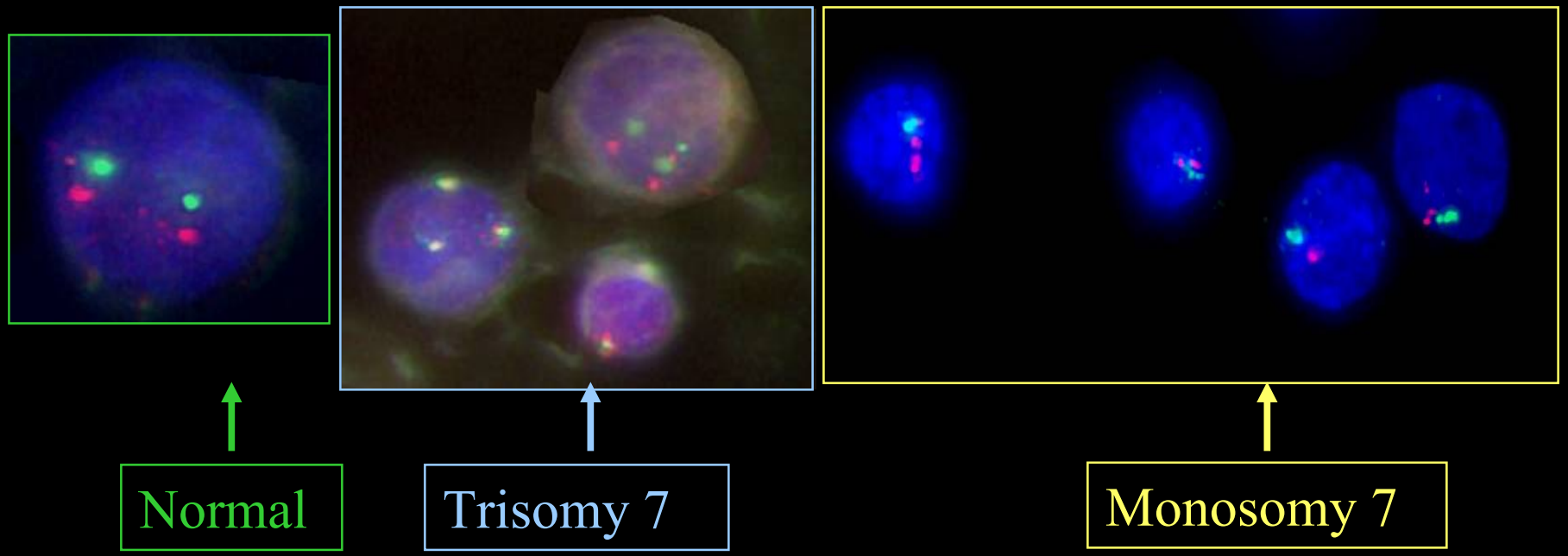
  (Cat. No. 32-191021)
  - *EGR1* ~ red
  - Control 5p15 ~ green





O'Connor & Barrans, HMDS (2005)

7q31 probe in AML



- Deletions of 7q31 (? Gene) are one of the commonest aberrations in myeloid malignancy.
- Interphase FISH is a rapid and cost effective way to screen large numbers of cells.
- LSI D7S522(7q31)/CEP 7 probe set
  (Cat. No. 32-191038)
  - 7q31 ~ red
  - Control alpha 7 ~ green

O'Connor & Barrans, HMDS (2005)

B-cell disorders:

Plasma cell disorders
B-CLL
Mantle cell lymphoma
Follicular lymphoma
Marginal zone lymphoma
Diffuse large B cell lymphoma
Burkitt's Lymphoma

O'Connor & Barrans, HMDS (2005)

Plasma cell disorders

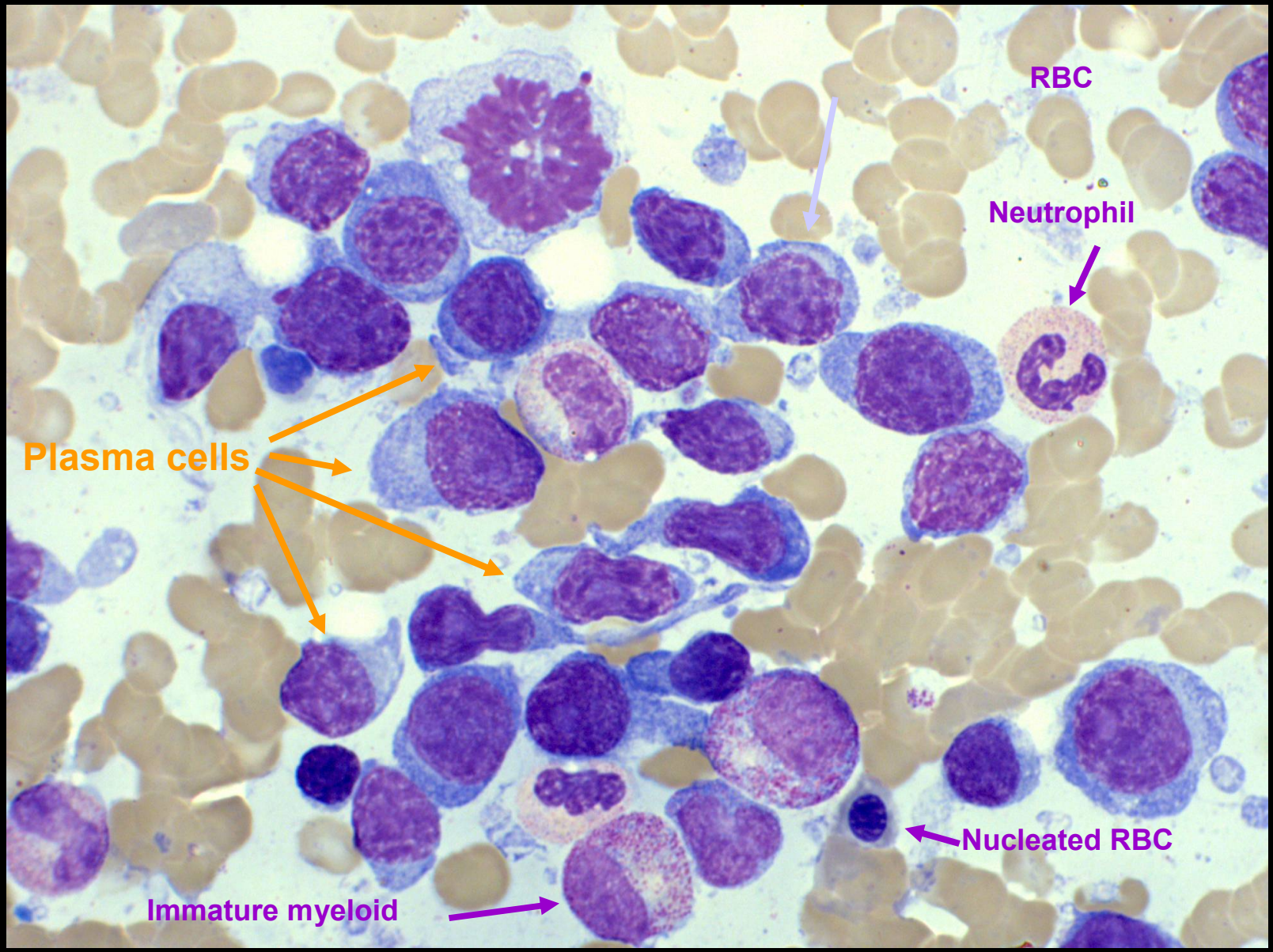
O'Connor & Barrans, HMDS (2005)

FISH as a diagnostic tool for plasma cell disorders

- Conventional cytogenetics is a poor technique for the identification of chromosome aberrations in plasma cell disorders ~ interphase FISH is an ideal technique for PC
  - Low proliferative index
  - Patchy infiltrate in bone marrow
  - MGUS is a particular problem
- Cytogenetic aberrations have prognostic significance in myeloma
  - Deletions of chromosome 13q ~ poor
  - Aneuploidy ~ ??
  - Translocations ~ ??

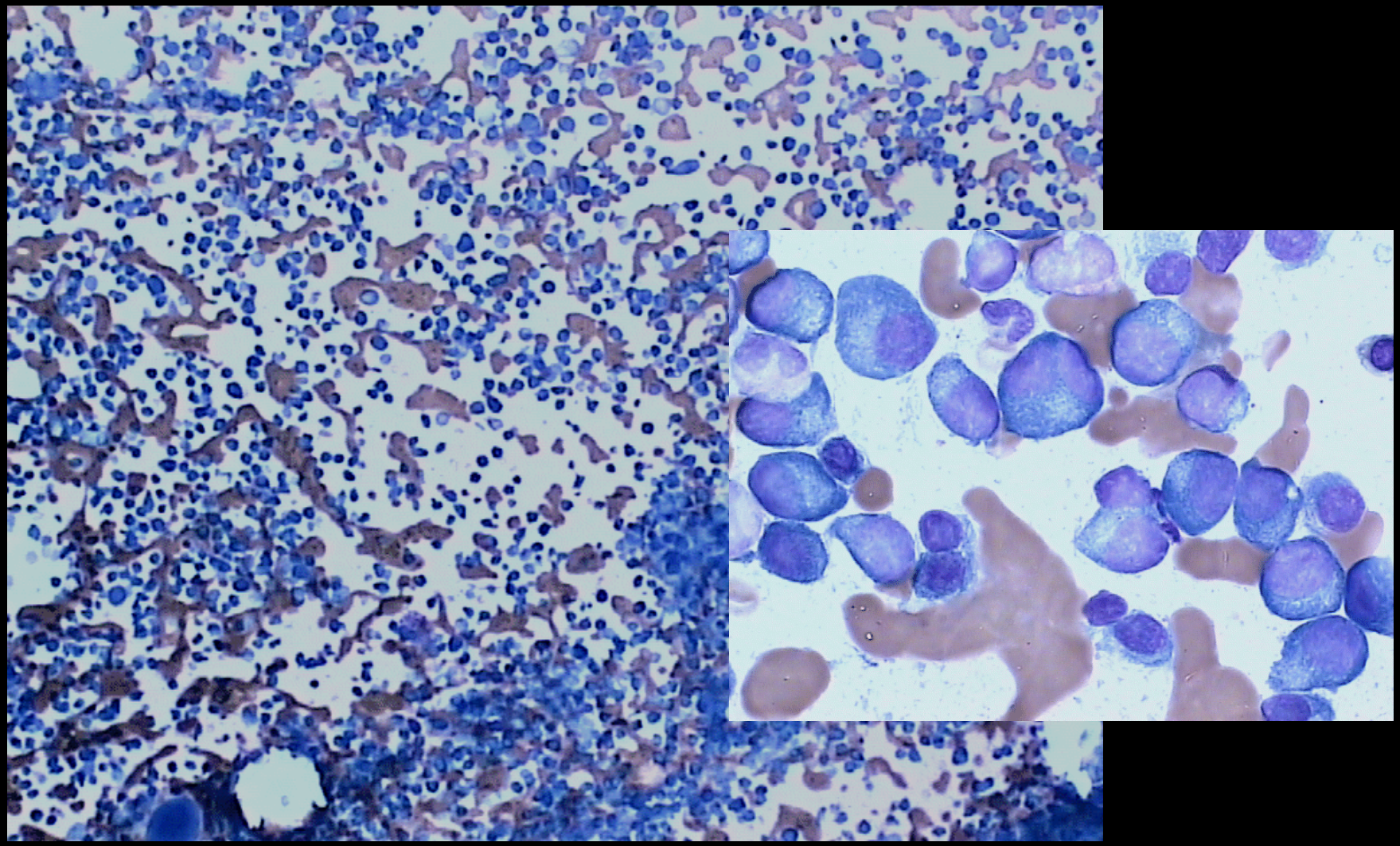
O'Connor & Barrans, HMDS (2005)

Identification of plasma cells



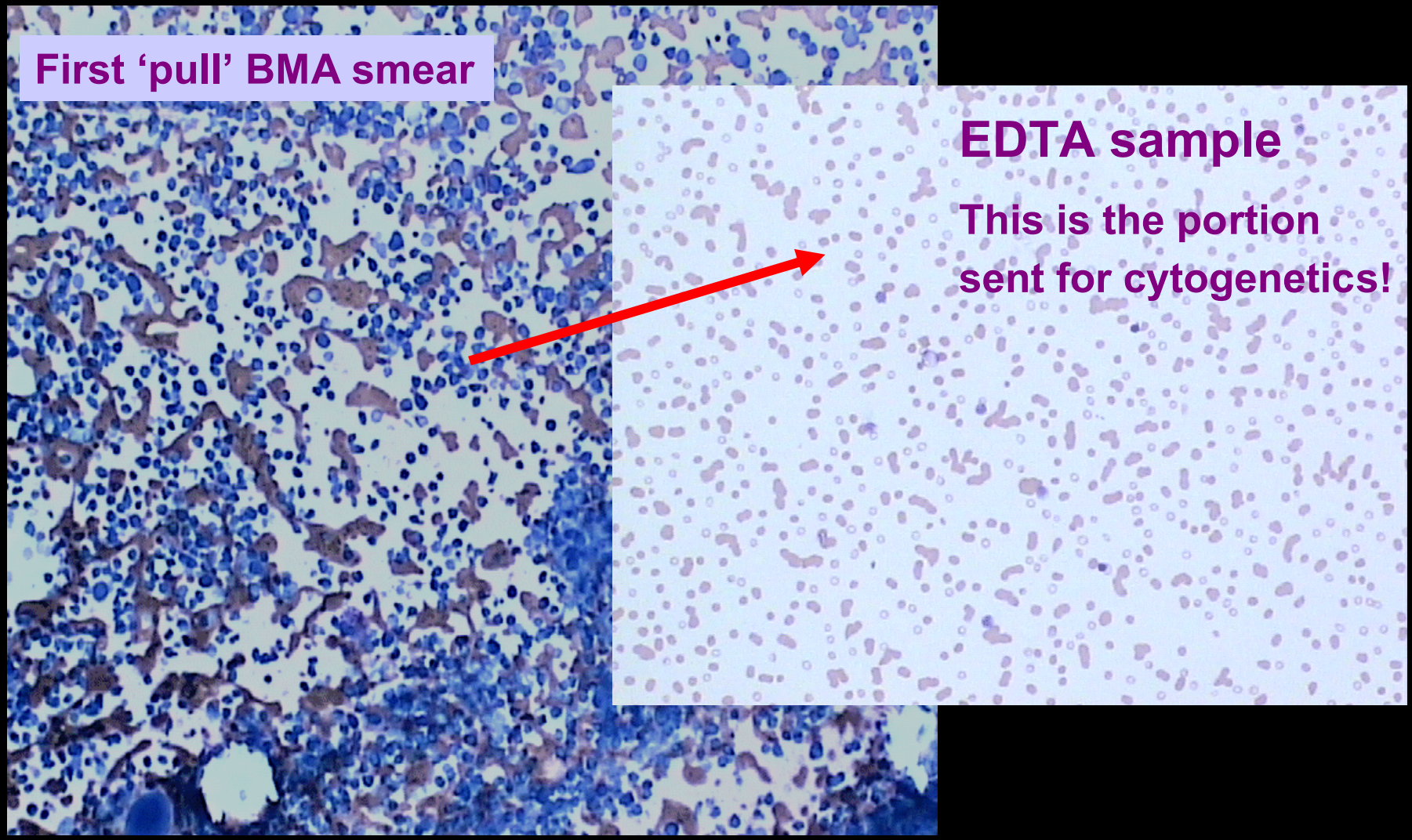
O'Connor & Barrans, HMDS (2005)

Quality of bone marrow!!



O'Connor & Barrans, HMDS (2005)

Quality of bone marrow!!

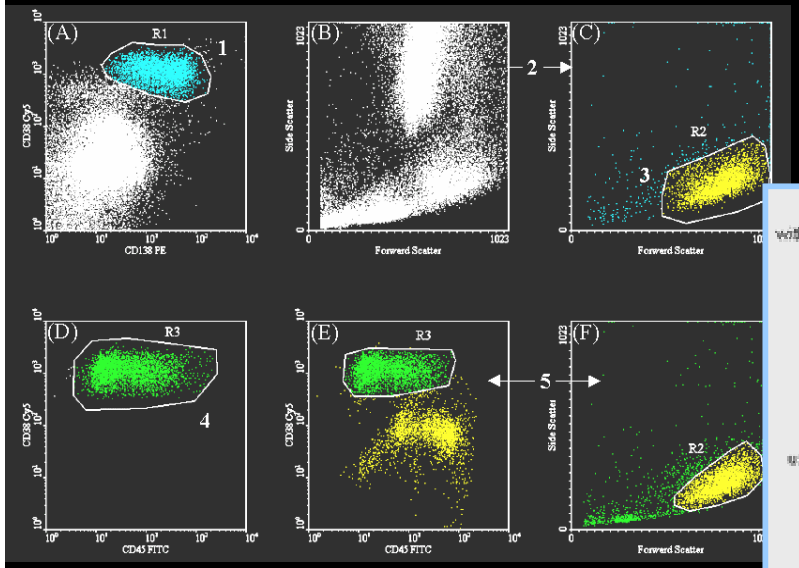


Both images are from same sample

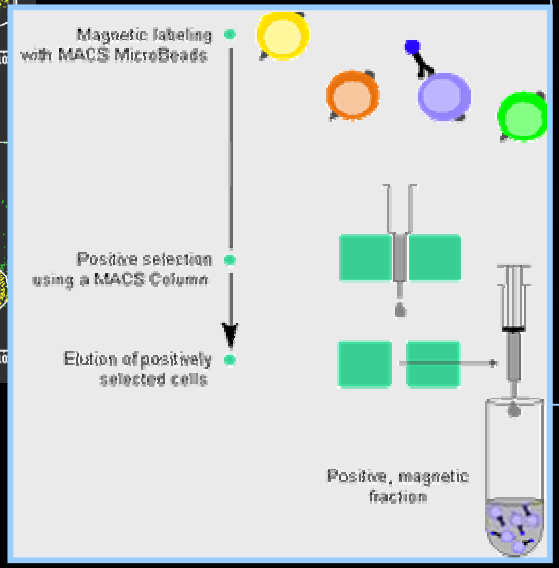
O’Connor & Barrans, HMDS (2005)

Methods – FISH on rare cells e.g.MGUS

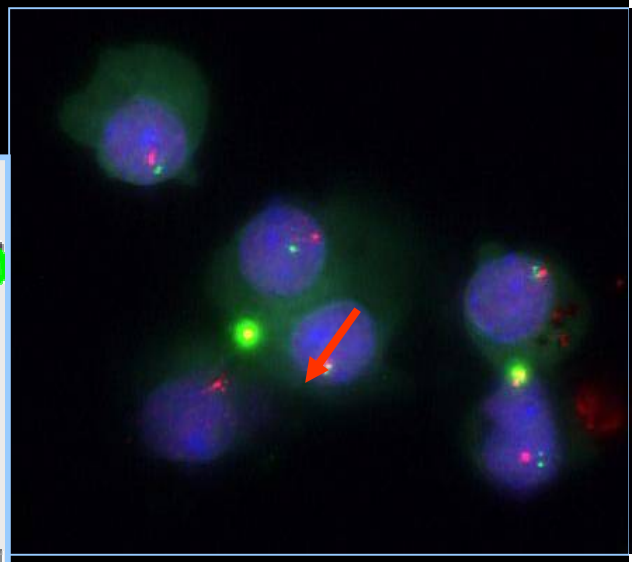
1. Flow cytometry



2. B cell selection



3. Interphase FISH



- Check purity of sort
- Cytospins ~ FISH
- Probe set 13q14/13q34
- Monosomy 13q14

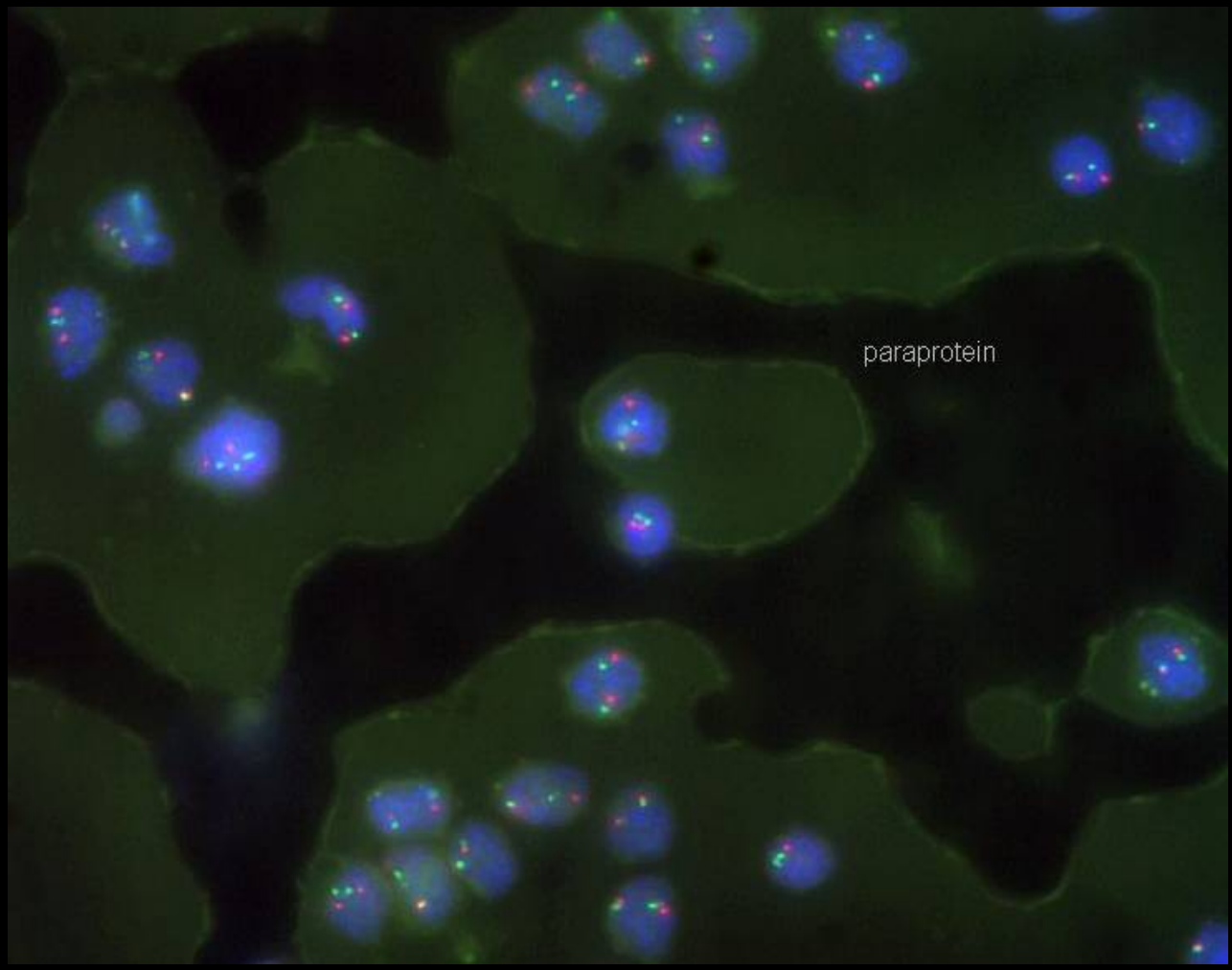
O'Connor & Barrans, HMDS (2005)

Interpretation of FISH: Artefacts

Paraprotein

- Plasma cell disorders
- May mask cellular morphology
- May block probe

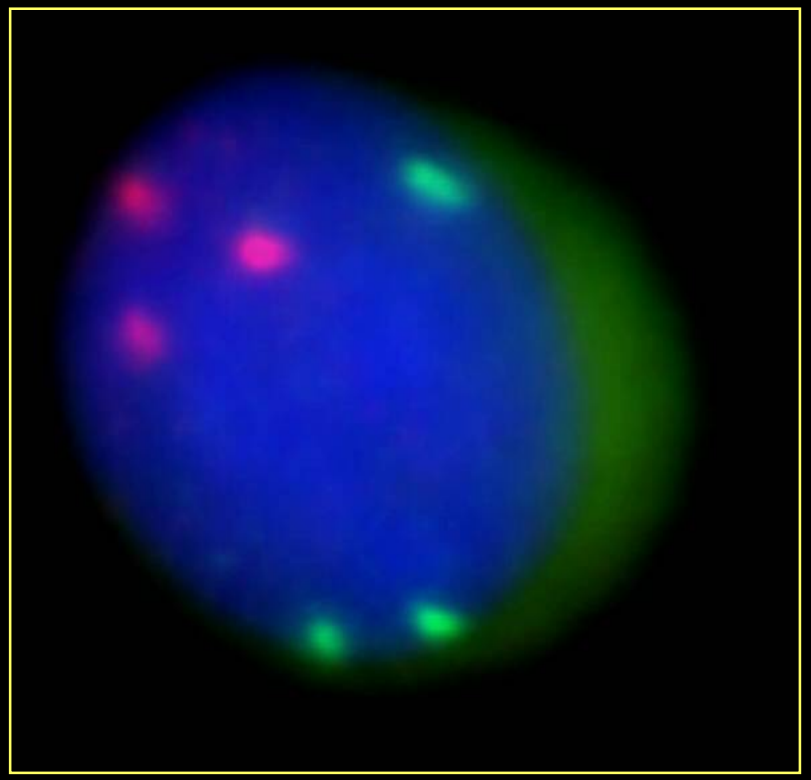
Use pre-treatment protocol



O'Connor & Barrans, HMDS (2005)

Aneuploidy is common in myeloma

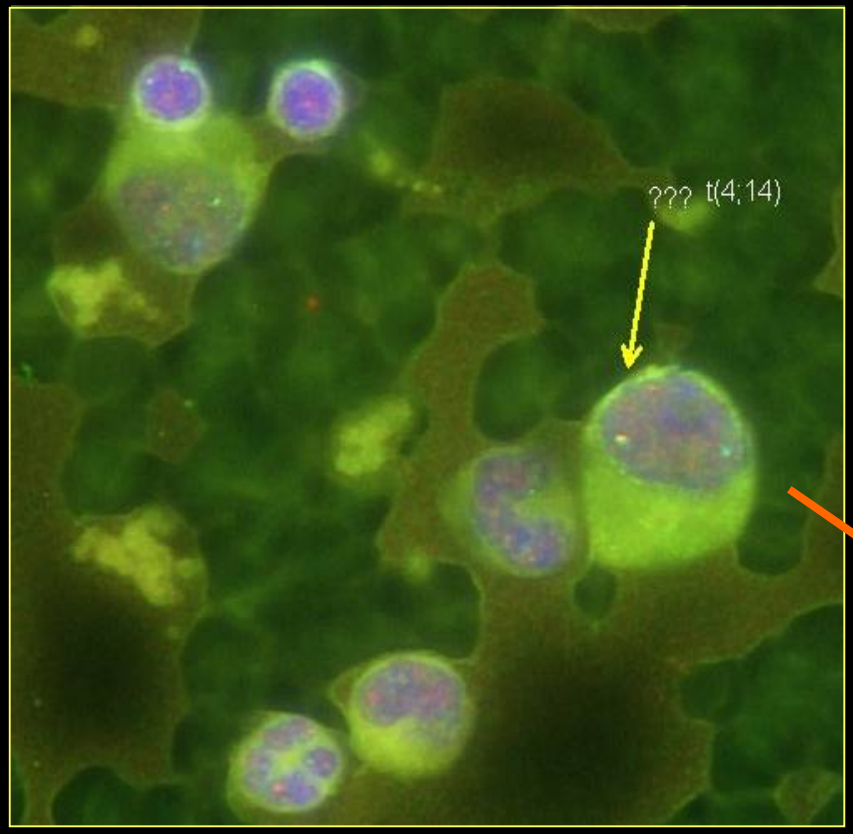
- Significance of aneuploidy is uncertain
- 2-colour FISH
  - Trisomy 3 ~ red
  - Trisomy 11 ~ green

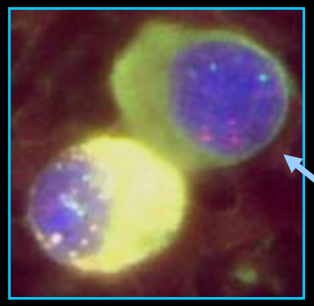


O'Connor & Barrans, HMDS (2005)

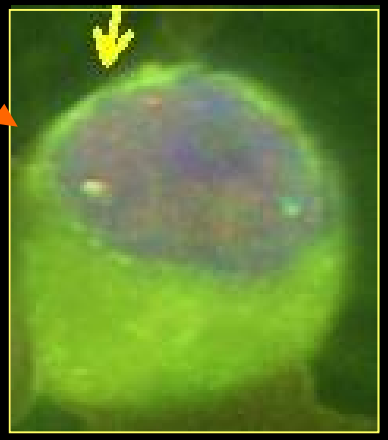
Myeloma ~ *FGFR3/IgH* probe set

- t(4;14) detected in an IgM Myeloma.
  - The *FGFR3* probe is not split therefore a single fusion is obtained (relatively insensitive FISH technique)



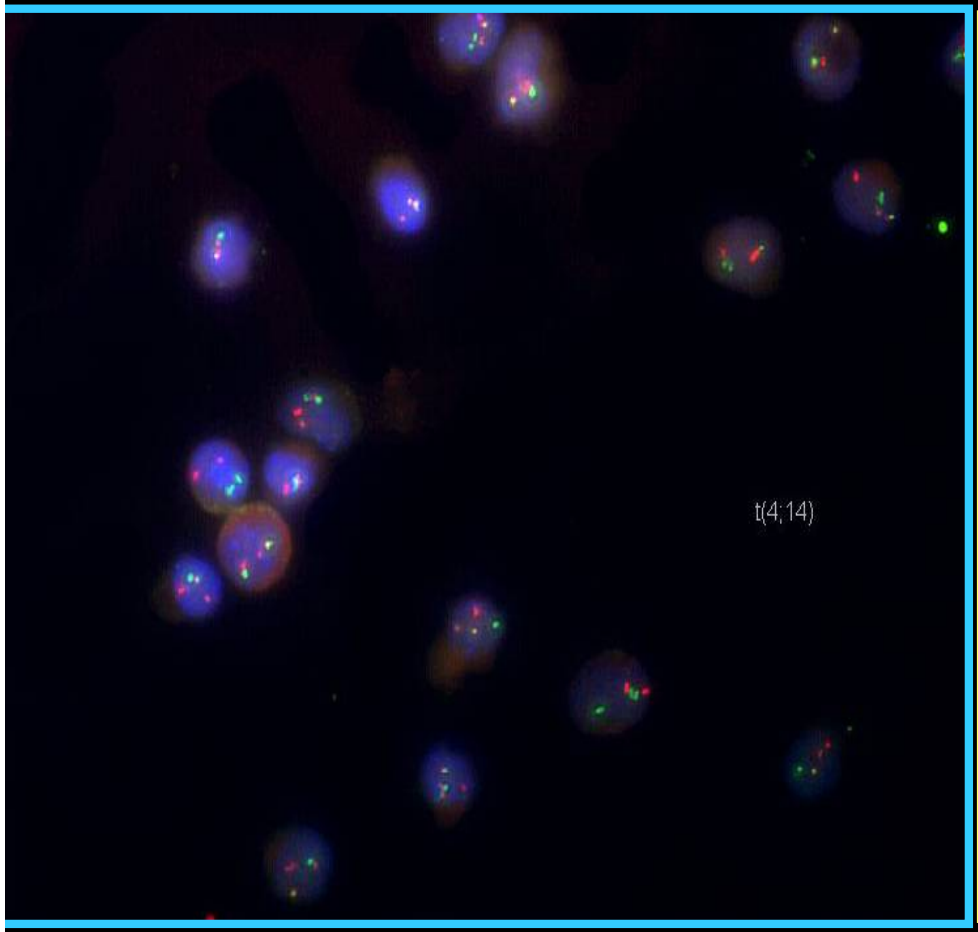


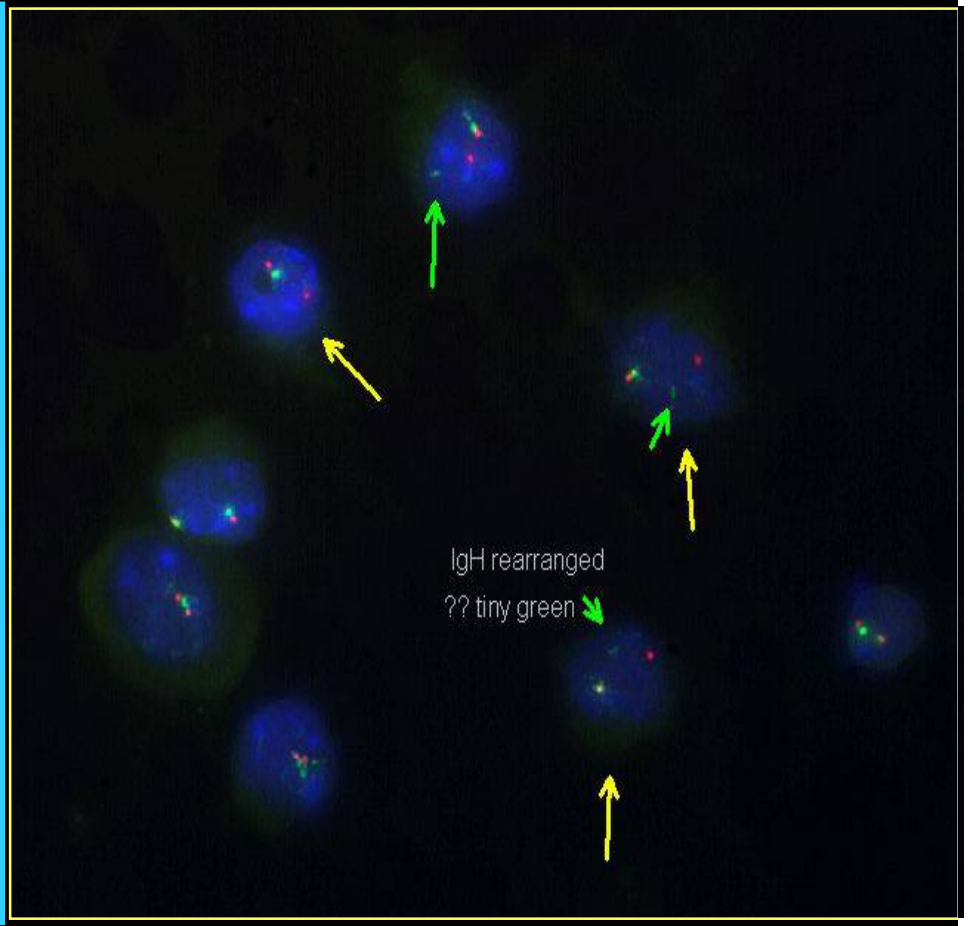
Plasma cells with a normal pattern for *FGFR3/IgH* were also seen. ? Normal PC or evidence of chimeric aberration



O'Connor & Barrans, HMDS (2005)

Myeloma cases: H1838/05



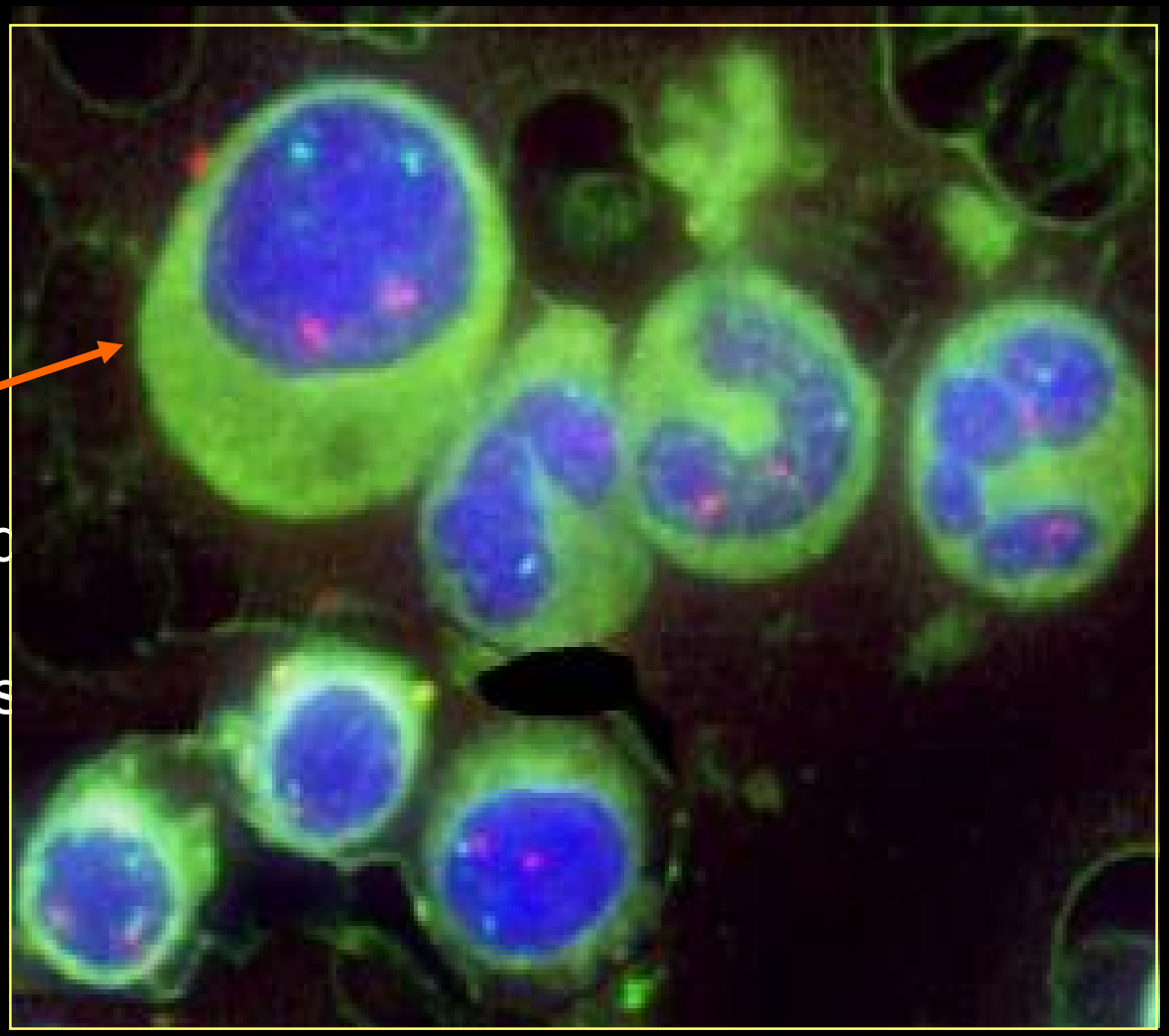


- 91% plasma cells (small) ~ t(4;14) positive
  - often unbalanced loss of Ig material

O'Connor & Barrans, HMDS (2005)

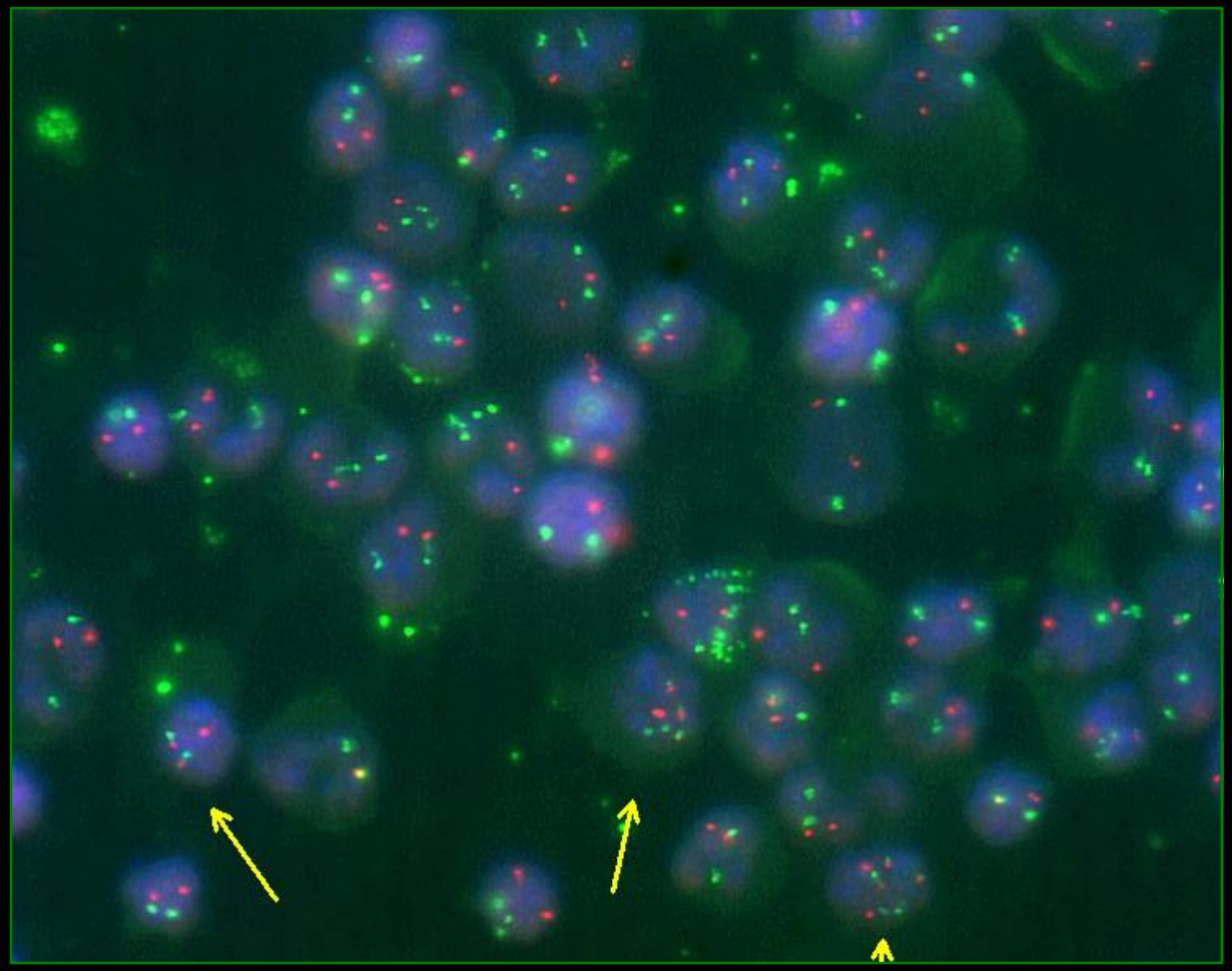
Myeloma ~ *BCL-1/IgH* probe set

- Normal pattern shown
  - Abnormal would be exactly the same pattern as for MCL i.e. 2 fusion signals.



O'Connor & Barrans, HMDS (2005)

Myeloma with t(11;14)

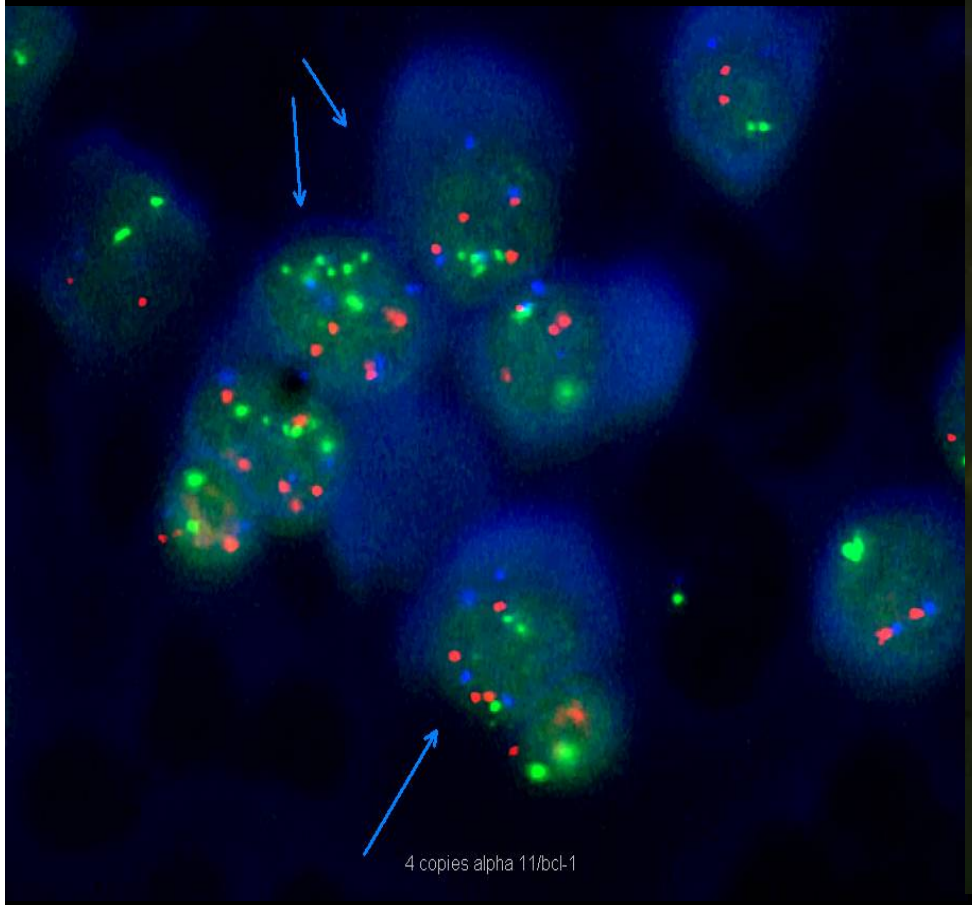


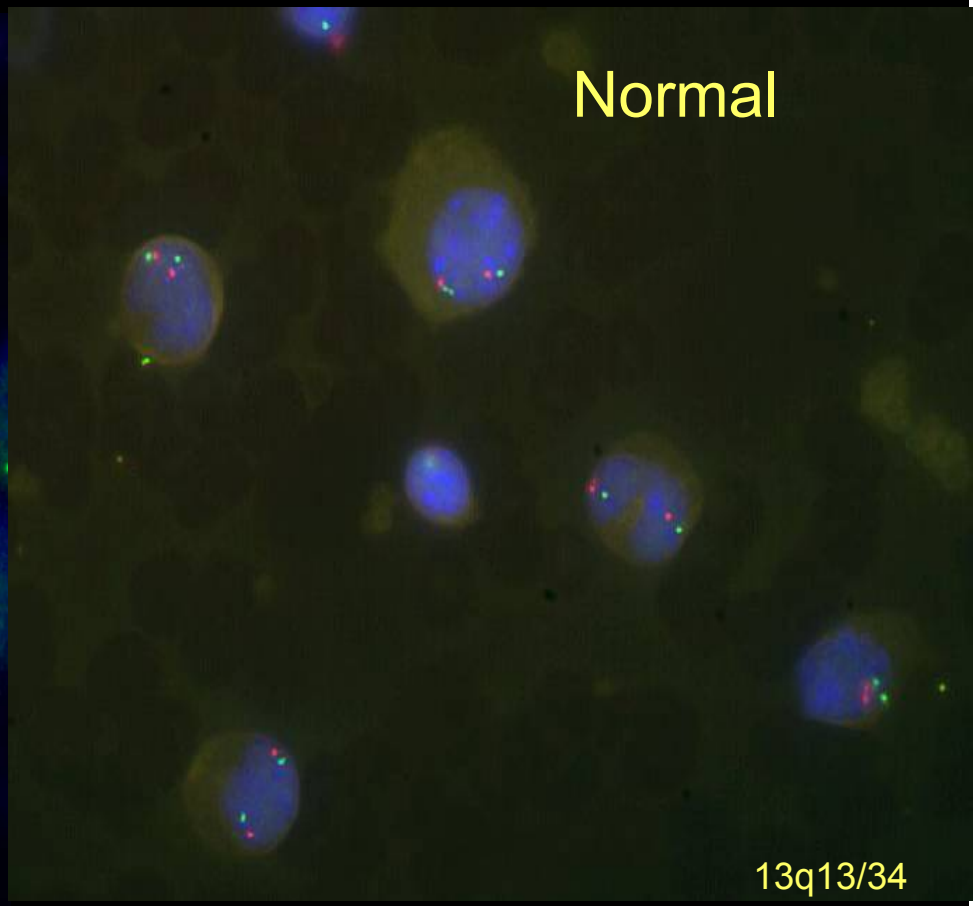
Majority of cells in image are normal

BUT plasma cells with the translocation can be identified.

O'Connor & Barrans, HMDS (2005)

Myeloma ~ *BCL-1/IgH* probe set

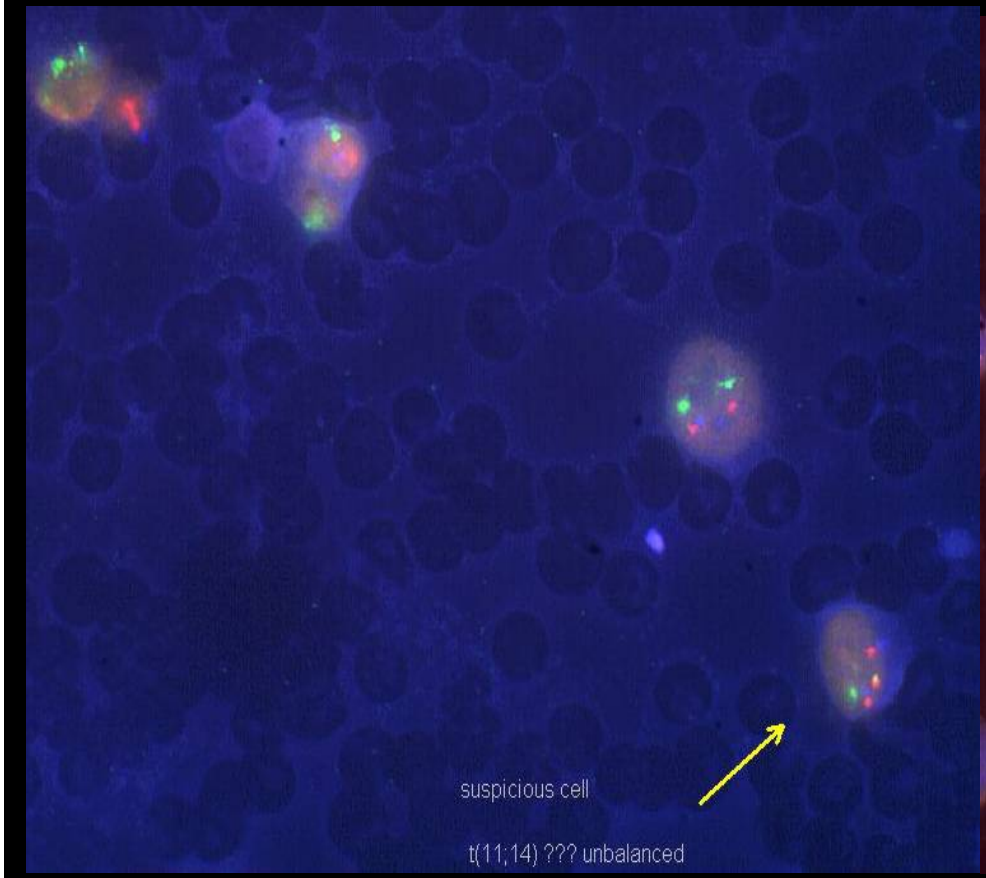


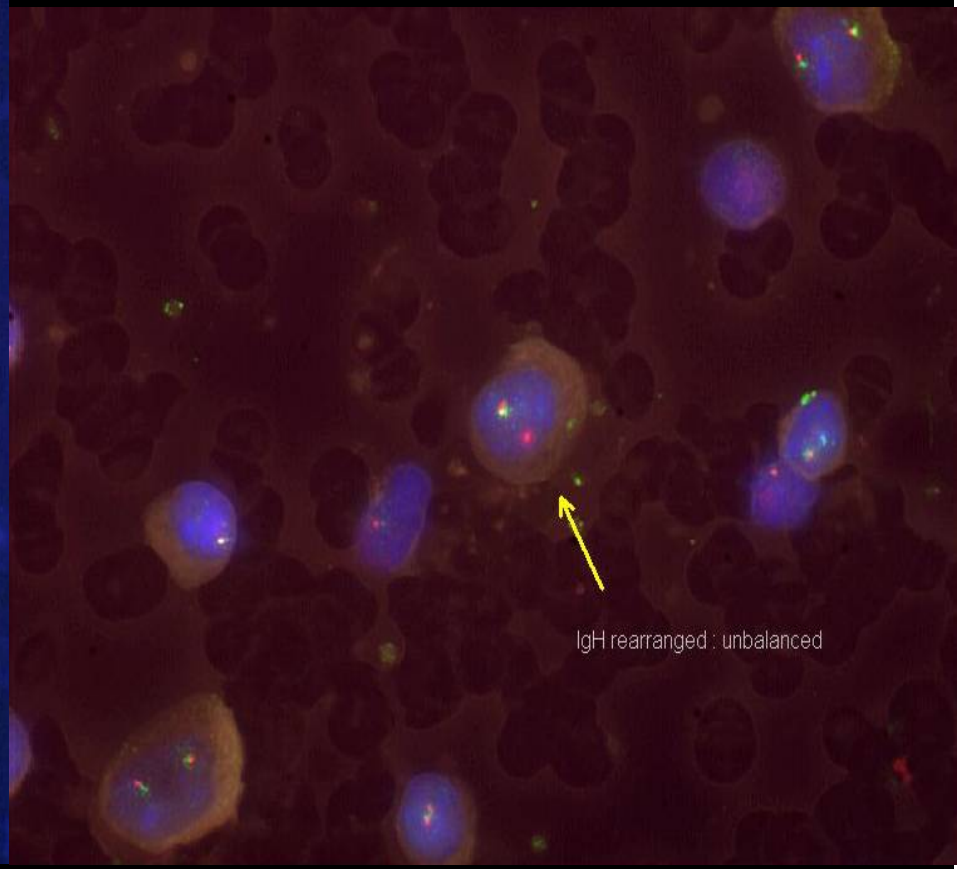


Multiple copies of probes

O'Connor & Barrans, HMDS (2005)

Myeloma case: H450/05

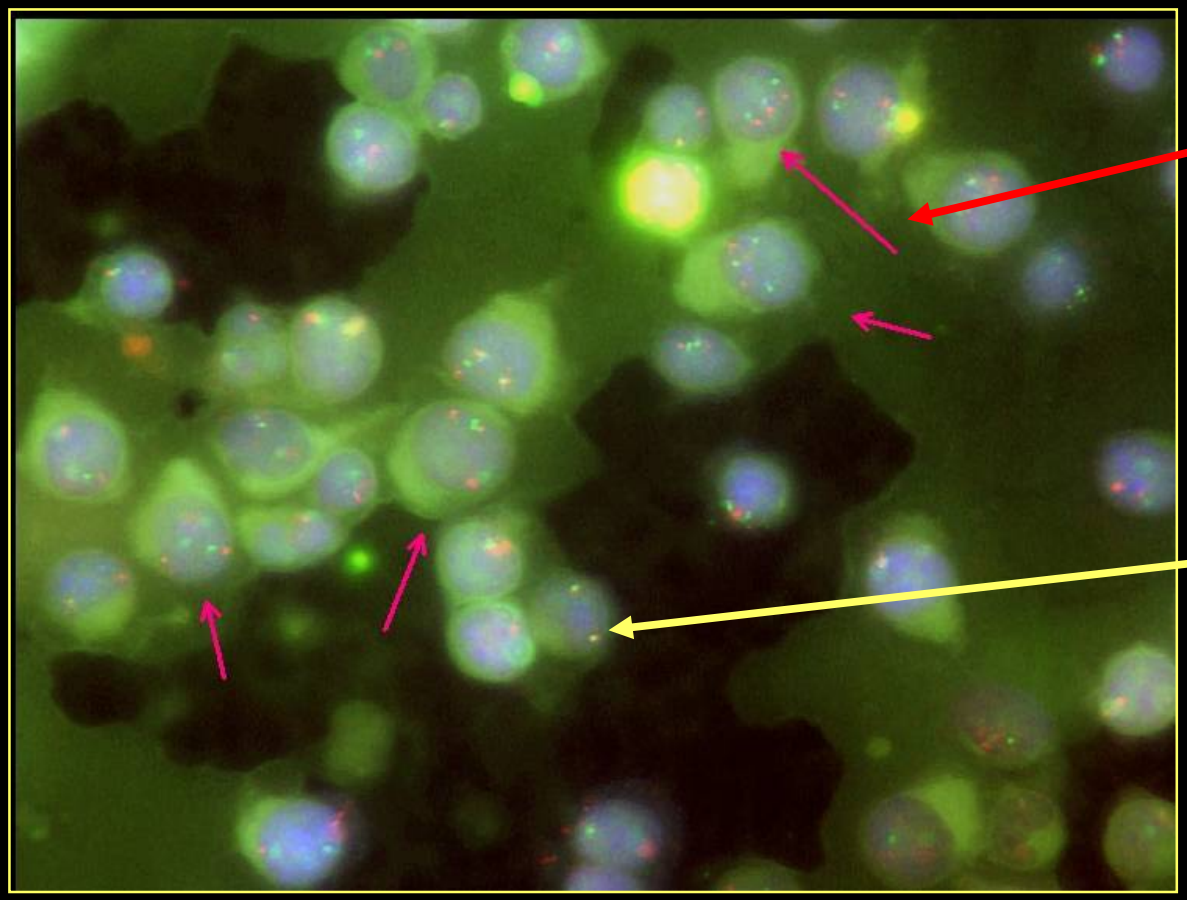




?? t(11;14) positive
odd pattern ~ 1 fusion, IgH unbalanced rearrangement

O'Connor & Barrans, HMDS (2005)

Myeloma ~ 13q14 & 13q34 probe set



Trisomy 13

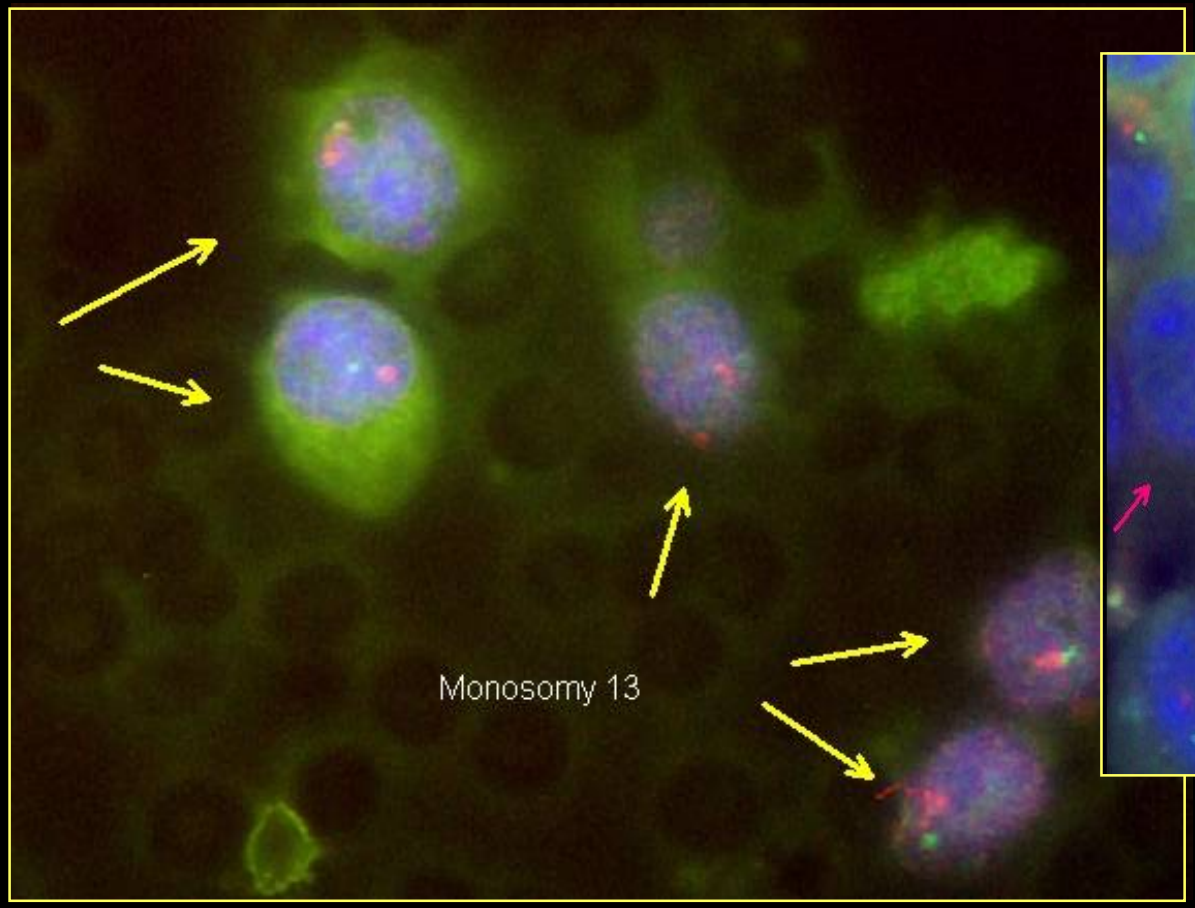
3R3G

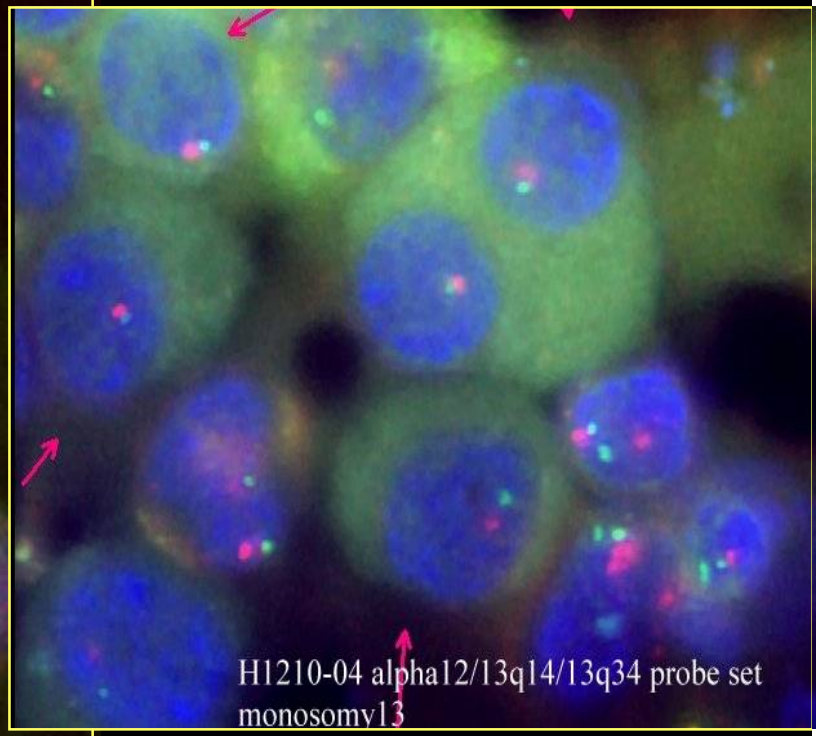
Cell with normal 13

2R2G

O'Connor & Barrans, HMDS (2005)

Myeloma ~ 13q14 & 13q34 probe set
Monosomy 13





Plasma cells with monosomy 13 (**1R1G** pattern). The binucleated plasma cell has **1R1G** in each nucleus. Occasional normal myeloid cells with **2R2G** pattern also present.

O'Connor & Barrans, HMDS (2005)

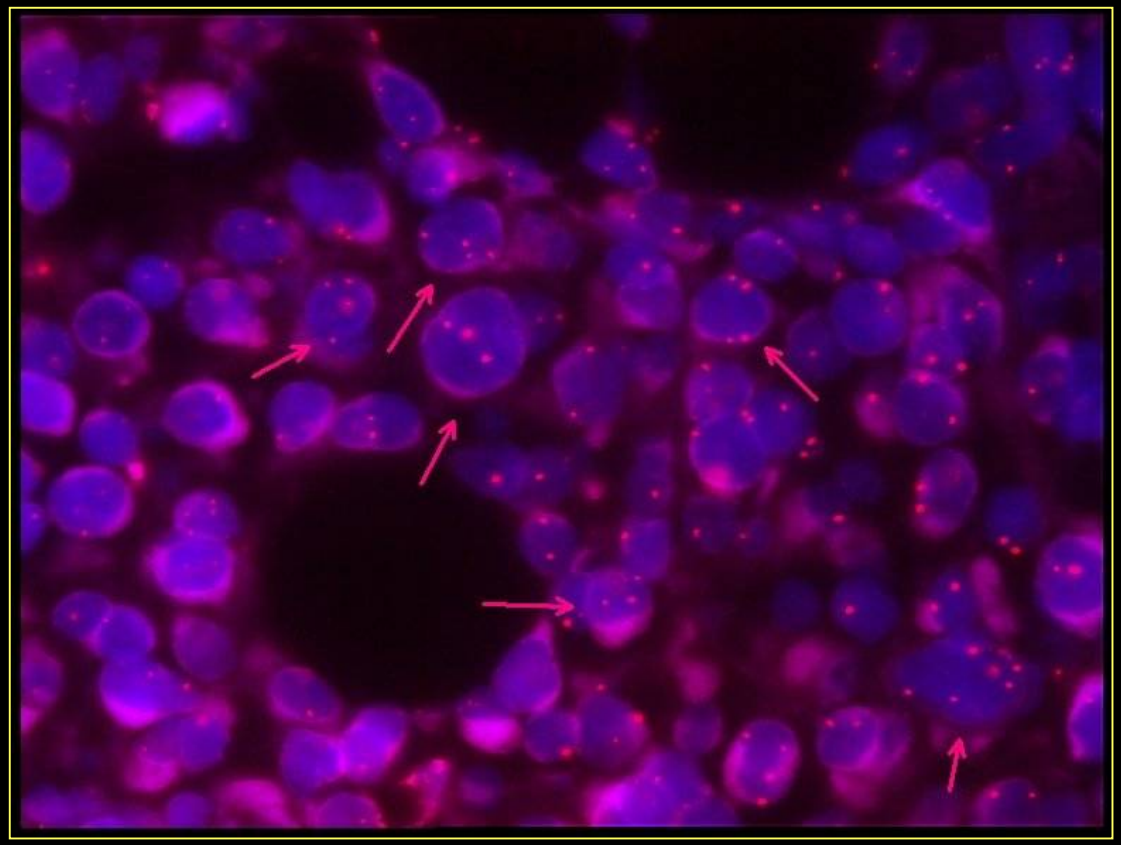
Summary – FISH in plasma cell disorders

Direct interphase FISH on bone marrow aspirate smears is rapid and relatively inexpensive and can be integrated into diagnostic laboratories……but

- Sample quality critical
  - use smears if plasma cells >10%
  - check MGG stain ~ cannot rely on flow % PC
- If plasma cells <10% may need cell selection
  - flow sorting ~ good for isolating different PC fractions
  - magnetic bead sorting ~ total PC population
- If cell selection is used in a diagnostic laboratory systems must be in place to identify and process samples rapidly…. ***DNA will degrade***

O'Connor & Barrans, HMDS (2005)

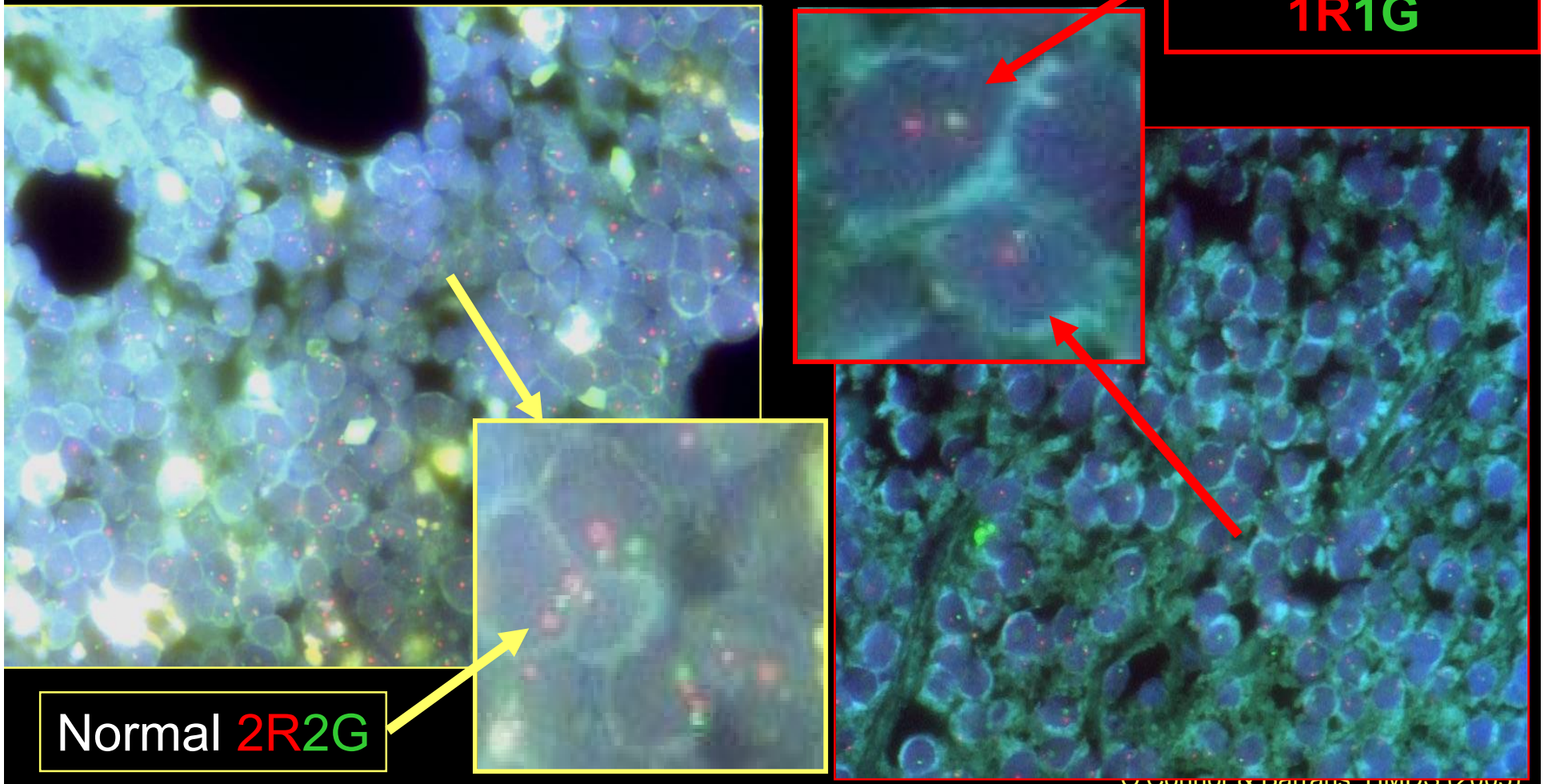
Plasmacytoma ~ Trisomy 11



O'Connor & Barrans, HMDS (2005)

Plasmacytoma ~ Monosomy 13

- 3μm thin sections cut from soft tissue plasmacytoma
- 13q14 red (critical region)
- 13q14 green (control probe)



B-CLL

O'Connor & Barrans, HMDS (2005)

Identification of CD5 positive B-LPDs for interphase FISH

- Clonal CD5+ B-cell population flow cytometry
  - PB, BMA and cell suspensions from fresh tissue samples
- Following histological diagnosis (incl panel of markers)
  - Where there was no material for flow or it was unrepresentative and FISH is required for confirmation of diagnosis

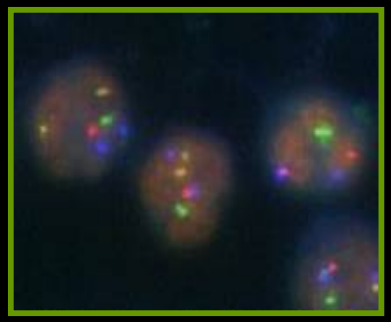
- Use of interphase FISH in differential diagnosis
  - Atypical t(11;14)
  - B-CLL vs MCL –
- Use of interphase FISH in predicting prognosis
  - **B-CLL favourable risk group**
    - Watch & Wait policy, may never require treatment
  - **B-CLL poor risk group**

O'Connor & Barrans, HMDS (2005)

WHO classification of MCL & B-CLL

Mantle cell lymphoma

- Monomorphic population of small to intermediate sized B lymphocytes
- Phenotype: sIg++/+++ (IgM & IgD), CD5+, CD19+, CD23-, CD20+
- BCL-1 expression /t(11;14)
- Blastic or large cell variant also recognised



B cell chronic lymphocytic leukaemia

- Monomorphic population of small B lymphocytes with clumped heterochromatin
- Phenotype: sIg+wk (IgM & IgD), CD5+, CD19+, CD23+, CD20+wk
- Pseudo-follicle formation in lymph node biopsies

O'Connor & Barrans, HMDS (2005)

B-cell chronic lymphocytic leukaemia

- B-CLL is a clinically heterogeneous disease
  - Biological parameters can segregate patients into risk-groups.
    - Morphology
    - immunophenotype
      - **Zap-70**
      - **CD38**
    - Cytogenetics
      - **13q14 deletion**
      - **11q23 (ATM) deletion**
      - **17p13 (p53) deletion**
      - **trisomy 12**
    - Ig V_H gene status
      - **germline (<2%)**
      - **mutated (>2%)**
- V_H gene status, cytogenetics and Binet clinical stage are independent prognostic markers.

O'Connor & Barrans, HMDS (2005)

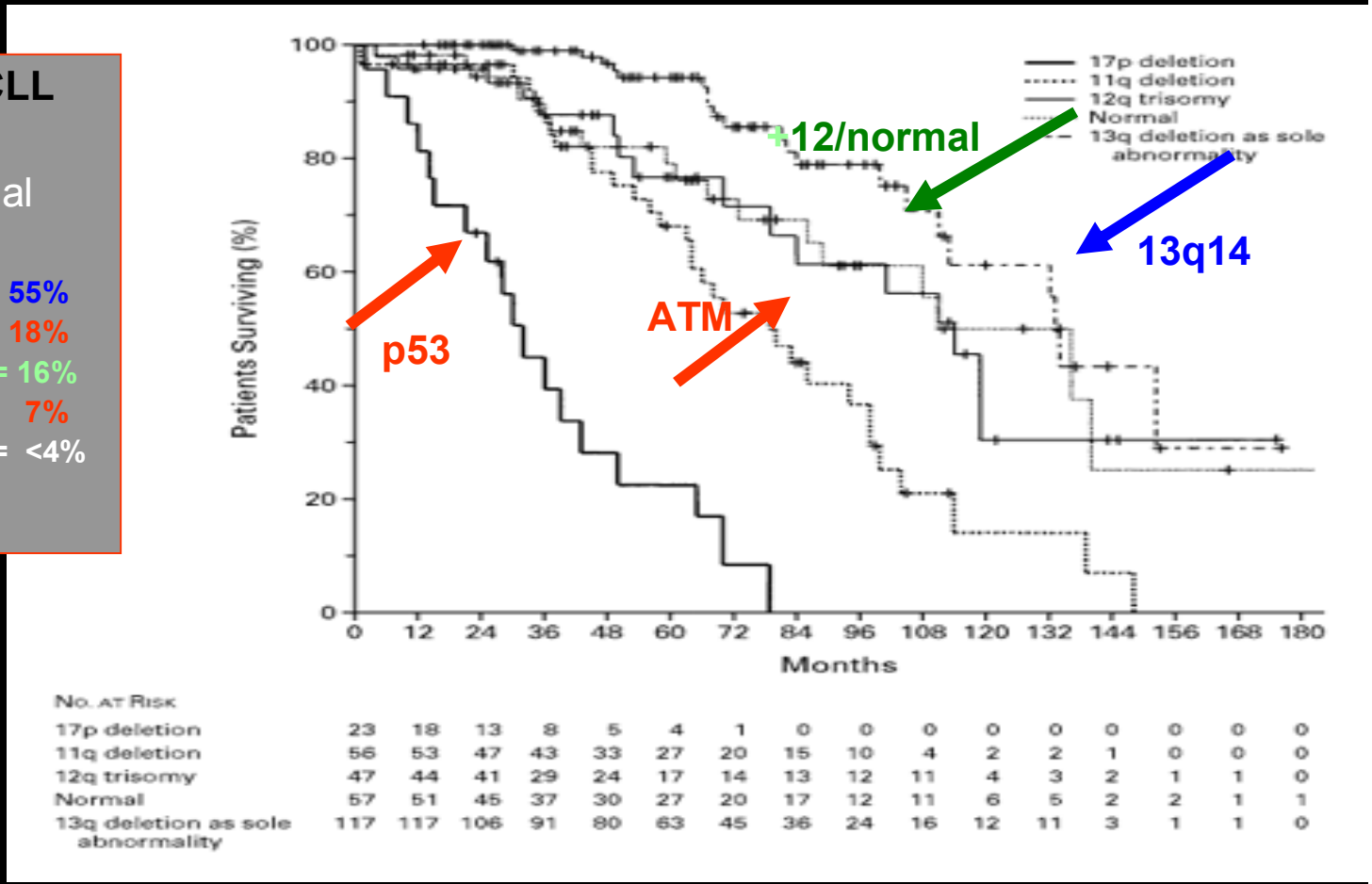
Cytogenetics of B-CLL

Dohner et al. *N Engl J Med.* 2000;343:1910-1916

Cytogenetics of B-CLL

HMDS >70% abnormal

13q14 deletion	= 55%
11q23 deletion	= 18%
Trisomy 12	= 16%
17p13 deletion	= 7%
t(14q32)	= <4%

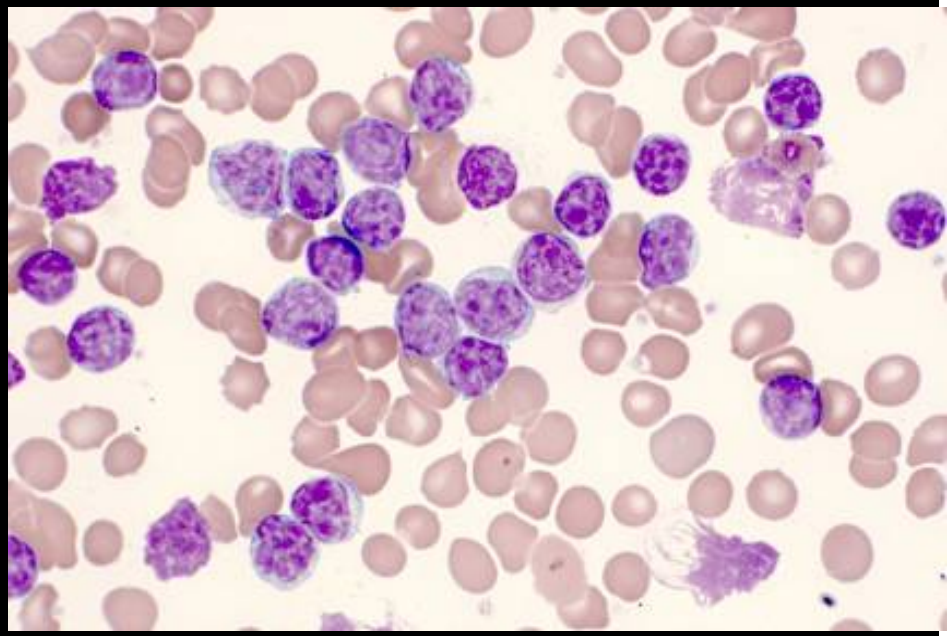


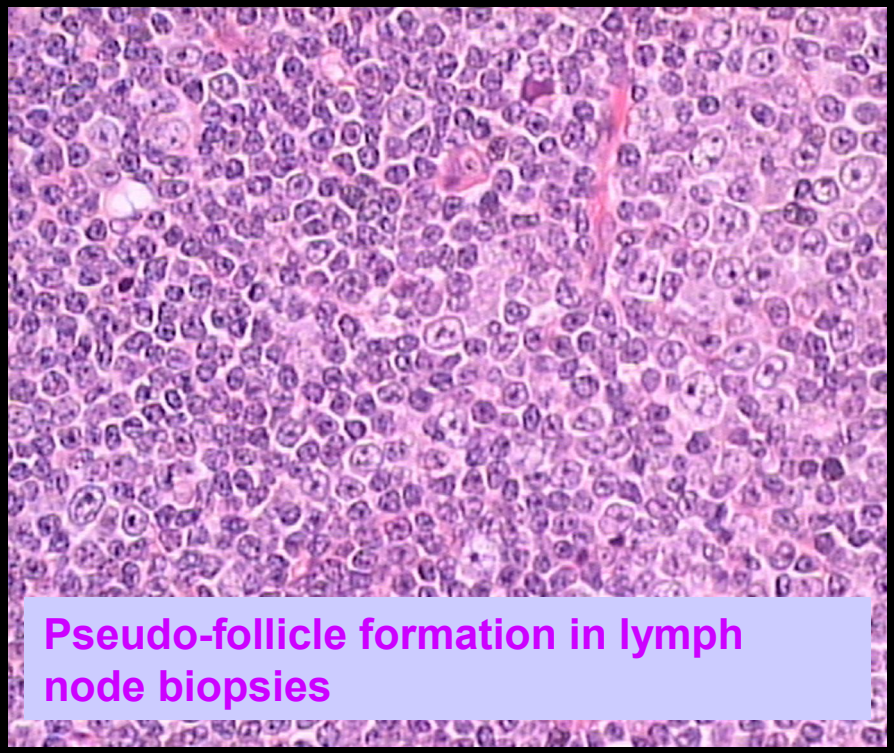
O'Connor & Barrans, HMDS (2005)

Typical B-CLL ~ favourable risk

- Monomorphic population of small B lymphocytes with clumped heterochromatin

- Phenotype:
  - sIg+wk (IgM & IgD), CD5+, CD19+, CD23+, CD20+wk





Pseudo-follicle formation in lymph node biopsies

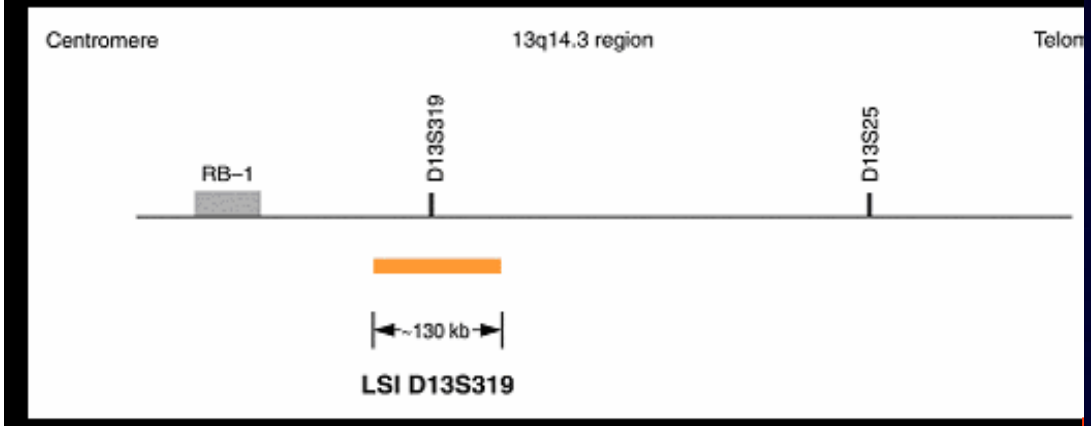
Favourable risk is defined as:

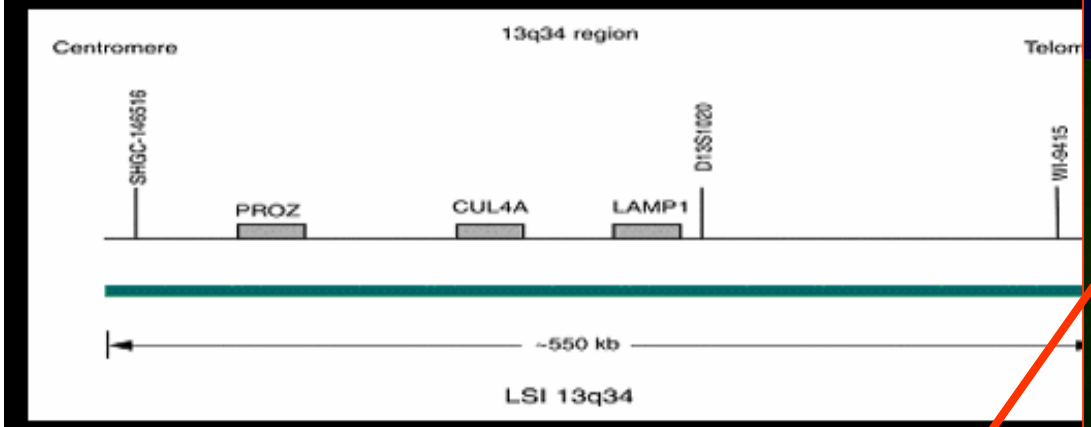
- Binet stage A
- typical morphology
- typical phenotype
- lack CD38 and Zap-70
- Isolated mono-allelic 13q14 deletion

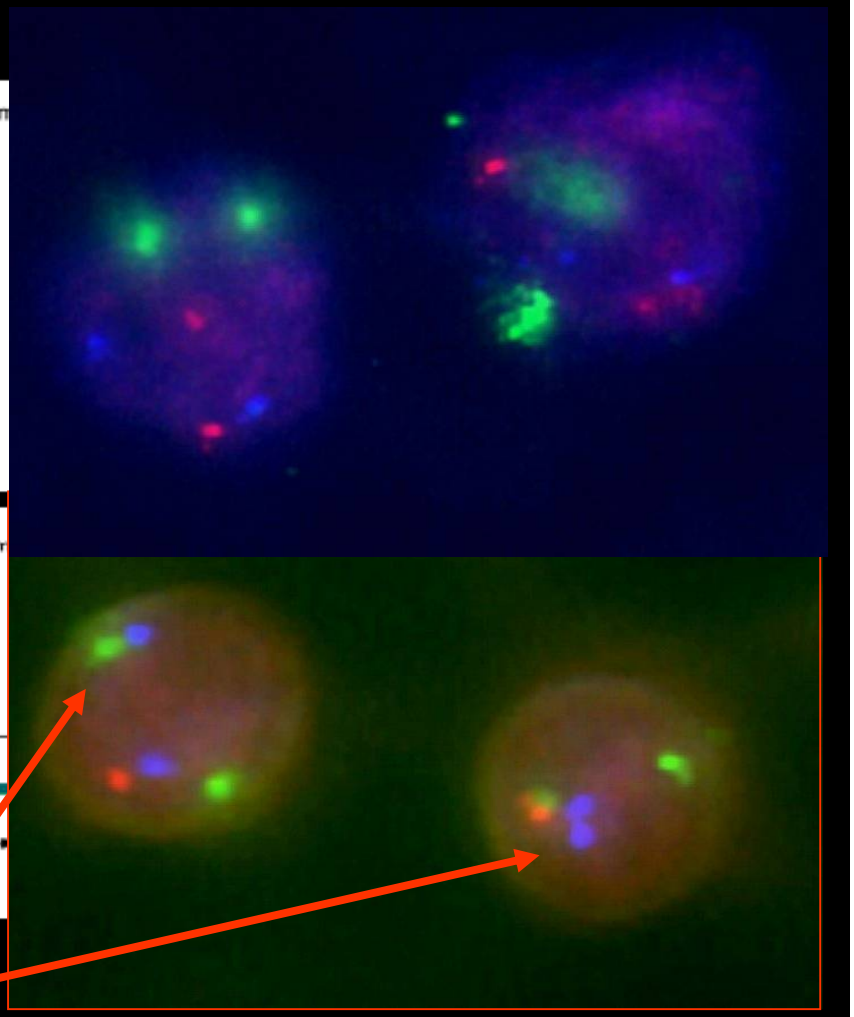
O'Connor & Barrans, HMDS (2005)

Typical B-CLL ~ 13q14 FISH

LSI D13S319 / LSI 13q34 / CEP® 12 Multi-color Probe Sets





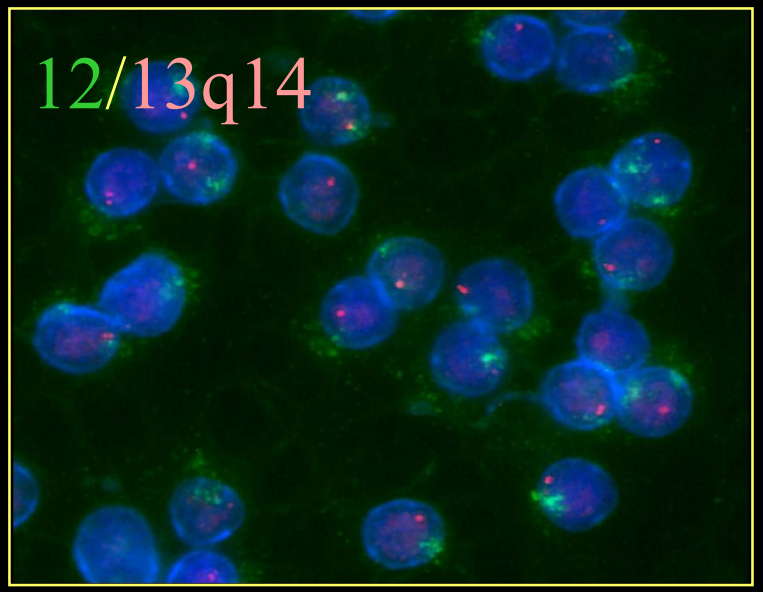


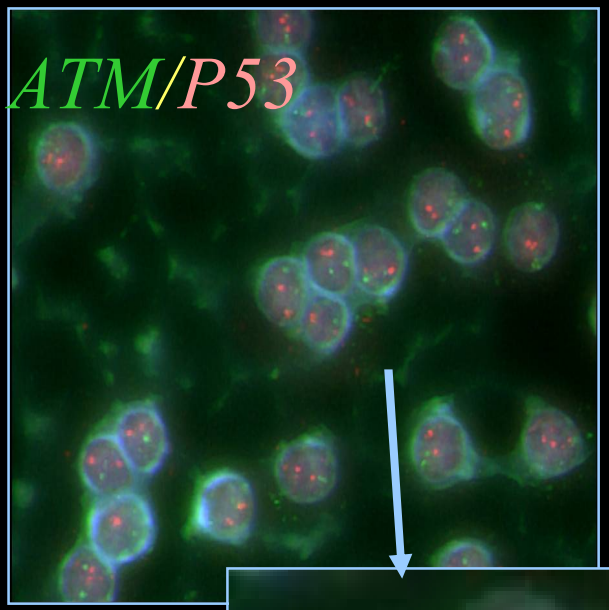
Mono-allelic 13q14 deletion
(top image = normal control)

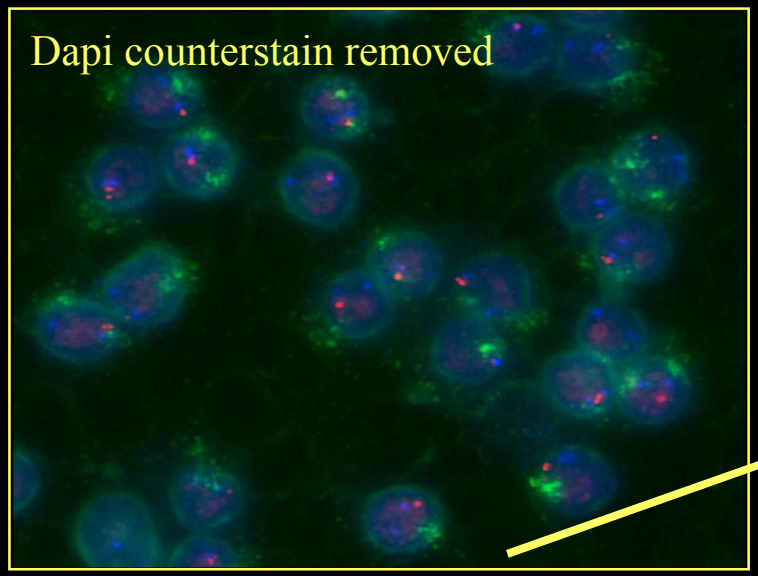
O'Connor & Barrans, HMDS (2005)

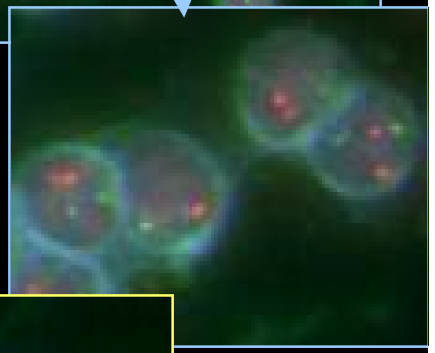
Typical B-CLL with deletion 13q14

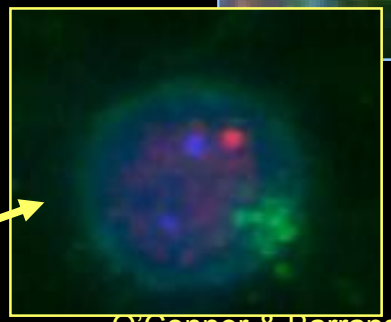
- Isolated 13q14 deletion are most frequently found in typical B-CLL cases.
- Good prognosis.
- Vysis probe set
  - 13q14 red
  - 13q34 aqua
  - Alpha 12 green





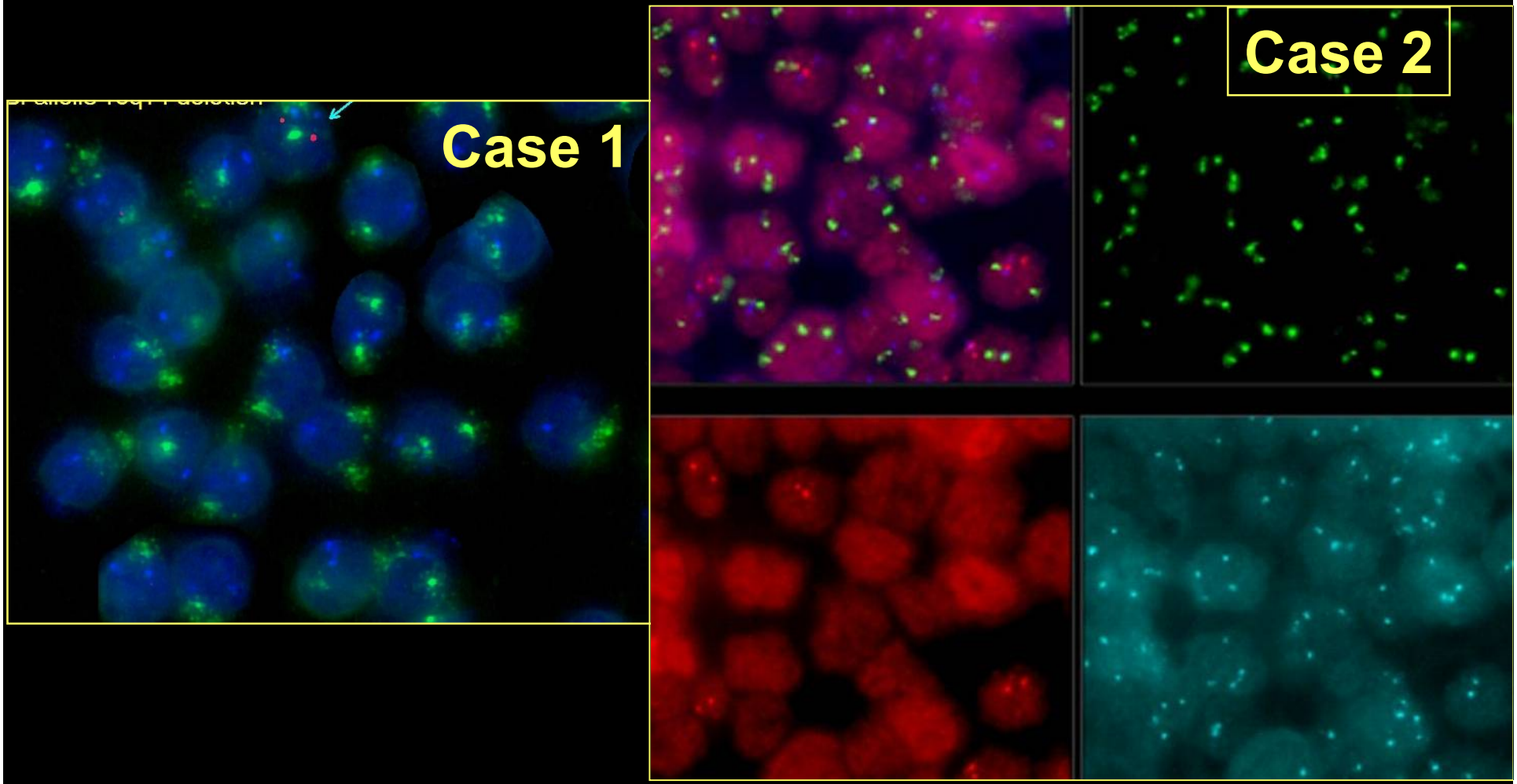






O'Connor & Barrans, HMDS (2005)

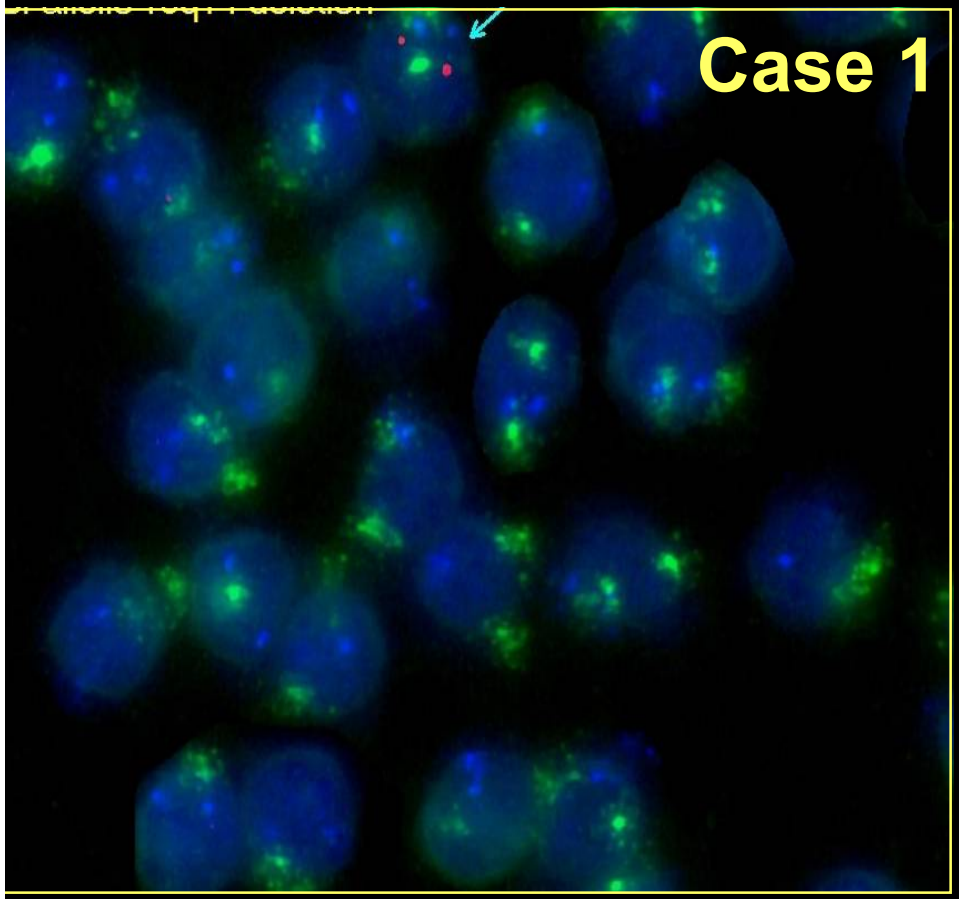
B-CLL with bi-allelic deletion 13q14

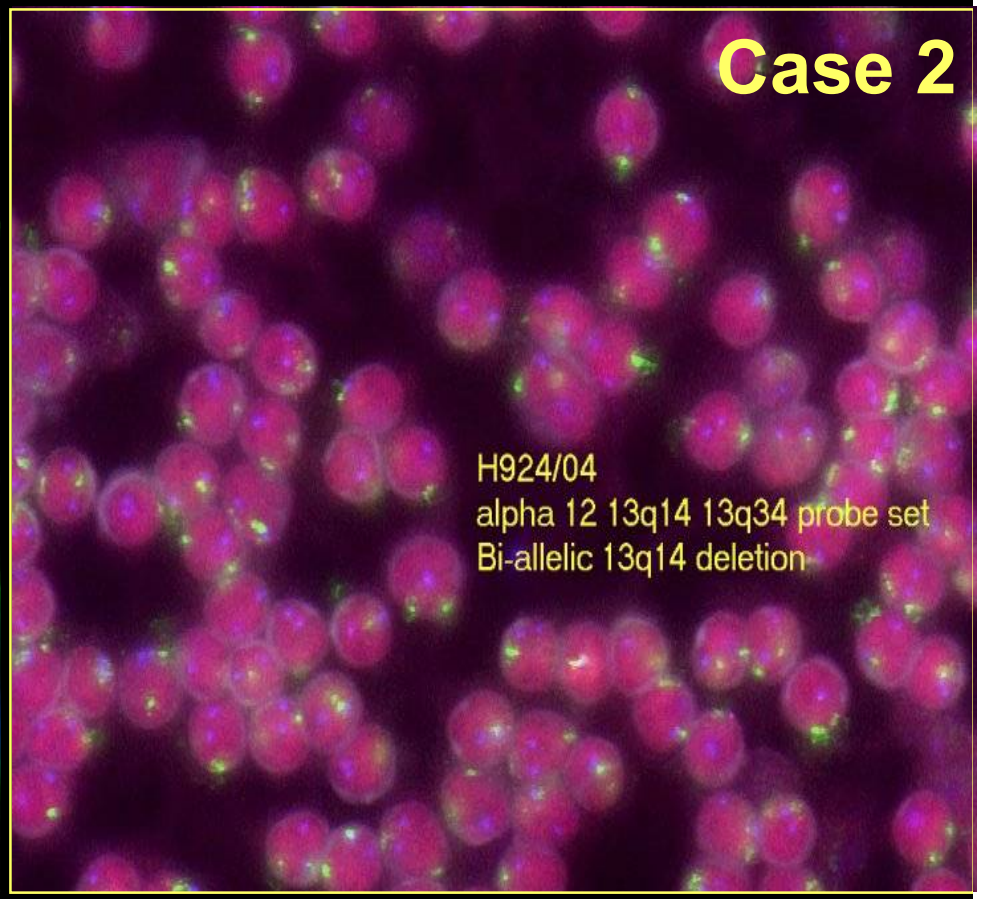


- Detected in ~ **3%-5%** of B-CLL cases
- Loss of the second 13q14 allele should probably be regarded as a second 'hit'
  - Higher incidence of associated ATM and p53 deletions in this group
  - Probably associated with disease progression, therefore poor prognosis.

O'Connor & Barrans, HMDS (2005)

B-CLL with bi-allelic deletion 13q14



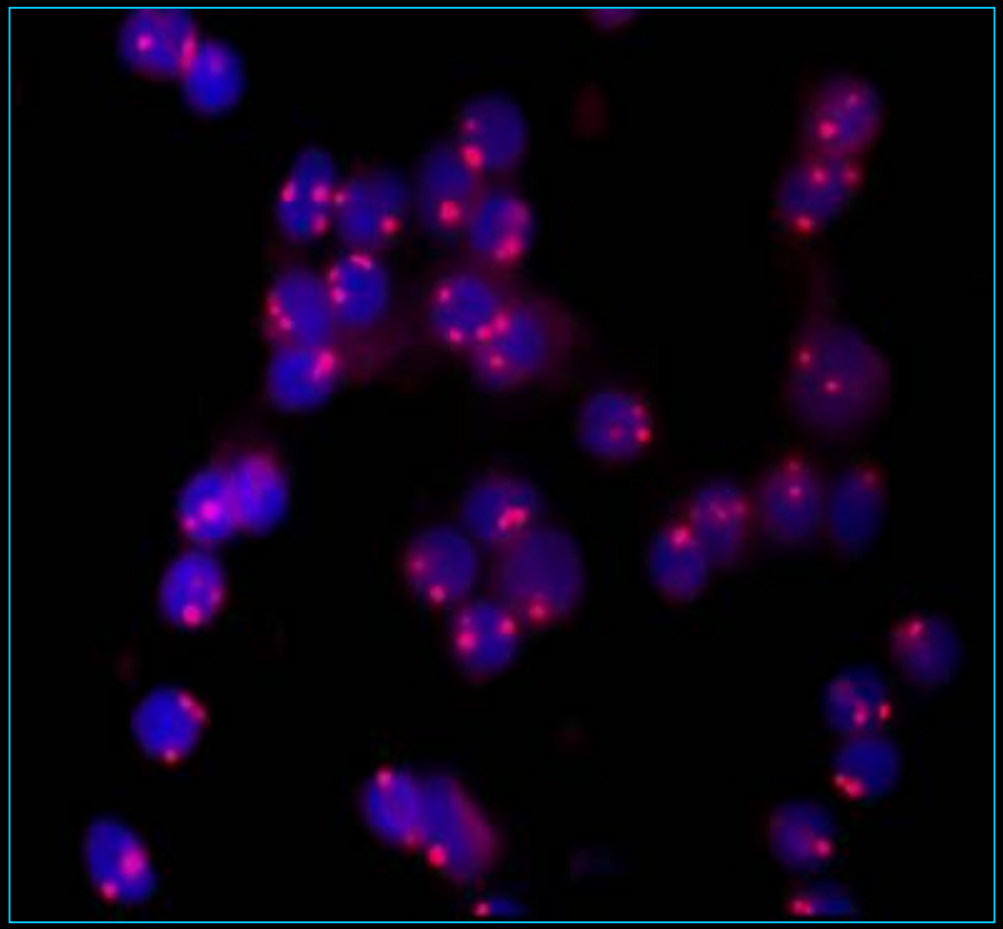


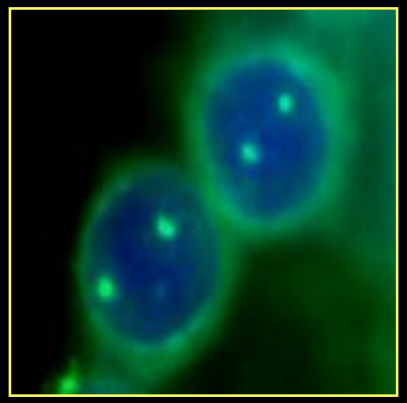
Bi-allelic 13q14

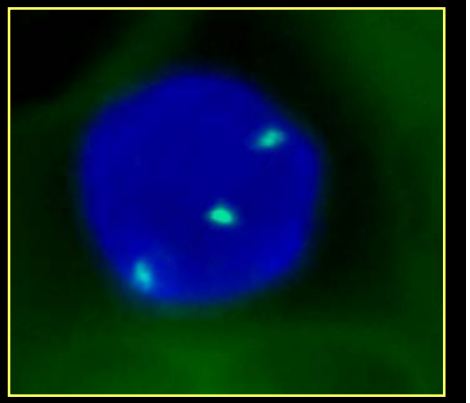
(Vysis Probe set - α12, 13q14, 13q34)
abnormal pattern 2G1R2B

O'Connor & Barrans, HMDS (2005)

Interphase FISH for trisomy 12







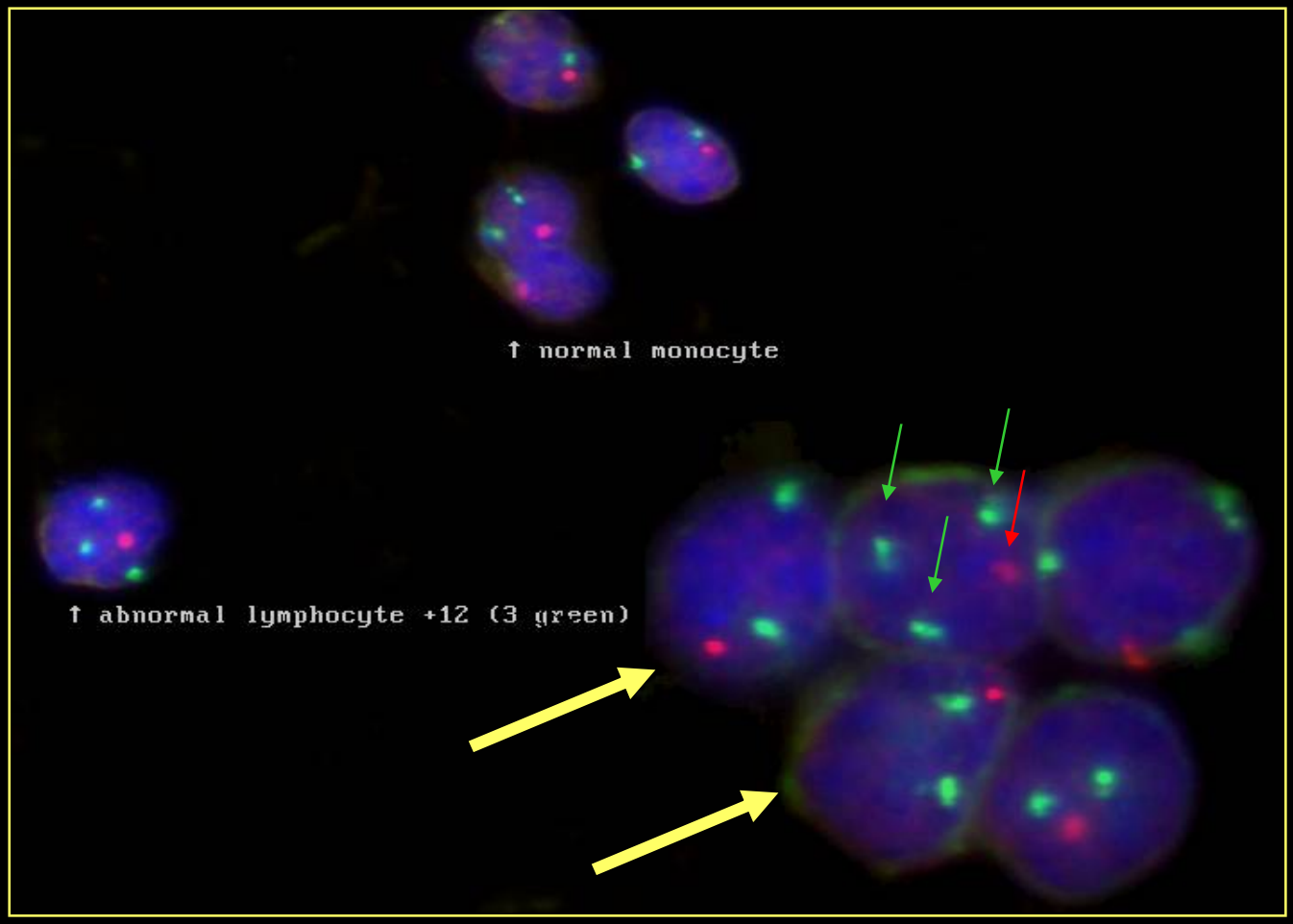
- Probes can be labelled with different labels for maximum flexibility.
- Trisomy 12 identified by the presence of 3 spots.

O'Connor & Barrans, HMDS (2005)

B-CLL with combined trisomy 12 & deletion 13q14

α sat 12 green

13q14 red
(in-house probes)



O'Connor & Barrans, HMDS (2005)

Demonstration of the genotypic relationship between CLUS and clinical B-CLL

- CLUS affects **3.5%** of adults over 40.

  Phenotypically indistinguishable from good-prognosis B-CLL

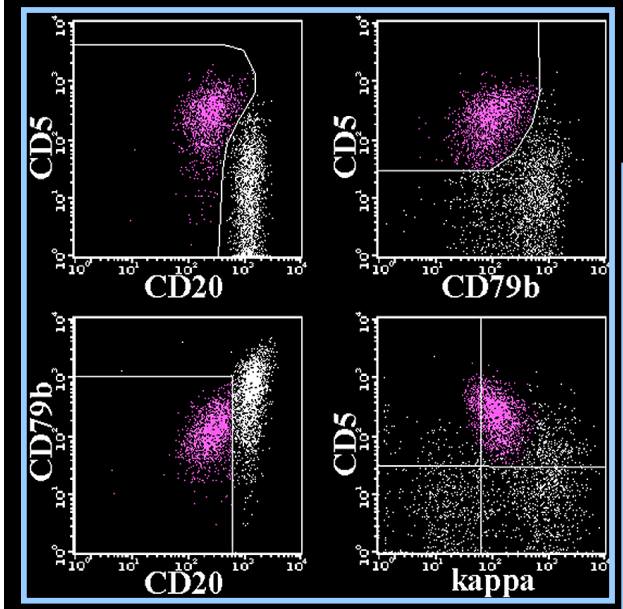
  - CD5/CD23 co-expression
  - weak CD20/ CD79b/CD22/sIg expression
  - absence of CD38 expression

- Independent studies have demonstrated a significantly increased relative risk of CLUS in healthy relatives from B-CLL families compared to the general population, confirming a clinical association between CLUS and B-CLL.

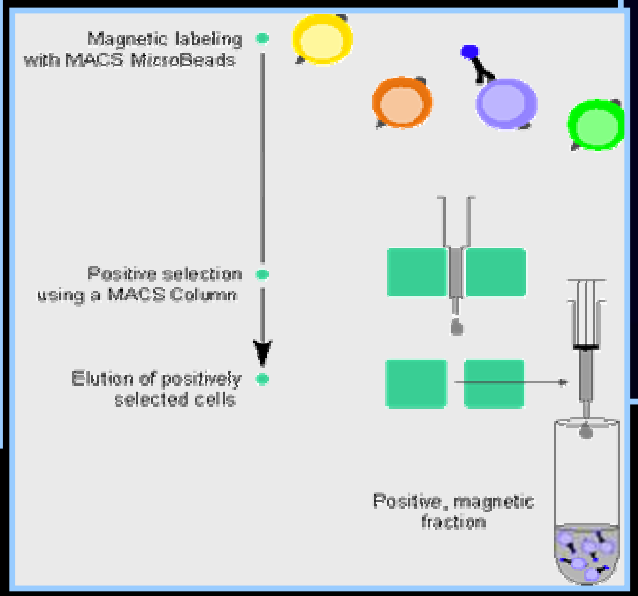
Reference. "Monoclonal B lymphocytes with the characteristics of "indolent" chronic lymphocytic leukemia are present in 3.5% of adults with normal blood counts'. Andy C. Rawstron, Michael J. Green, Anita Kuzmicki, Ben Kennedy, James A. L. Fenton, Paul A. S. Evans, Sheila J. M. O'Connor, Stephen J. Richards, Gareth J. Morgan, Andrew S. Jack, and Peter Hillmen.
Blood, 15 July 2002, Vol. 100, No. 2, pp. 635-639

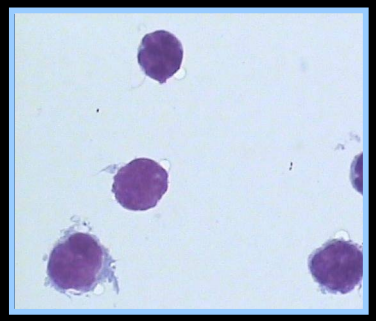
Methods – FISH on rare cells

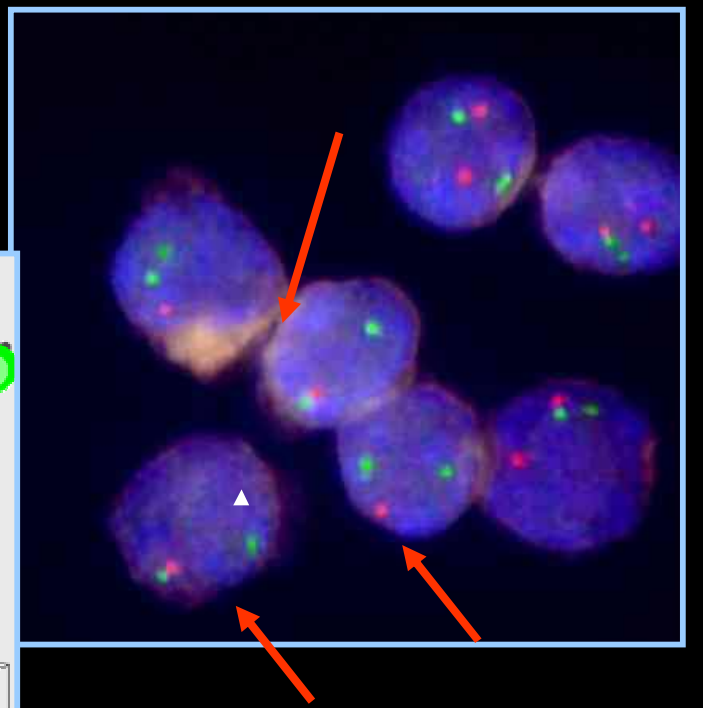
1. Flow cytometry



2. B cell selection





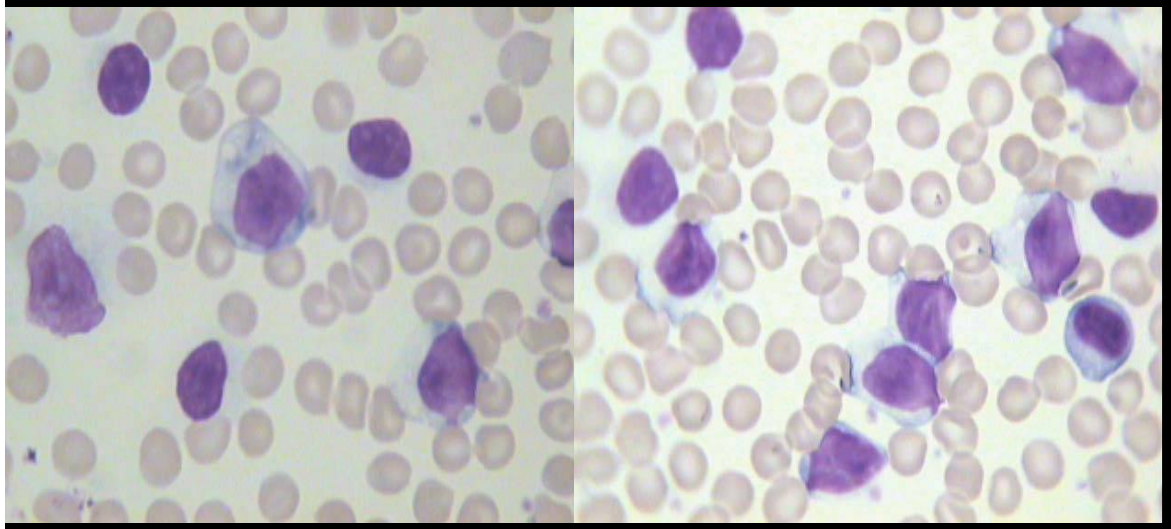


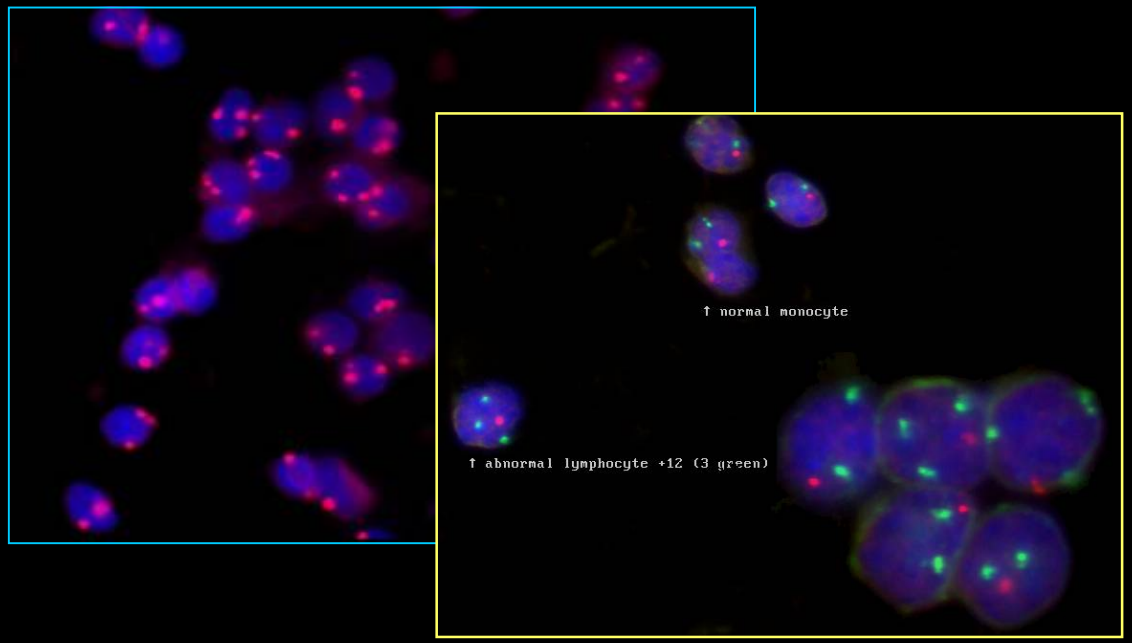
3. Interphase FISH

- Probe set 13q14/13q34
- Deletion 13q14

O'Connor & Barrans, HMDS (2005)

B-CLL ~ Intermediate risk

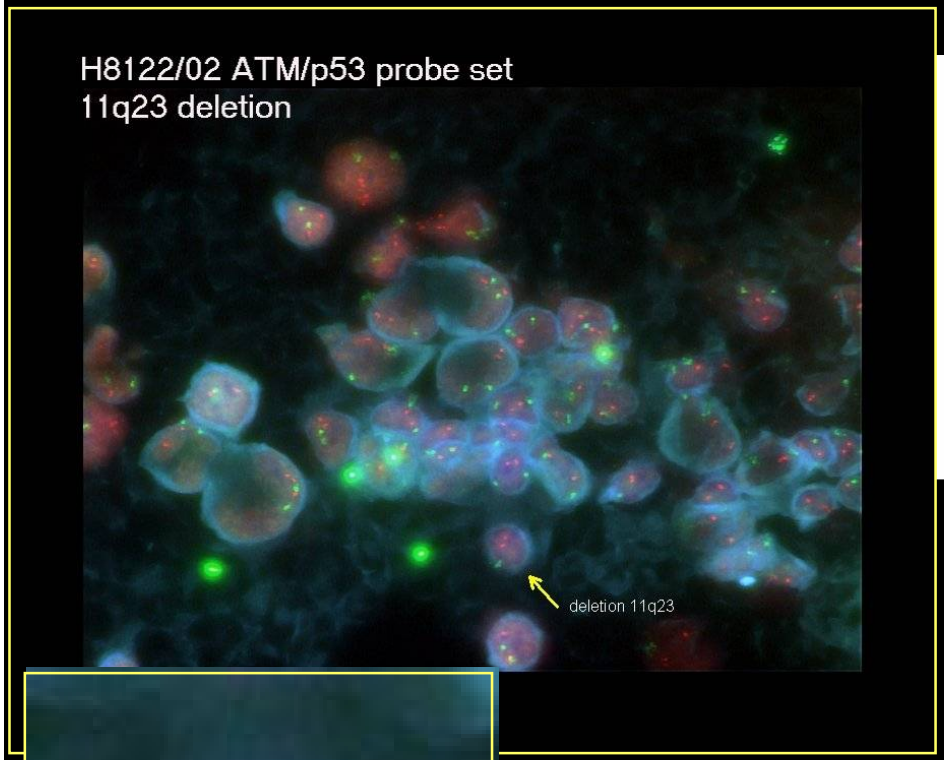


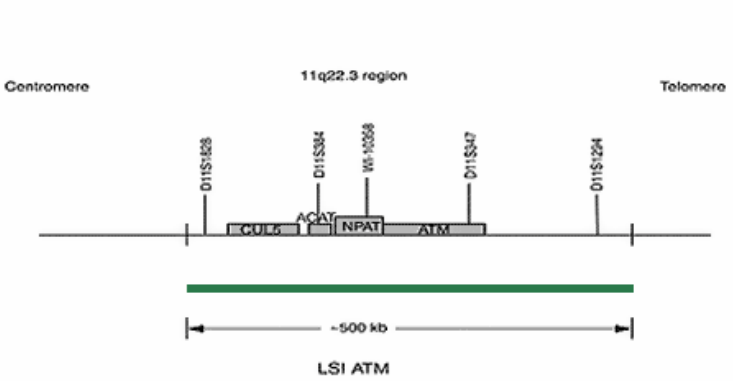


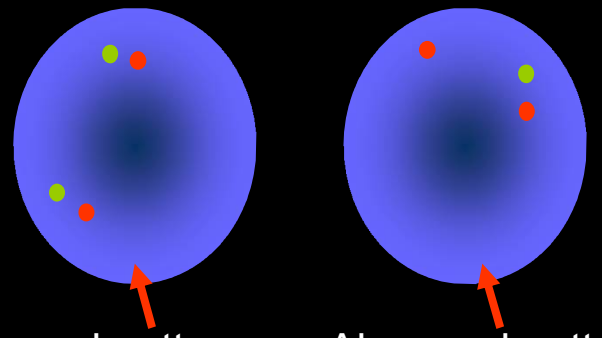
- **Variable morphology**
  - Small to intermediate B cells
  - May have indistinct nucleoli
  - 'Prolymphocyte morphology'
- Phenotype:
  - sIg+moderate (IgM & IgD), CD5+, CD19+, CD23+, CD20+moderate
  - CD11a+
  - likely to express CD38 & Zap-70
- Pattern of BM infiltration
  - Nodular
  - Interstitial
  - Diffuse
  - Pseudo-follicle formation in lymph node biopsies

O'Connor & Barrans, HMDS (2005)

B-CLL ~ Adverse risk: ATM deletion



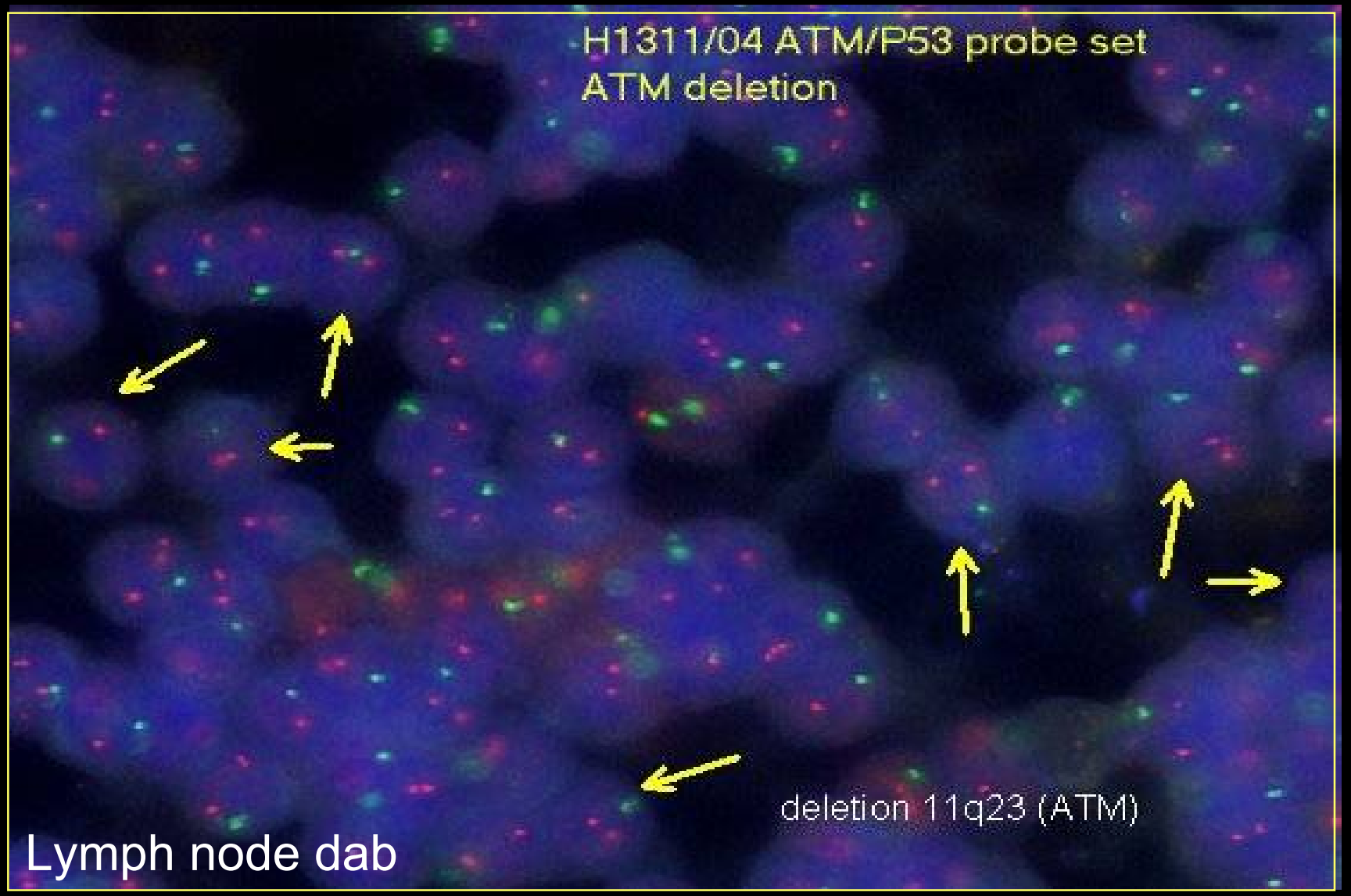




B CLL cells may be very small !

O'Connor & Barrans, HMDS (2005)

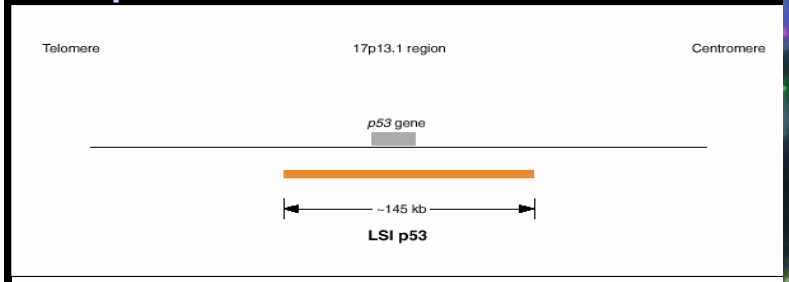
B-CLL ~ Adverse risk: ATM



O'Connor & Barrans, HMDS (2005)

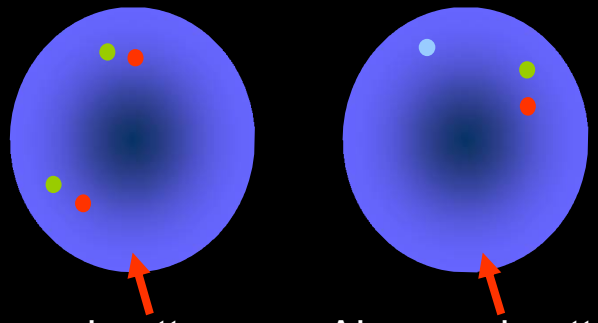
B-CLL ~ Adverse risk: p53 deletion

LSI® p53 / LSI ATM

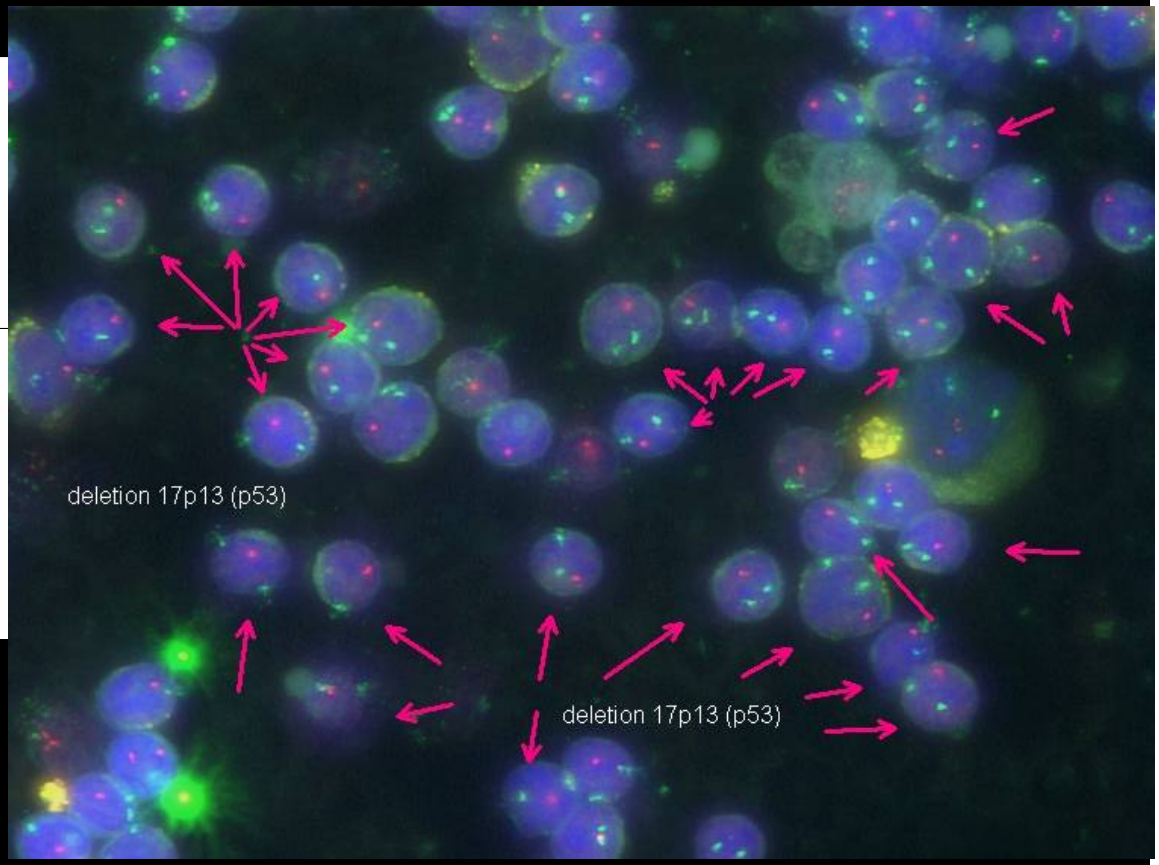


17p13 (P53) red

Alpha sat 17 (green)

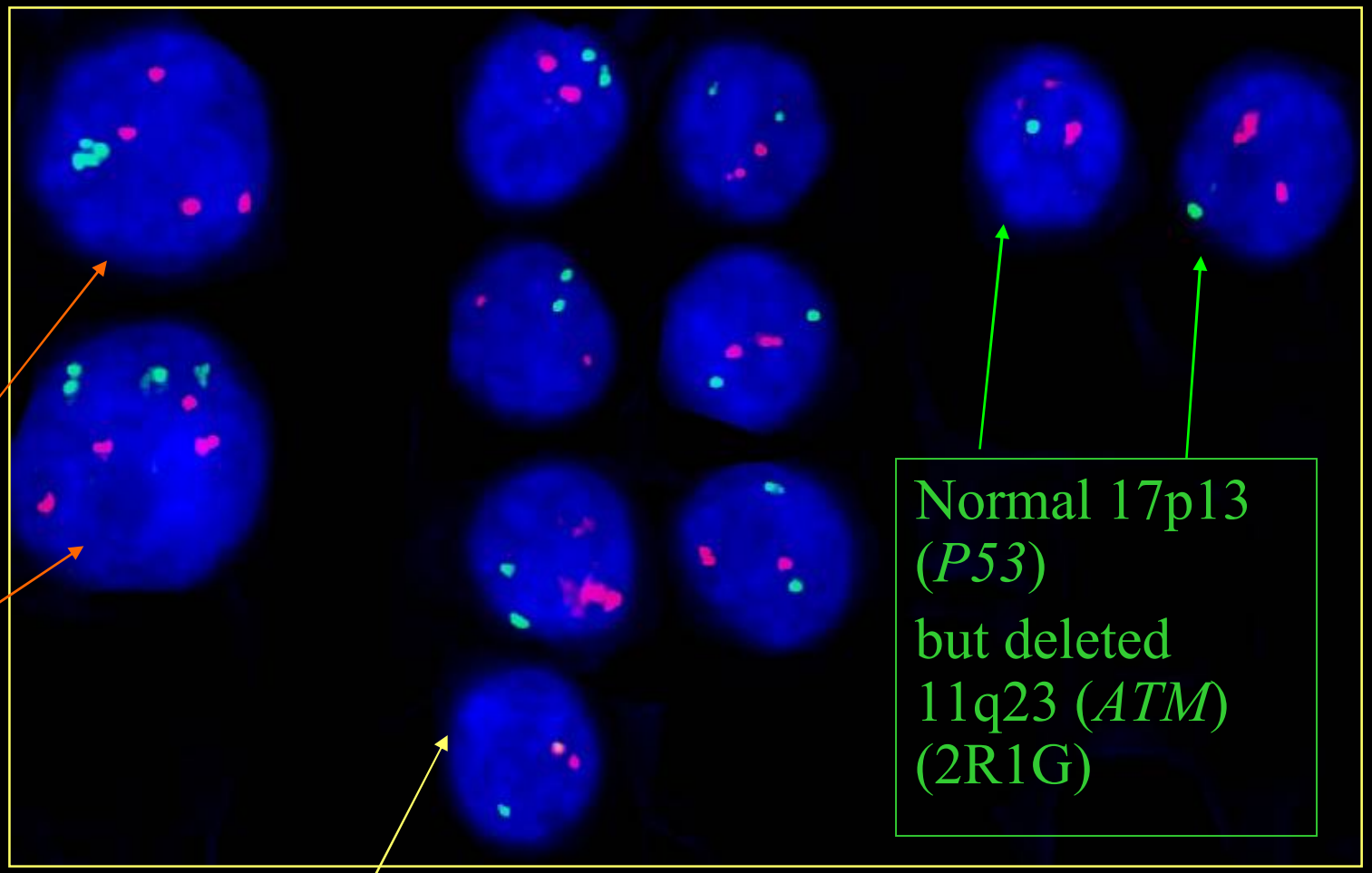


Normal pattern Abnormal pattern



O'Connor & Barrans, HMDS (2005)

B-CLL with deletion 11q23 (*ATM*)



Possible normal G2 cells or tetraploid tumour cells (4R4G)

Normal 17p13 (*P53*) but deleted 11q23 (*ATM*) (2R1G)

All normal (2R2G)

O'Connor & Barrans, HMDS (2005)

B-CLL with deletion 11q23 (*ATM*)

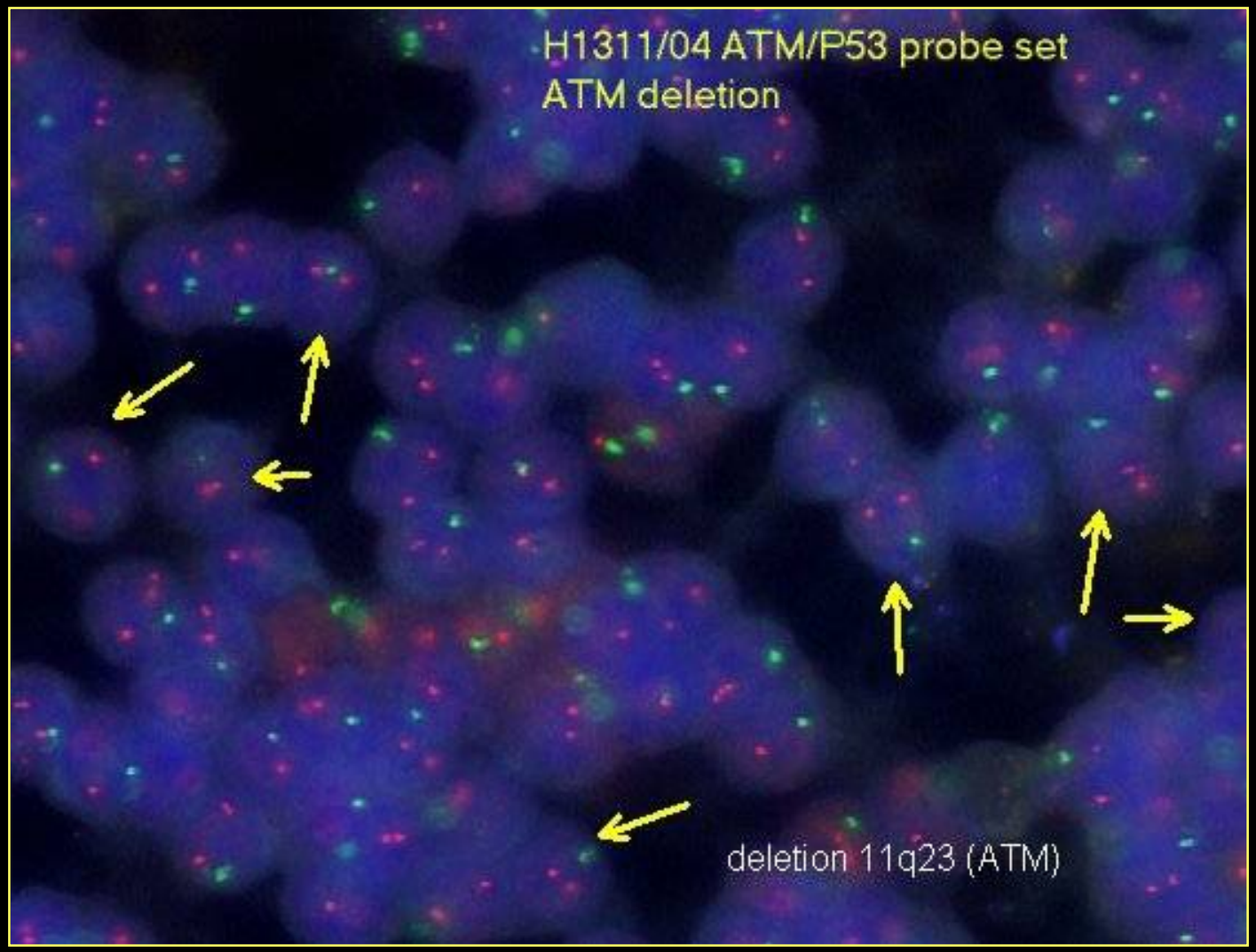
Vysis 11q23 (*ATM*)/17p13 (*P53*) probe set

(*ATM* ~ green, *P53* ~ red)

(Cat. No. 30-191025)

O'Connor & Barrans, HMDS (2005)

B-CLL with deletion 11q23 (*ATM*)



Vysis
ATM)/*p53*
probe set

ATM ~ green,

p53 ~ red

O'Connor & Barrans, HMDS (2005)

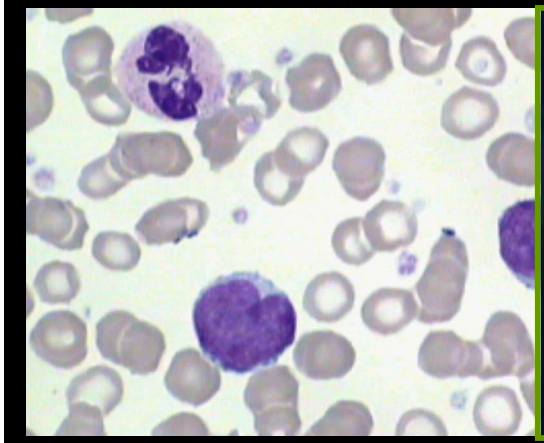
Mantle cell lymphoma

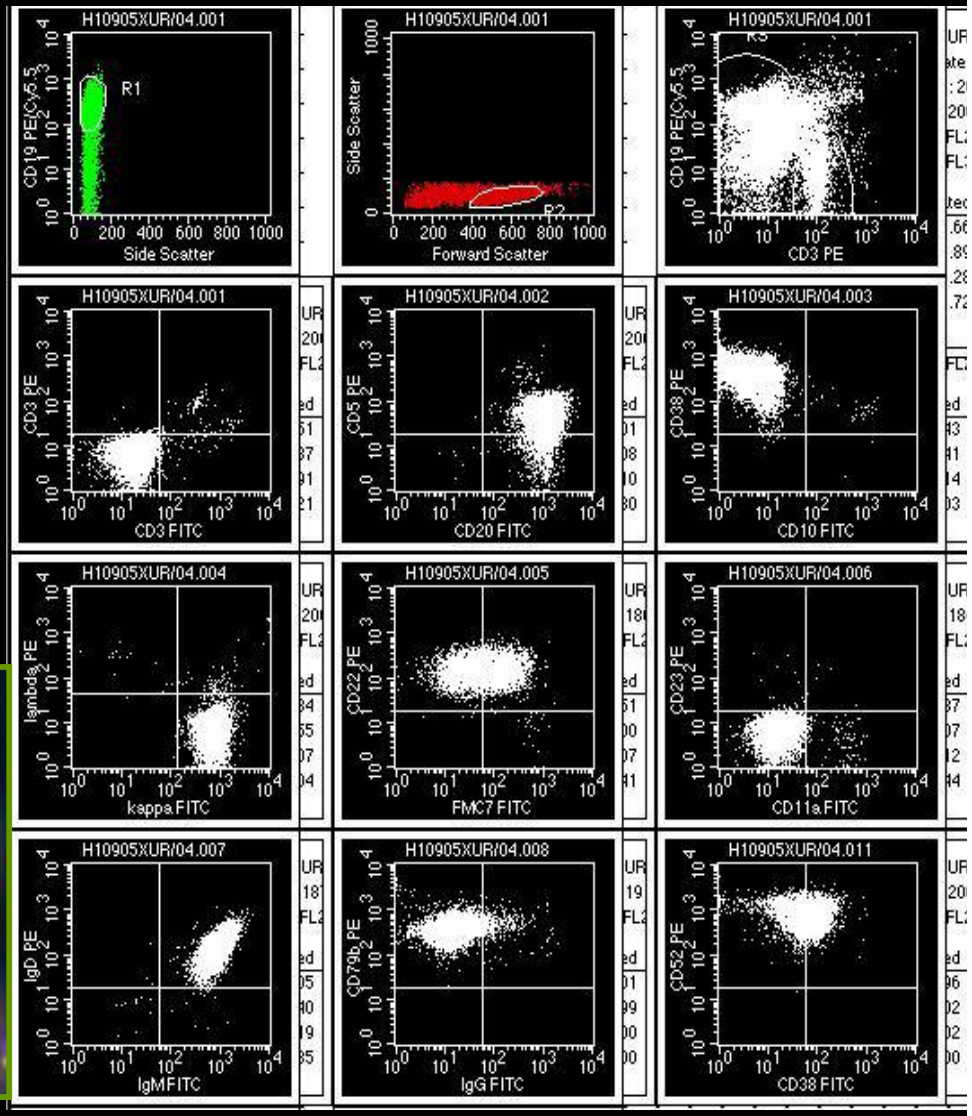
O'Connor & Barrans, HMDS (2005)

Mantle cell lymphoma

Diagnostic Criteria

- ***Typical Cases***
  - Monomorphic population of small to intermediate B-cells
  - Phenotype: sIg++/+++ (IgM & IgD), CD5+, CD23-, CD20+
  - bcl-1 expression / t(11;14)
- ***Variants***
  - Blastic or large cell variant associated aggressive clinical course





O'Connor & Barrans, HMDS (2005)

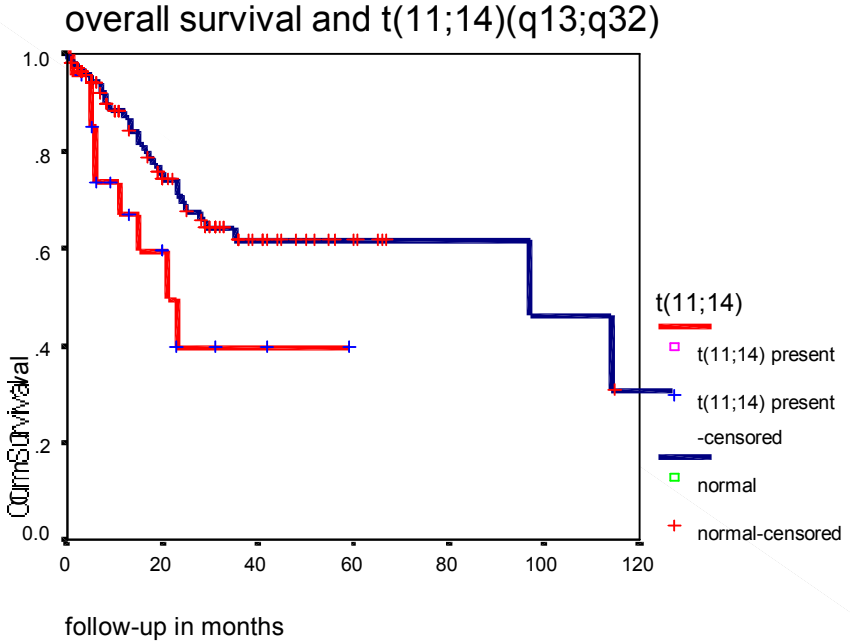
Cytogenetics of Mantle cell lymphoma

Mantle cell lymphoma is characterised by deregulated over-expression of *Cyclin D1* (*BCL-1*) caused by the t(11;14)(q32;13).

- PCR will at best detect 40% to 50% of cases
- FISH >95% of cases
- Variant translocations may occur

Overlap between B-CLL and MCL

- Immunophenotype
  - CD5+ CD23-
- V_H gene status
- mutated and germline
- Cytogenetics
- deletions 11q23, 13q14, 17p13, trisomy 12



Overall survival and t(11:14)(q13;q32)(*p* = .037)

Favourable risk MCL ?

Low clinical stage?

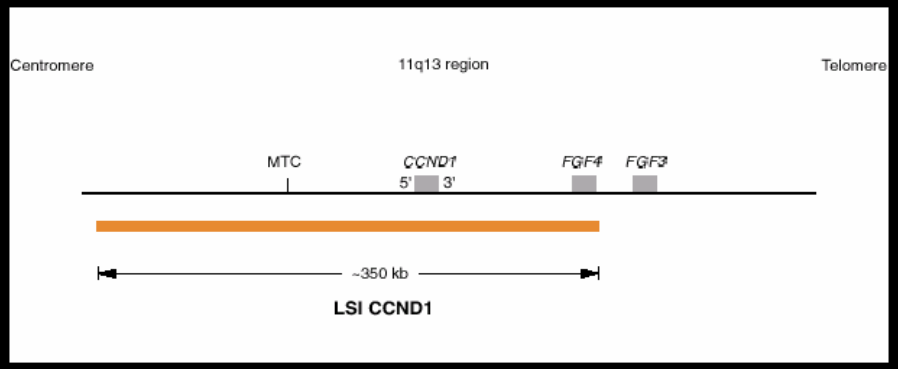
Mutated?

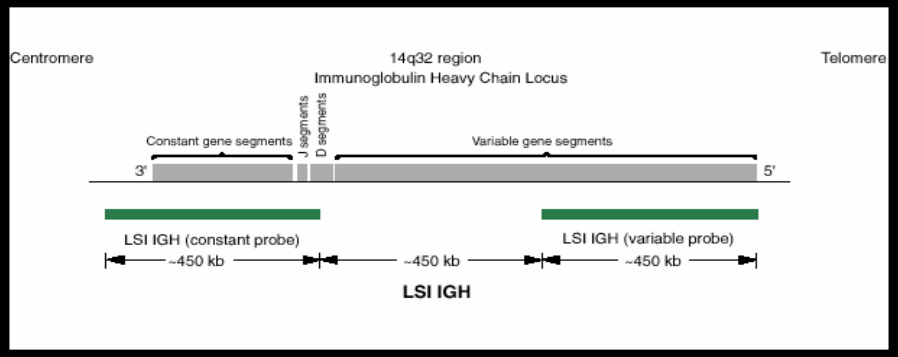
Normal ATM and p53?

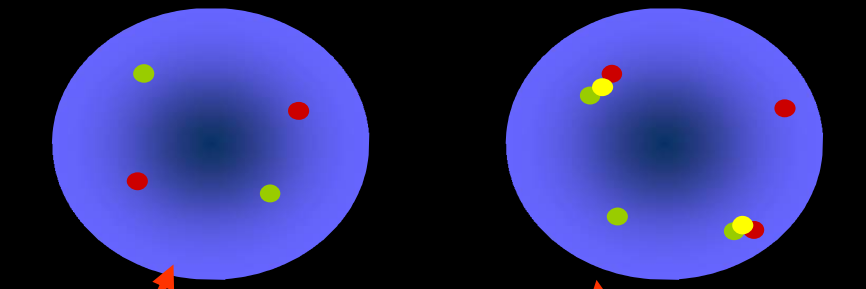
O'Connor & Barrans, HMDS (2005)

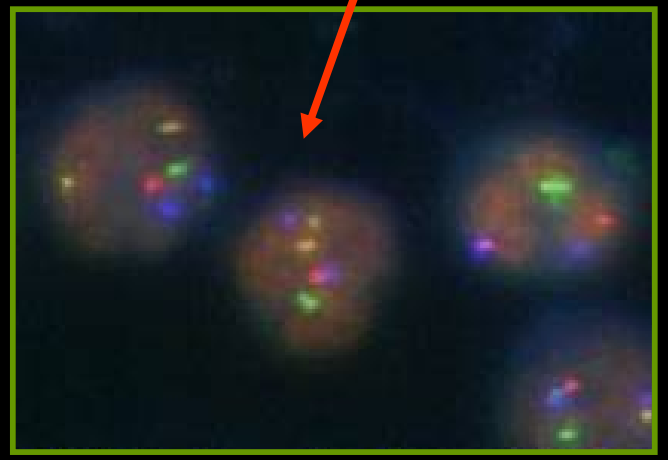
Mantle cell lymphoma ~ t(11;14)

LSI® IGH/CCND1 Dual Color, Dual Fusion Translocation Probe (32-91017)







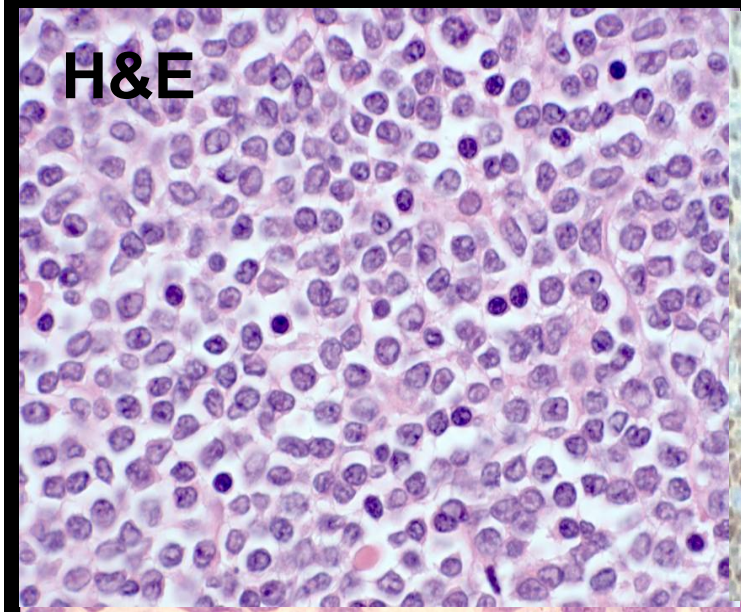


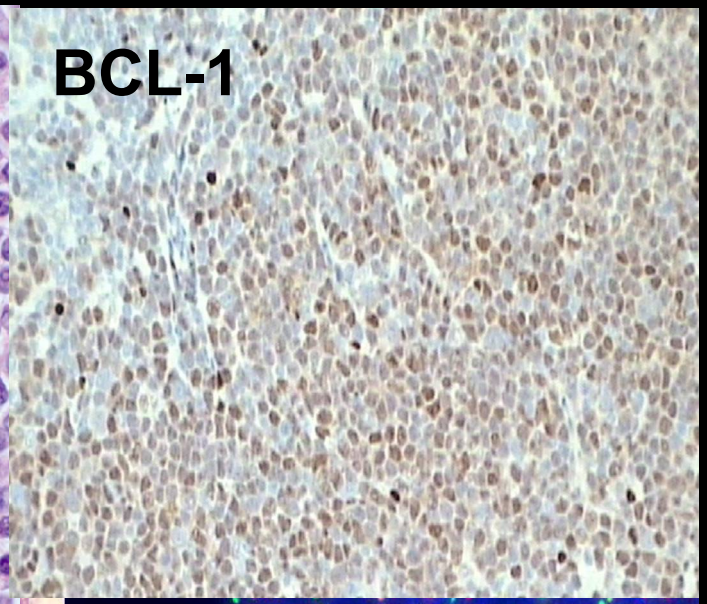
Dual Fusion protocol

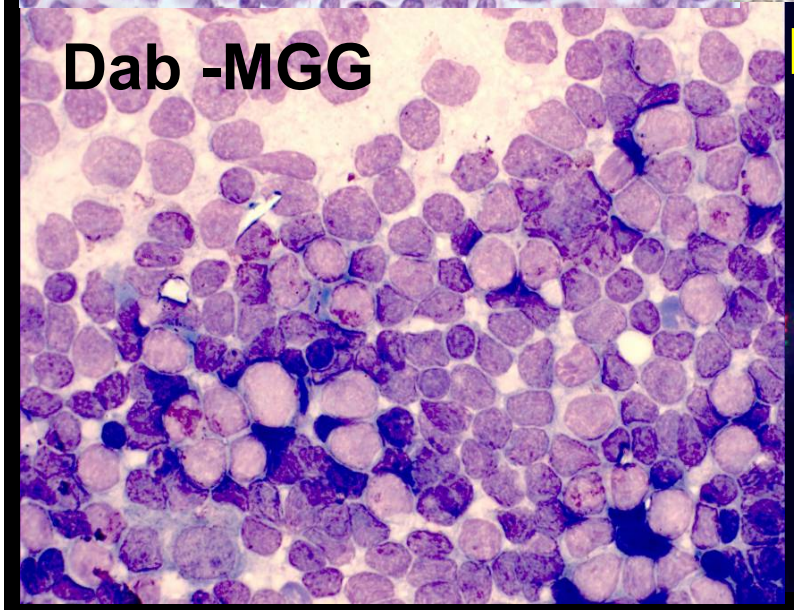
Most sensitive strategy for translocations when partner chromosome is known.

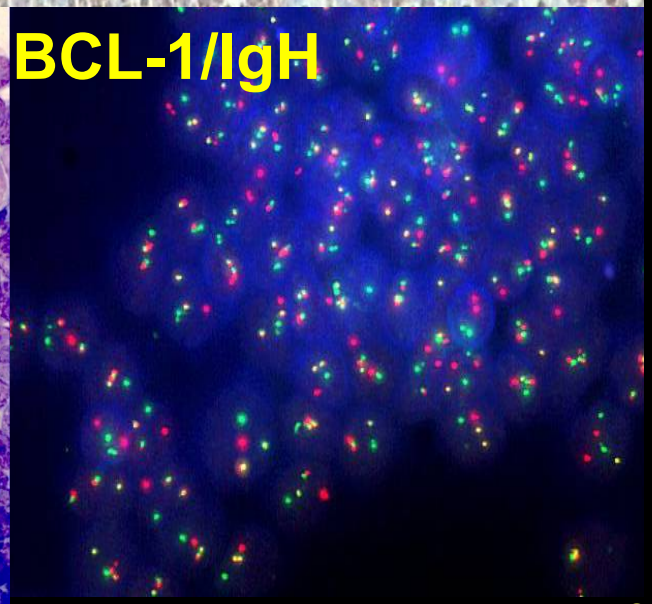
O'Connor & Barrans, HMDS (2005)

Mantle cell lymphoma ~ t(11;14)







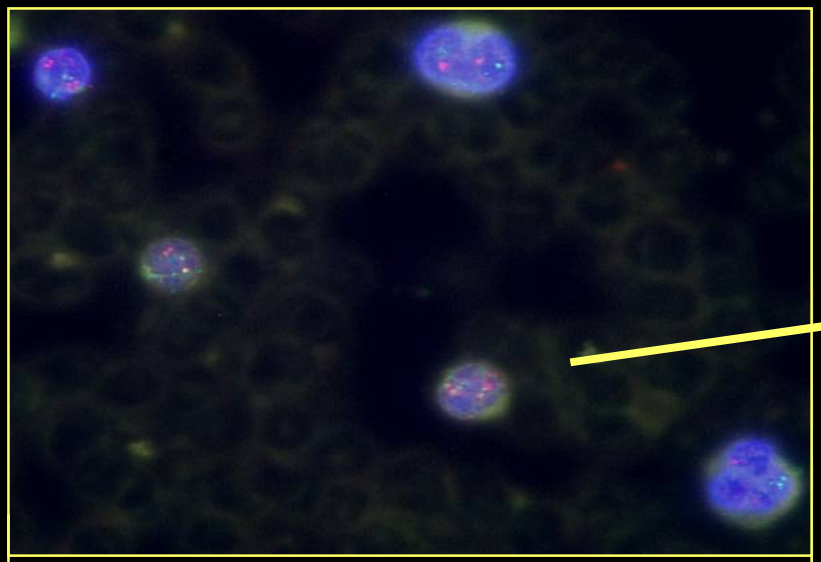


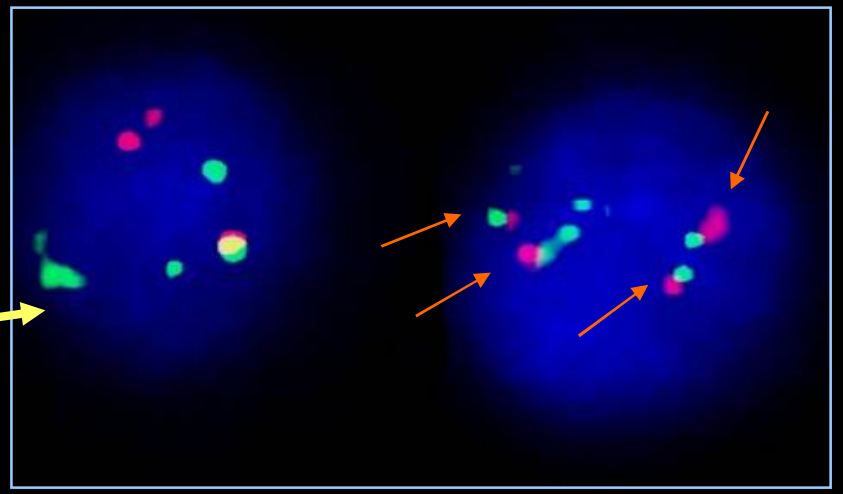
Representative case:

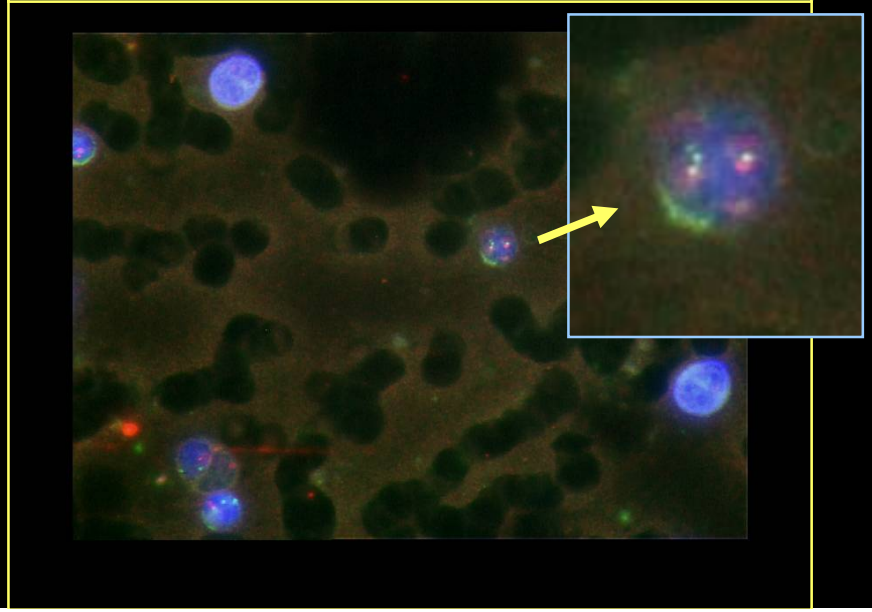
- Lymph node biopsy
- CD5+CD23-
- Strong CD20
- Strong SIg
- BCL-1 positive
- t(11;14) positive

O'Connor & Barrans, HMDS (2005)

Mantle cell lymphoma ~ t(11;14)







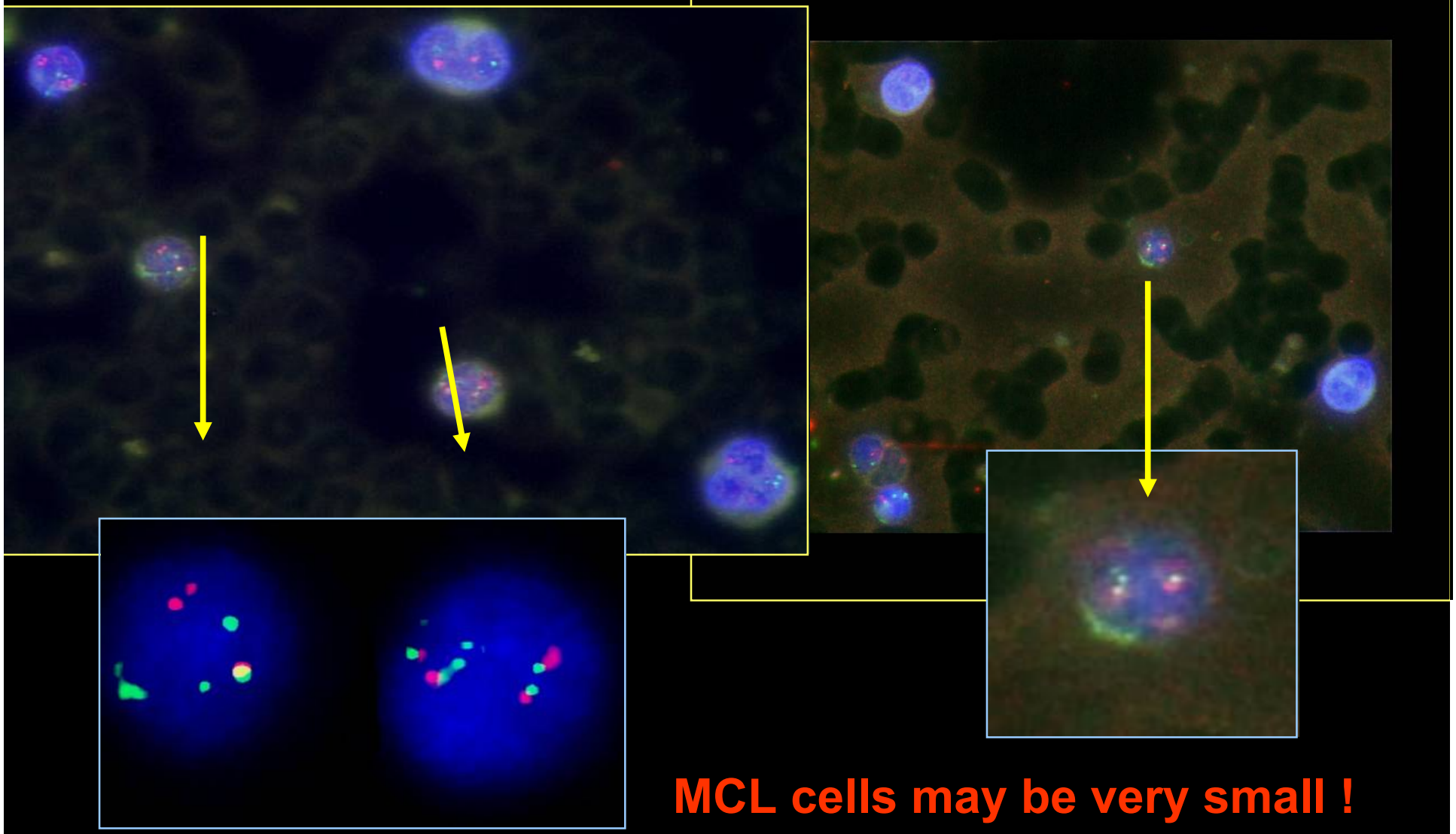
- Probe set prepared in-house (see earlier diagram).
- Multiple fusions occur.
- Practical problems are low cell counts and very small cells.

11q13 *(BCL-1)* ~ red

14q32 *(IgH)* ~ green

O'Connor & Barrans, HMDS (2005)

Mantle cell lymphoma ~ t(11;14)

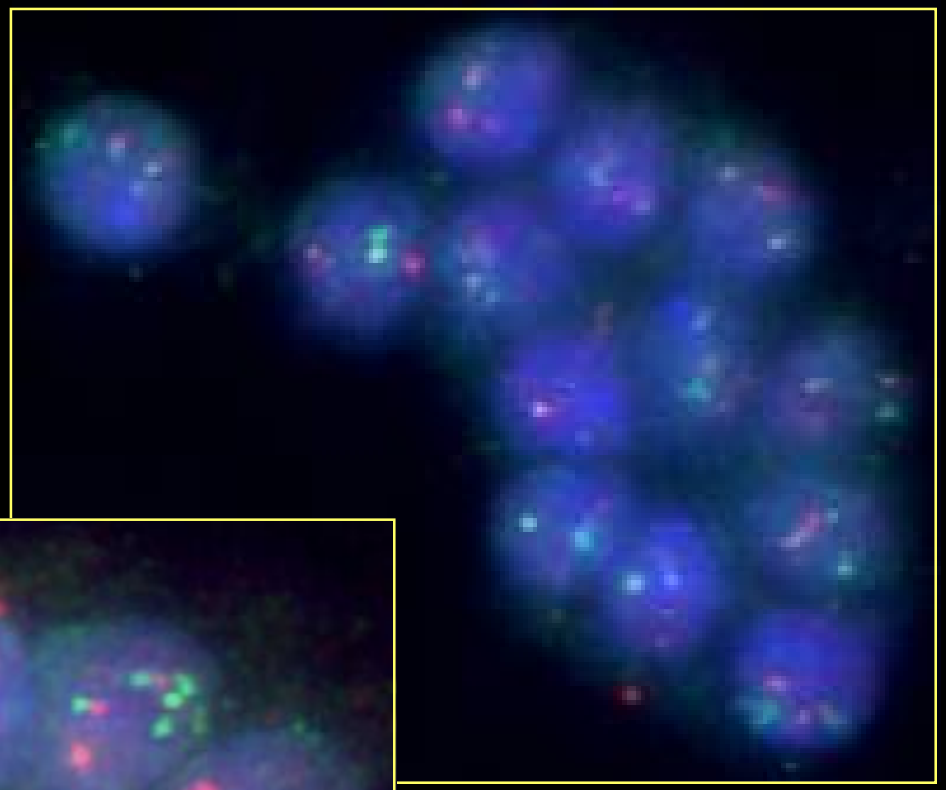


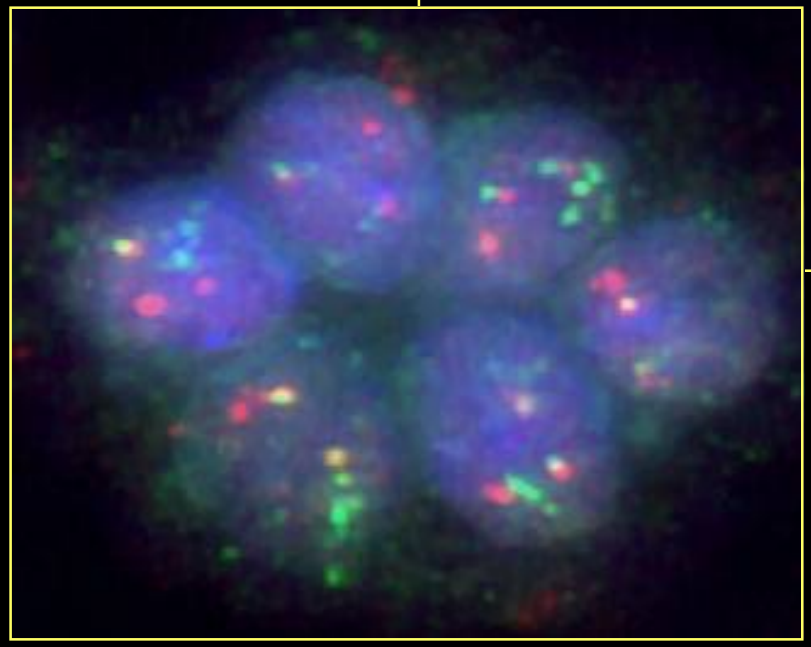
MCL cells may be very small !

O'Connor & Barrans, HMDS (2005)

Mantle cell lymphoma ~ t(11;14)

- Bone marrow with moderate infiltrate of MCL cells
  - Occur in small clumps
  - ? Artefact
  - ?? Any significance



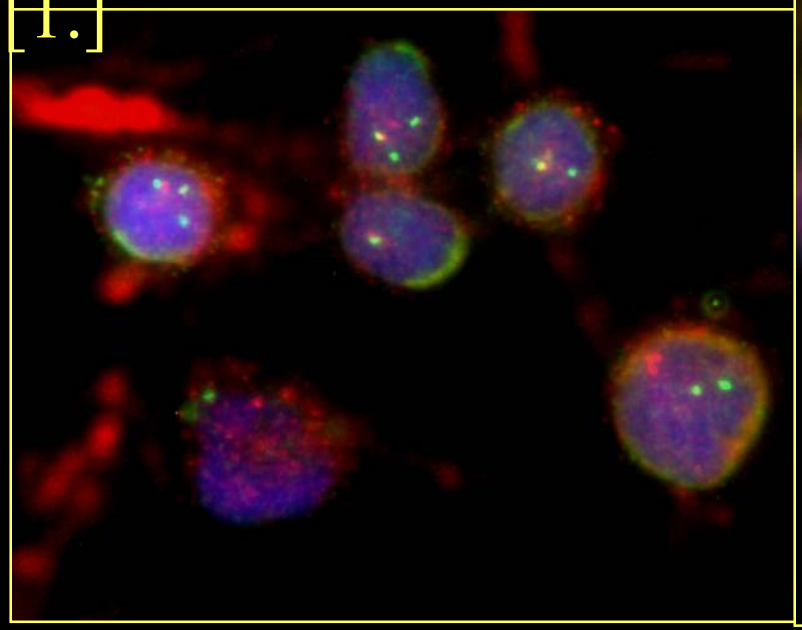


O'Connor & Barrans, HMDS (2005)

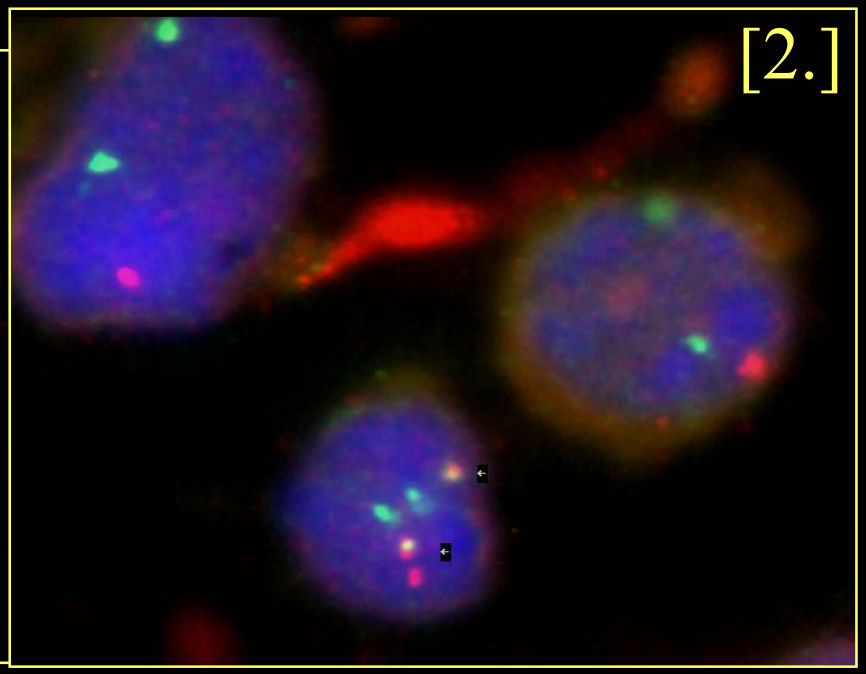
Relapsed mantle cell lymphoma

Patient RT (50 year old male) awaiting CABG found to have Hb of 4g/dl with leuco-erythroblastic film. BMA infiltrated with abnormal lymphs.
Immunophenotype: CD5+CD23- with strong CD20 and SIg+
2-colour FISH at presentation showed 30% t(11;14).

[1.]



[2.]



relapsed at 8 months; 65% t(11;14) [fig.1] with 10% of these being double t(11;14) [fig.2] ? significance of this.

O'Connor & Barrans, HMDS (2005)

Differential diagnosis of MCL & atypical B-CLL

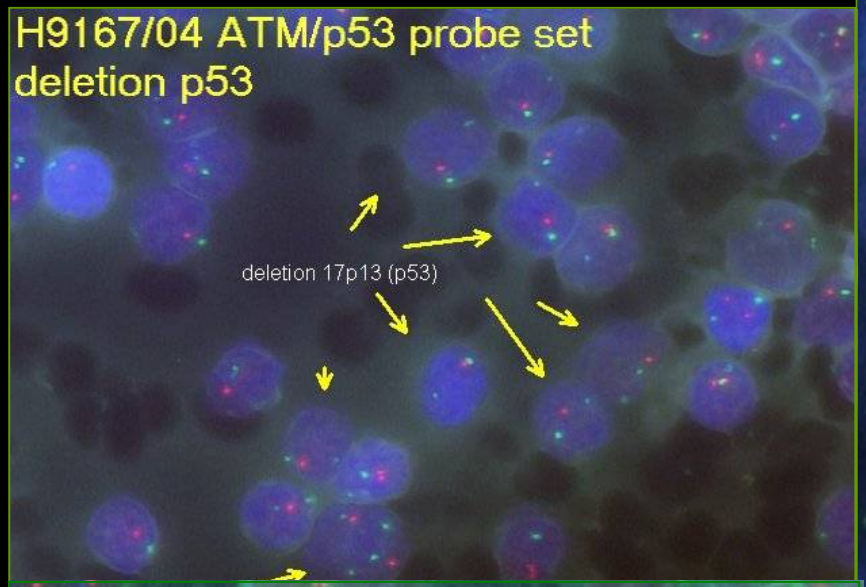
- Approximately 5-8% of CD5+CD23- B-cell LPDs
  - Phenotypically identical to MCL
  - Translocation negative and BCL-1 protein negative

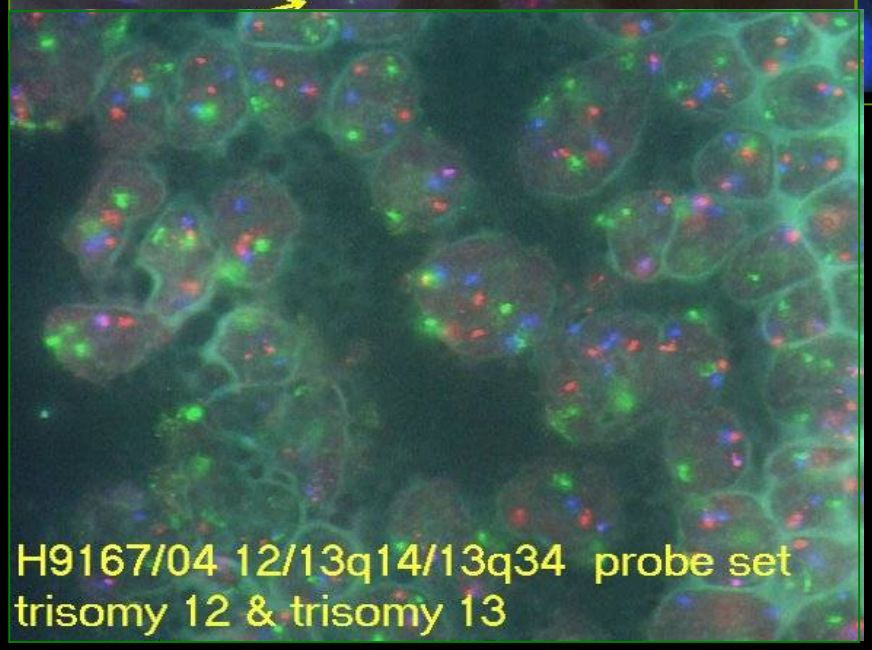
Clinical Characteristics of MCL v B-CLL v atypical CD23- B-CLL

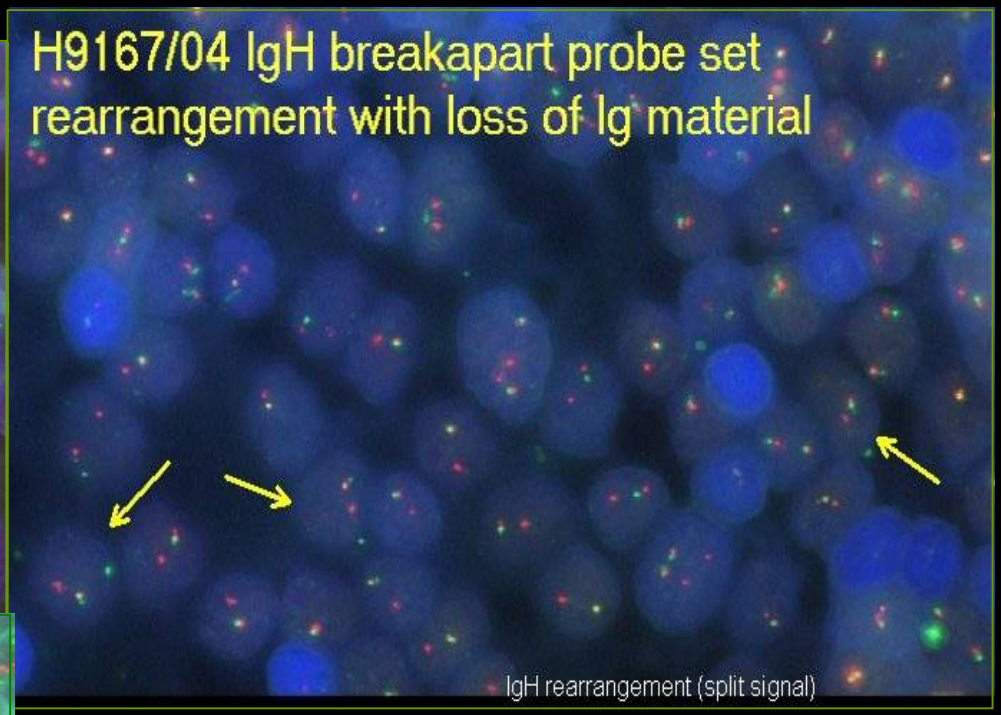
	MCL	B-CLL	CD5+CD23-	
Male:Female	2.6:1	1.7:1	1.15:1	
Age range	36-83	36-95	51-96	
Binet A	13%	56%	57%	OS not reached
Binet B	42%	26%	27%	OS 69 months
Binet C	46%	18%	16%	OS 25 months
Extra-nodal	36%	2%	12%	
Trisomy 12	8%	15%	35%	

O'Connor & Barrans, HMDS (2005)

Representative CD5+CD23- B-LPD ~ FISH







Patient HP, male age 81 years

- CD5+CD23-CD20+wkCD38+CD79b+mod IgMDkappa+
- BCL-1 negative
- BCL-1 not rearranged
- BUT IgH is rearranged

O'Connor & Barrans, HMDS (2005)

Key points ~ MCL/B-CLL

- CD5+CD23- phenotype does **not** predict for the presence of t(11;14) or BCL-1 protein expression.

- Diagnosis of MCL requires the demonstration of t(11;14) or BCL-1 protein.
  - FISH ~ best pick-up in B-cell LPDs
    - PCR or cytogenetics ~ poor compared to FISH
  - Immunohistochemistry ~ BCL-1 over-expression

- Translocation negative **CD5+CD23- B-LPD** should be regarded as **atypical B-CLL**.
  - Poor risk variant

O'Connor & Barrans, HMDS (2005)

Prognostic factors in CD5+ B-LPD

	Good prognostic indicator	Intermediate prognostic indicator	Poor prognostic indicator
Histology	Nodular pattern Proliferation centres	Interstitial Diffuse	Interstitial Diffuse
Immunophenotype	Strong CD23 Weak CD20 Zap-70 negative	CD38 CD11a FMC7	Absent CD23 Strong CD20 Zap-70 positive
Cytogenetics	Deletion 13q14	Trisomy 12	Deletion 17p13 Deletion 11q23 t(11;14)(q13;q32)
VH status	mutated	-	germline

O'Connor & Barrans, HMDS (2005)

Summary – CD5+ B-cell LPD

Integrated approach to diagnosis

Use interphase FISH to diagnose Mantle cell lymphoma

Use of interphase FISH in differential diagnosis

- Atypical B-CLL v MCL ~ t(11;14)

Use interphase FISH to predict prognosis

- Favourable risk B-CLL ~ watch & wait strategy
- Poor risk B-CLL ~ treat early

Continual audit – only use published / validated data
- Must be adaptable to future developments

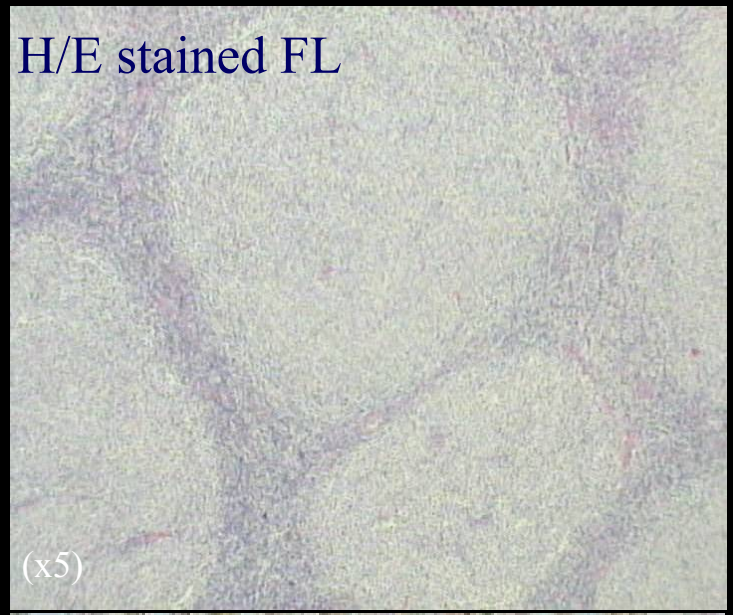
O'Connor & Barrans, HMDS (2005)

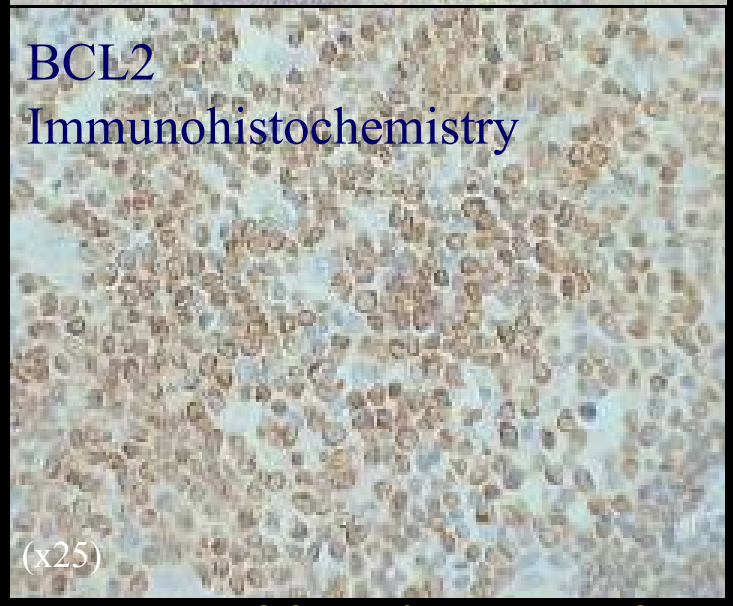
Follicular Lymphoma

O'Connor & Barrans, HMDS (2005)

Features of follicular lymphoma

- Follicular lymphoma (FL) is a CD5 neg B-LPD
- FL is characterised by multiple relapses and progressive resistance to therapy. A proportion of cases transform to diffuse large B cell lymphoma (DLBCL).
- FL is characterised by the t(14;18)(q32;q21), a germinal centre phenotype and a low proliferative index.
- The t(14;18) results in deregulation of *BCL2* and aberrant expression of the protein
- The presence of a t(14;18) can be used in the differential diagnosis between FL and MZL

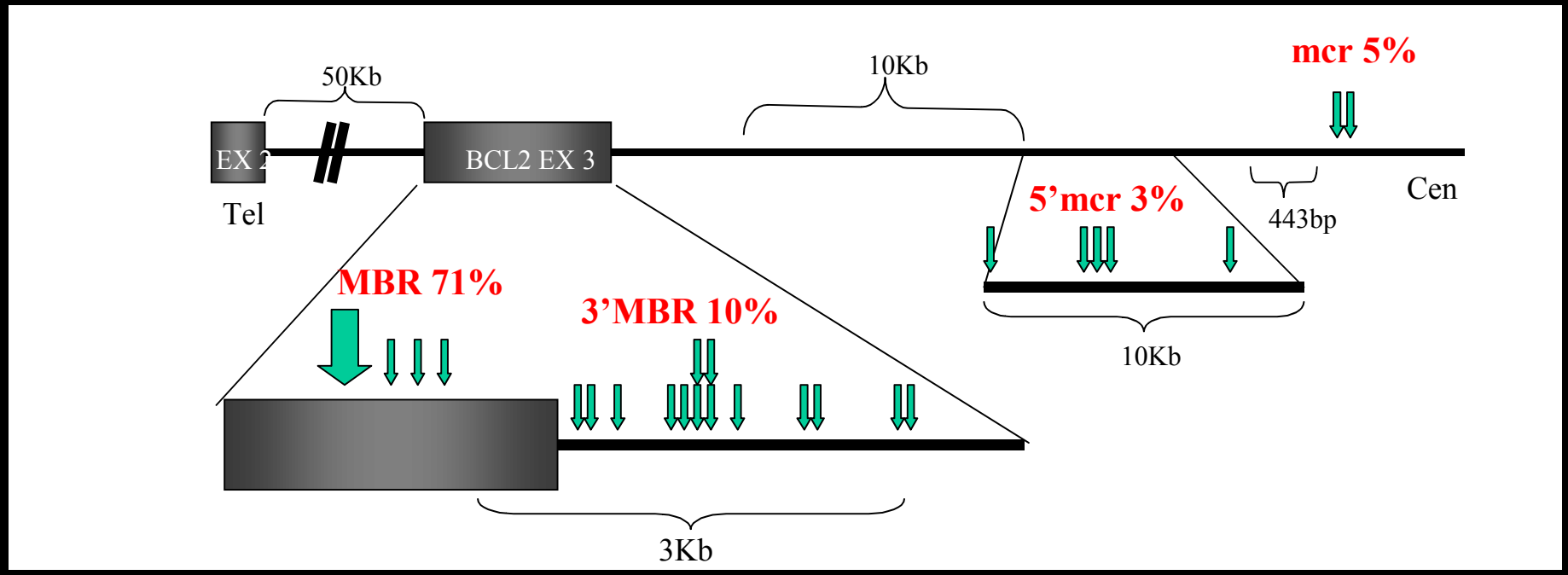




O'Connor & Barrans, HMDS (2005)

DETECTION OF THE t(14;18)

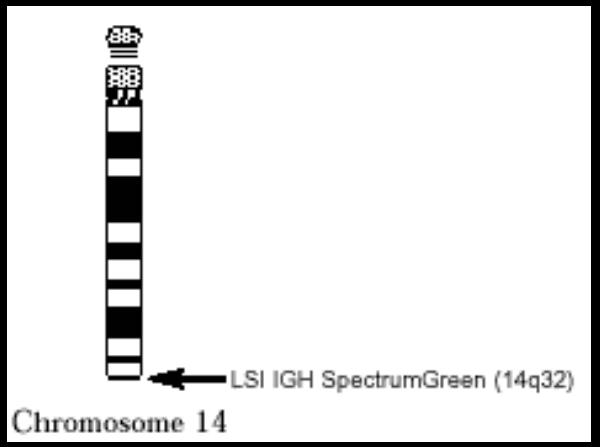
- Approximately 25% of breakpoints are not detected using PCR strategies for the MBR and mcr alone
- Gold Standard PCR involves a multiplex reactions that includes MBR, mcr and 3'MBR (using the BIOMED II t(14;18) primers)
- Using PCR the detection rate of translocations in fixed tissue is significantly reduced due to poor quality DNA
- FISH is the best approach, particularly in translocations with variable breakpoints.
- For retrospective studies, and due to the limited store of fresh material, it is vital that molecular techniques used for the detection of translocations are applicable to paraffin embedded tissue.

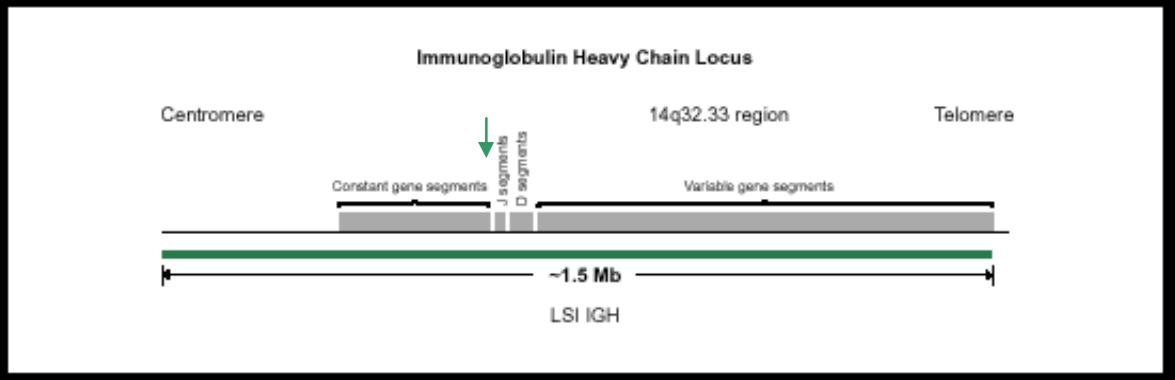


O'Connor & Barrans, HMDS (2005)

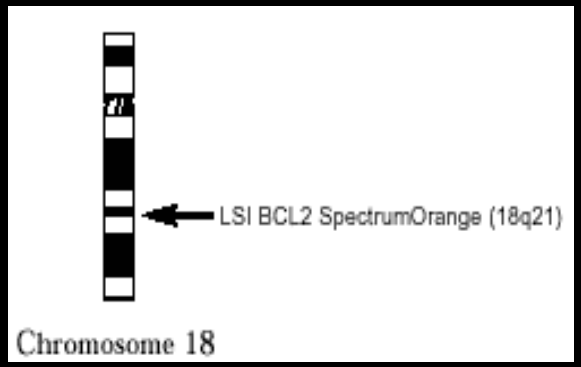
Probe Map Of The Vysis LSI *IgH/BCL2* Dual Colour Probe

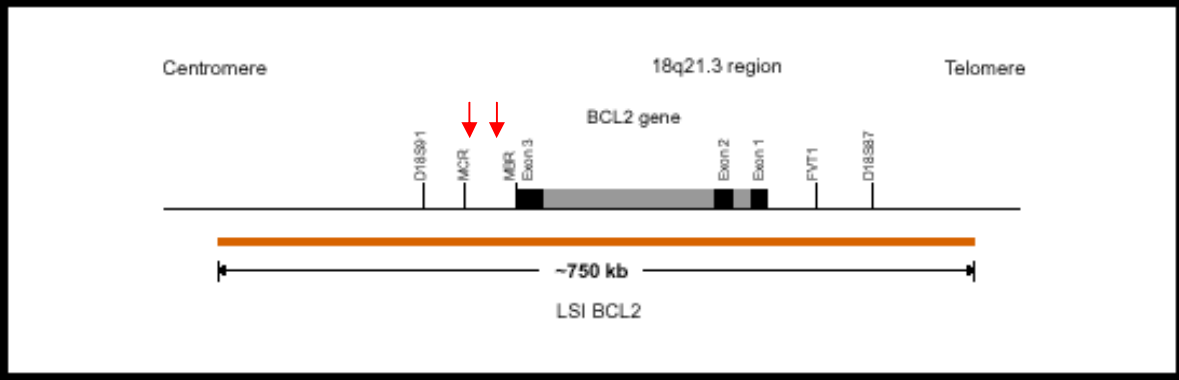
(Cat No: 32-191018)





The *IgH* probe spans approximately 1.5Mb and contains sequences homologous to essentially the entire *IgH* locus, as well as sequences extending about 300kb beyond the 3' end of the *IgH* locus.
The green line indicates the span of the IgH probe, and the arrow indicates the main breakpoint region.



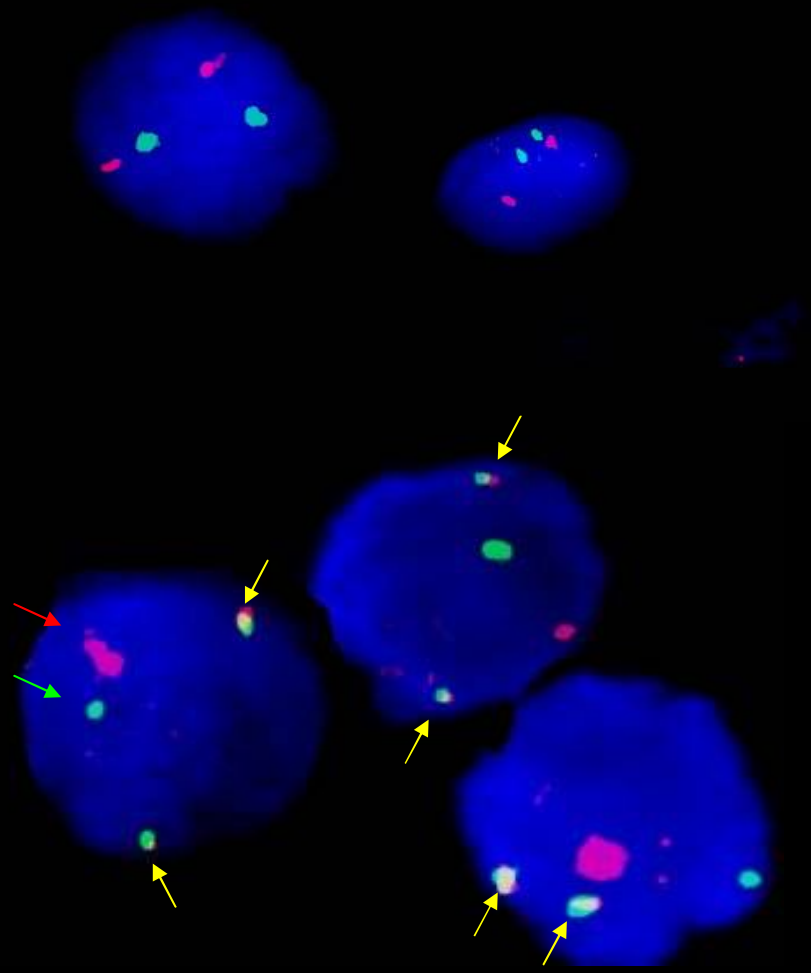


The *BCL2* probe covers an approximate 750kb region, including the entire *BCL2* gene with additional sequences extending approximately 250kb both distal and proximal to the gene.
The span of the *BCL2* probe is indicated by the orange line, the arrows indicate the breakpoint regions.

Diagrams reproduced from the Vysis Website (http://www.vysis.com)

O'Connor & Barrans, HMDS (2005)

Representative FISH images of the *IgH/BCL2* probe set



(paraffin nuclei)

a) The normal pattern of staining of the *IgH/BCL2* probe set

•2 red (*BCL2*) and 2 green (*IgH*) FISH signals, one for each copy of the chromosome.

b) A case with a t(14;18)

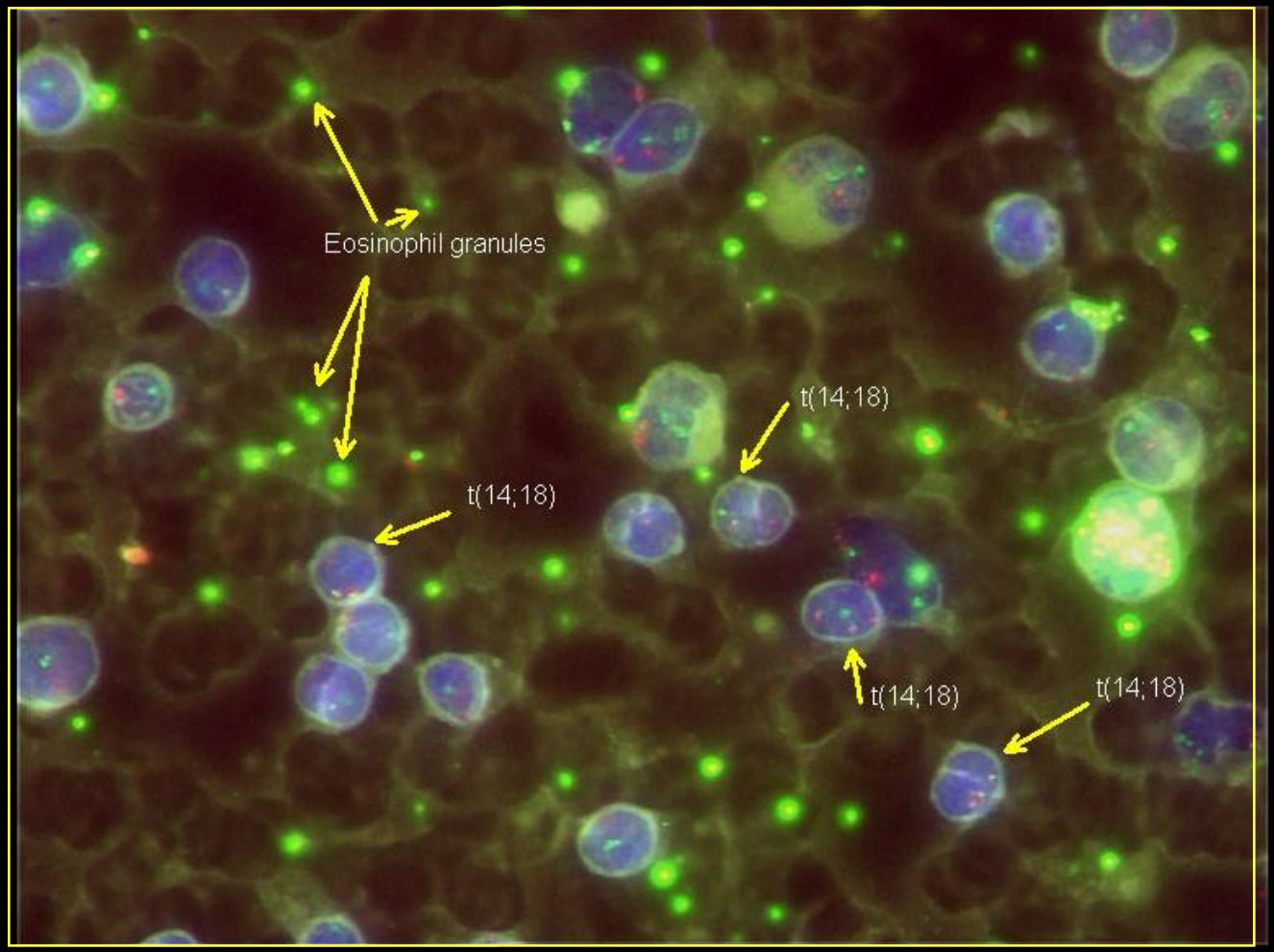
•3 signals with both *BCL2* and *IgH* probe sets, indicating a 'split' in one each of the copies of the genes.

• The reciprocal translocation, t(14;18), is demonstrated by the presence of 2 fusion signals in each cell (indicated by yellow arrows), along with a residual normal copy of each gene.

O'Connor & Barrans, HMDS (2005)

Follicular Lymphoma

t(14;18) positive Bone Marrow smear



t(14;18) useful in differential diagnosis between MZL and FL

O'Connor & Barrans, HMDS (2005)

Molecular abnormalities of Follicular lymphoma

- Up to 20% of cases of FL lack the t(14;18)
  - Rearrangement of *BCL6* is reported to be common in this group (30% in our series)
  - BCL2 protein is negative in up to 5% of cases (always t(14;18) neg)
- Deletion and mutation of *P53*, rearrangement of *BCL6*, and multiple *BCL2*/*IgH* fusions are associated with transformation of FL to DLBCL
  - These are also acquired within the indolent phase of FL, without evidence of transformation, and are present with increased incidence at relapse.
  - 20% of patients have a *BCL6* rearrangement in addition to the t(14;18) (13% at presentation and 31% at relapse)
  - 14% of cases have a P53 deletion (4% at presentation, 17% at relapse)

O'Connor & Barrans, HMDS (2005)

Deletion of *P53* in FL

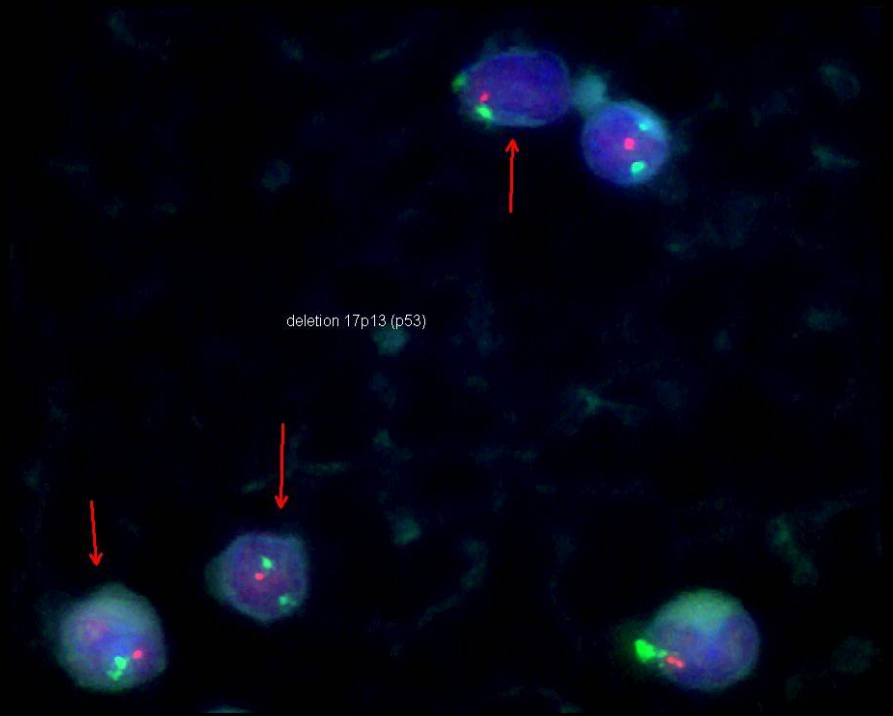
Q-Biogene *P53* (17p13) / Alphasat 17 Probe Cocktail, Dual-Colour (PONC1753)

- 17p13 labelled red, alpha 17 control probe labelled green
- Normal pattern 2G2R
- P53 deletion pattern 2G1R

17p13 (P53) red

Alpha sat 17 (green)

Chromosome 17



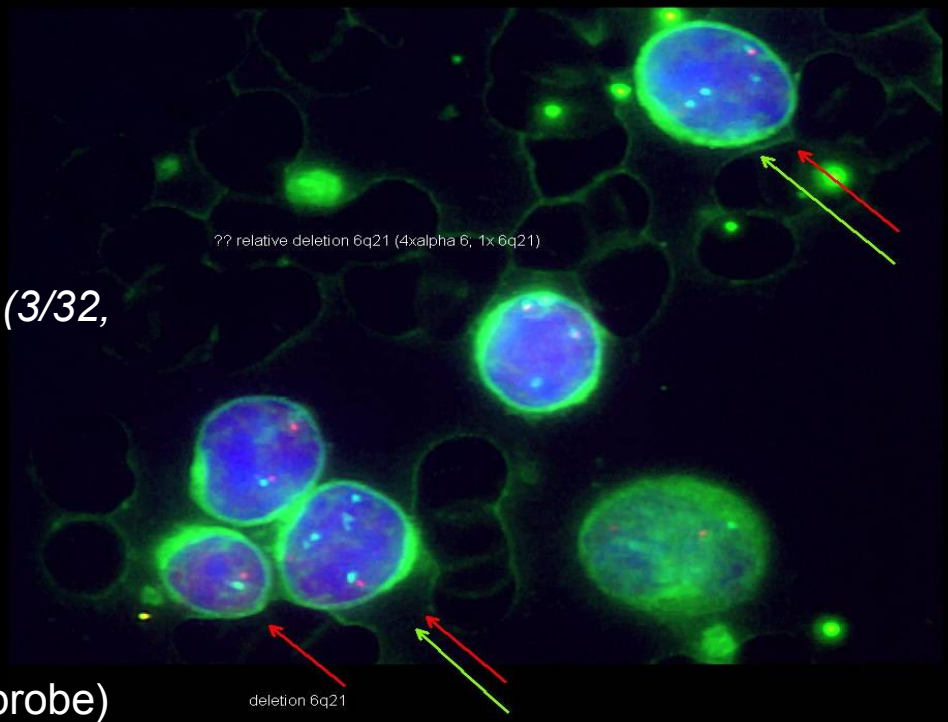
O'Connor & Barrans, HMDS (2005)

Marginal Zone Lymphoma

O'Connor & Barrans, HMDS (2005)

Cytogenetics of Marginal Zone Lymphoma

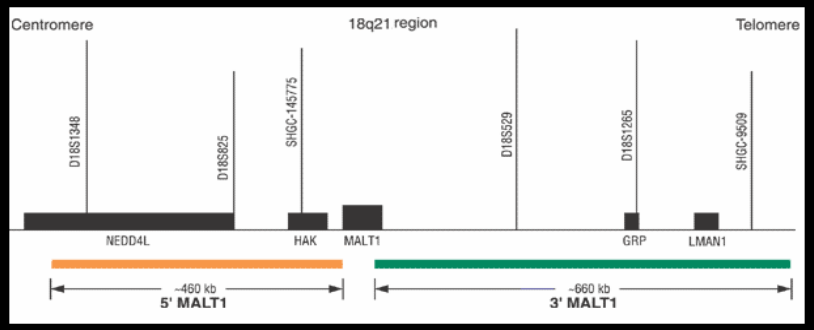
- Trisomy 3 and 18 common in all types MZL
  - *+18: 15% in SMZL, 18% ExMZL*
- IgH rearrangements
  - *5% in SMZL*
- Extranodal MZL
  - t(11;18) .
    - API2-MALT1 fusion gene
    - commonest structural abnormality *(3/32, 9%)*
    - only seen in EMZL
  - t(1;14)
    - BCL-10 and IgH
- Splenic MZL
  - del 7q (*0/25 – Trisomy seen*)
  - Del 6q21 (*10/23* – 2 borderline, poor probe)
  - CDK6, t(2;7) – CDK6
  - t(9;14) and variants – PAX-5 (no commercial probe)
  - t(6;14) – cyclin D3



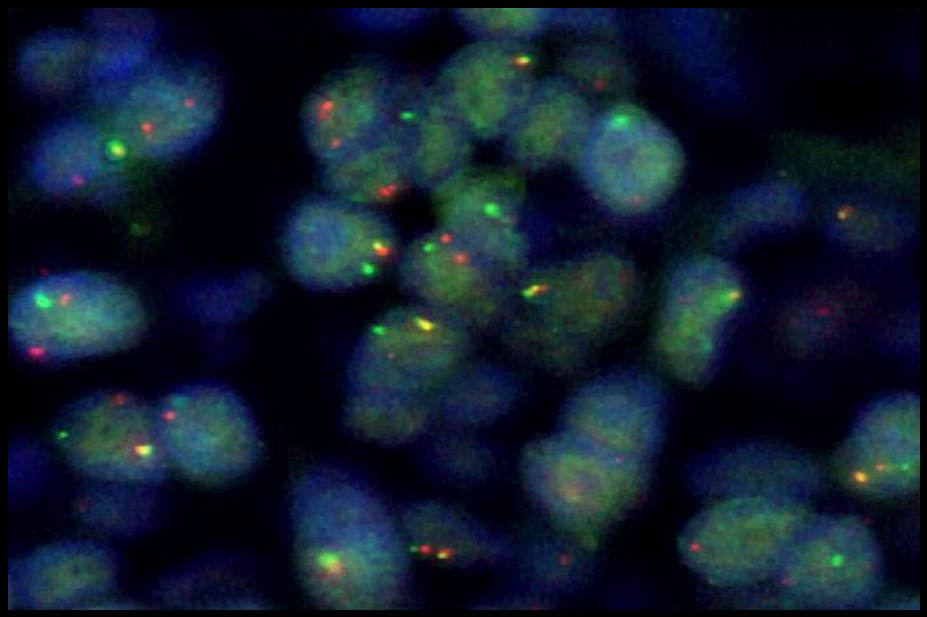
O'Connor & Barrans, HMDS (2005)

MALT-1 in gastric MZL

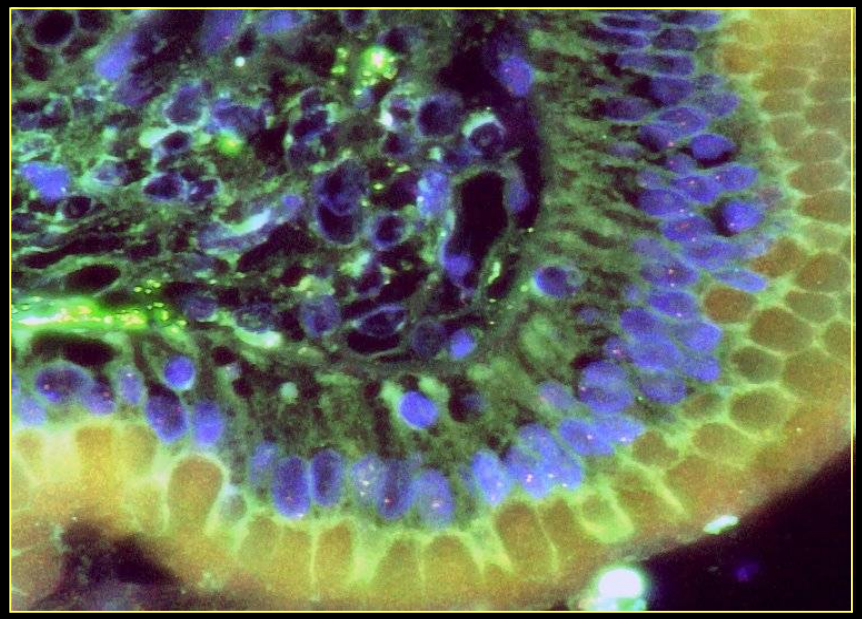
Vysis LSI® MALT1 (18q21) Dual Color, Break Apart Rearrangement Probe (32-190055)



Gastric MZL– *MALT-1* rearrangement



Normal gastric biopsy – normal *MALT-1*

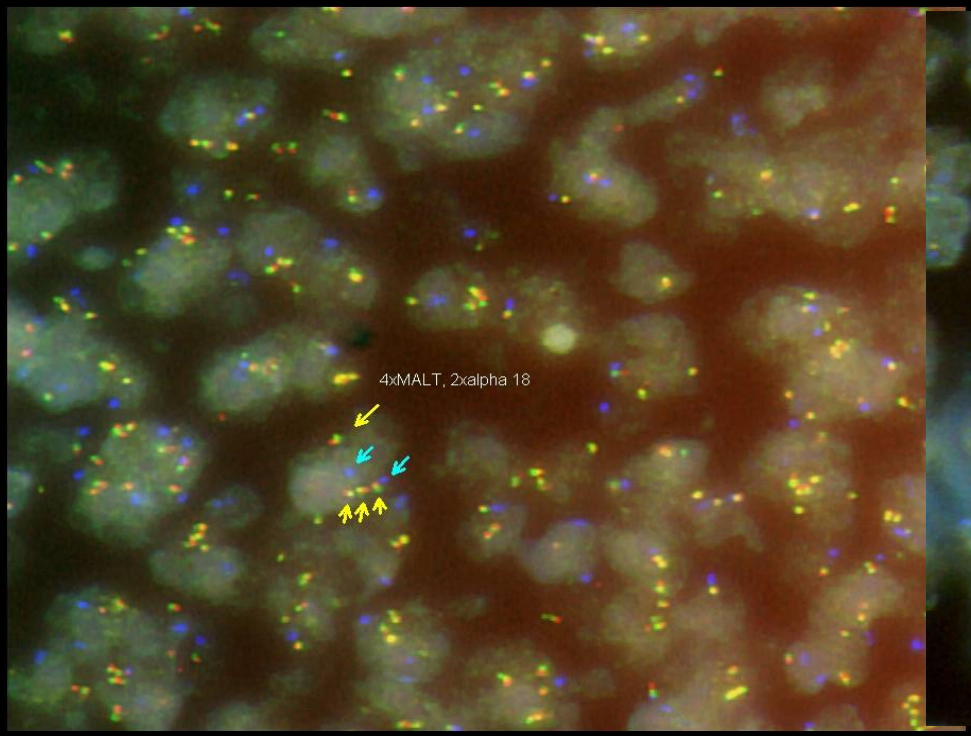


O'Connor & Barrans, HMDS (2005)

MALT-1 in gastric MZL
Alternative signals patterns

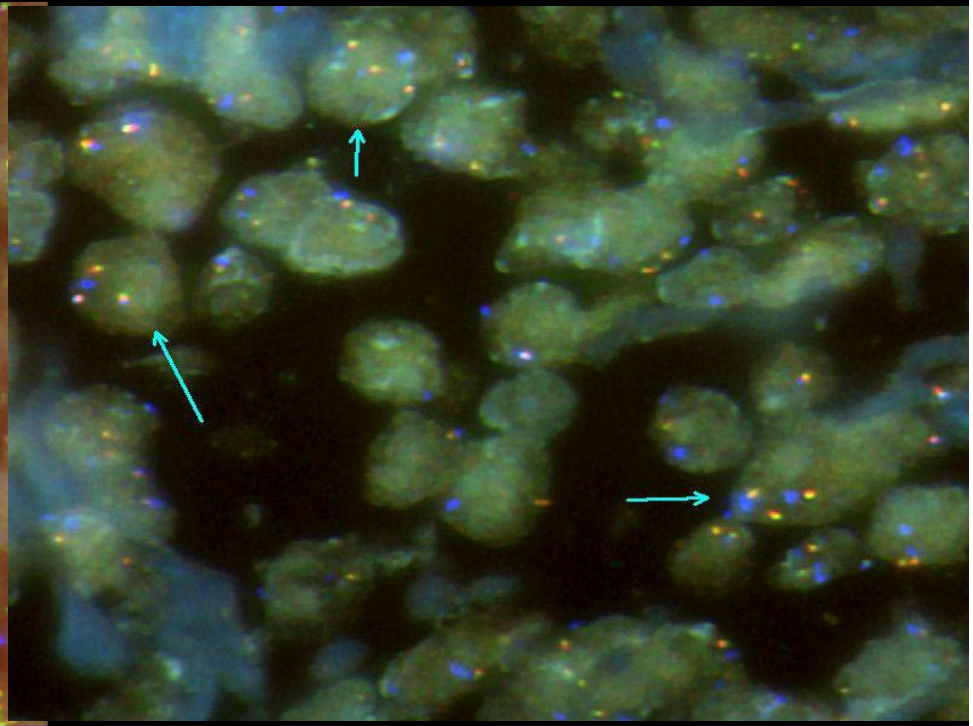
4x *MALT-1*, 2x alpha 18

?isochromosome 18 / ?partial tetrasomy



3x *MALT-1*, 3x alpha 18

Trisomy 18



O'Connor & Barrans, HMDS (2005)

Burkitt Lymphoma
and
Diffuse Large B-cell Lymphoma

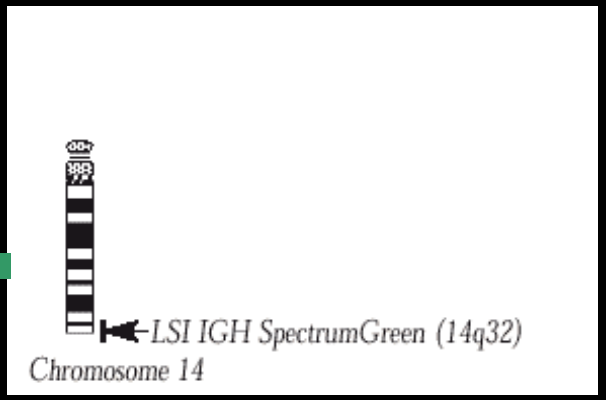
O'Connor & Barrans, HMDS (2005)

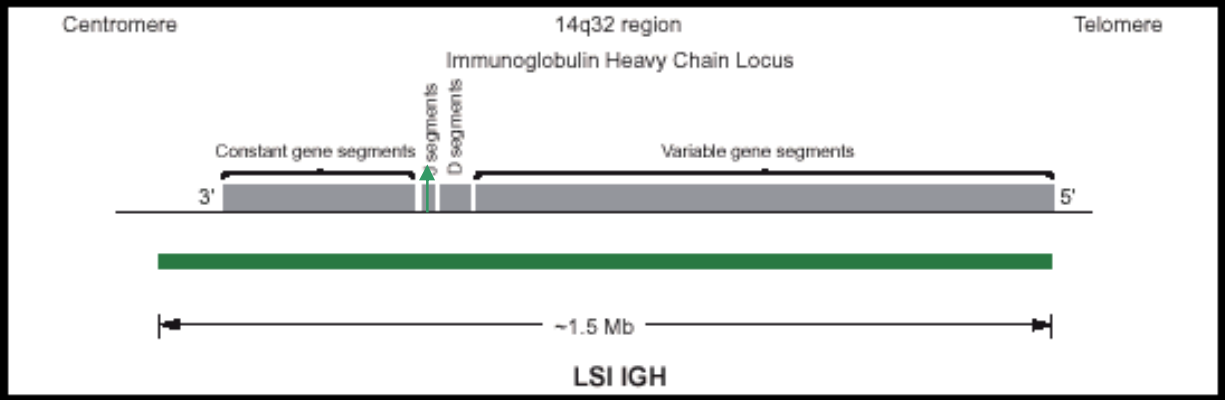
Diagnosis of Burkitt Lymphoma (BL)

- Germinal centre (GC) derived tumour
- *cMYC* rearrangement is the diagnostic feature and must be demonstrated before a diagnosis of BL is made
  - Deregulation of *cMYC* results in very high rates of proliferation and apoptosis
  - Cytogenetics often fails due to high levels of apoptosis
  - FISH is often the only way of detecting translocation
- Cases with cMYC rearrangement in the context of a complex Karyotype or t(14;18) / BCL2 expression are more likely to be transformed FL and should be diagnosed as DLBCL

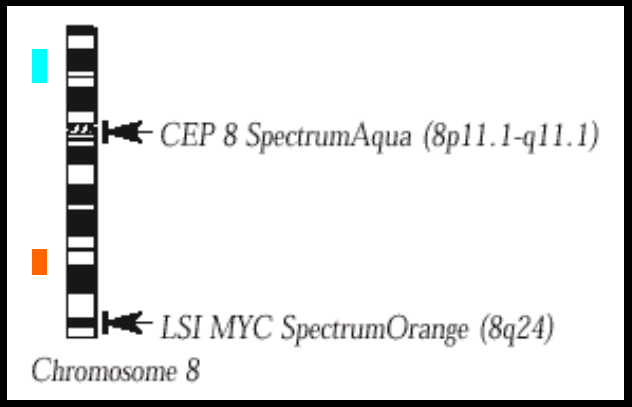
O'Connor & Barrans, HMDS (2005)

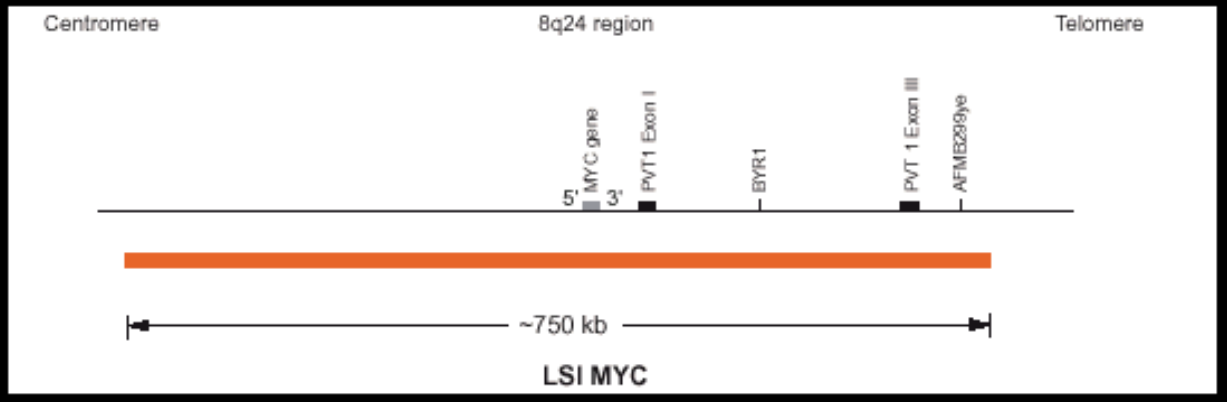
Probe Map Of The Vysis LSI® IGH/MYC, CEP® 8 Tri-color, Dual Fusion Translocation Probe (Cat No: 32-191020)





The *IgH* probe spans approximately 1.5Mb and contains sequences homologous to essentially the entire *IgH* locus, as well as sequences extending about 300kb beyond the 3' end of the *IgH* locus.
The green line indicates the span of the IgH probe, and the arrow indicates the main breakpoint region.





The *cMYC* probe covers an approximate 750kb region, including the entire *cMYC* gene with additional sequences extending both distal and proximal to the gene.
The span of the *cMYC* probe is indicated by the orange line

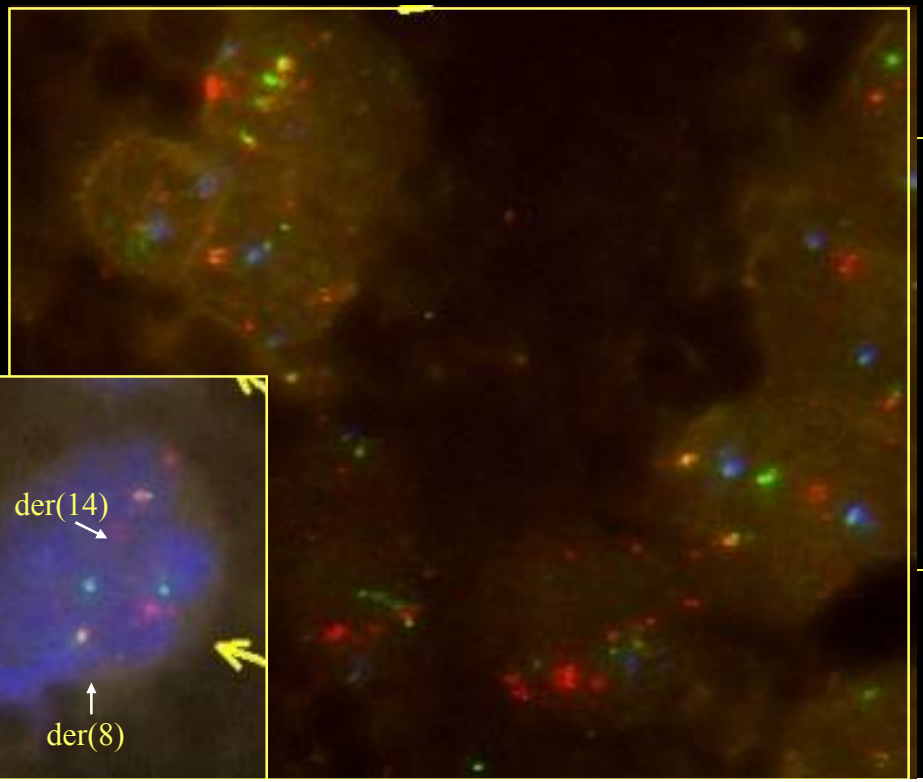
Diagrams reproduced from the Vysis Website (http://www.vysis.com)

O'Connor & Barrans, HMDS (2005)

IGH/MYC, CEP® 8 Tri-color, Dual Fusion Translocation Probe

Normal pattern (2G,2R,2B)

- *cMYC*/*IgH*/CEP 8 Aqua probe
  t(8;14) negative



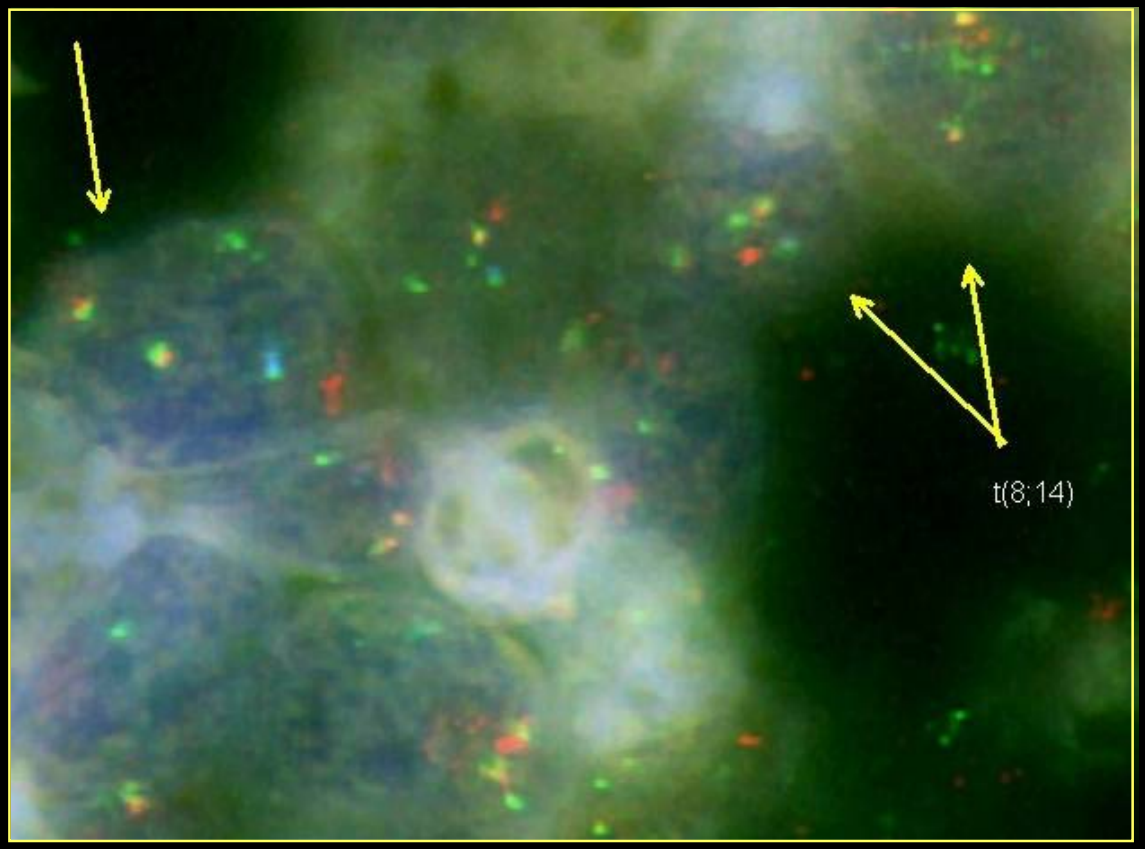
t(8;14) positive (2F,1G,1R,2B)

- Classic Burkitt
- 2 chromosome 8
- 2 fusions
- Residual *BCL2* & *IgH*

O'Connor & Barrans, HMDS (2005)

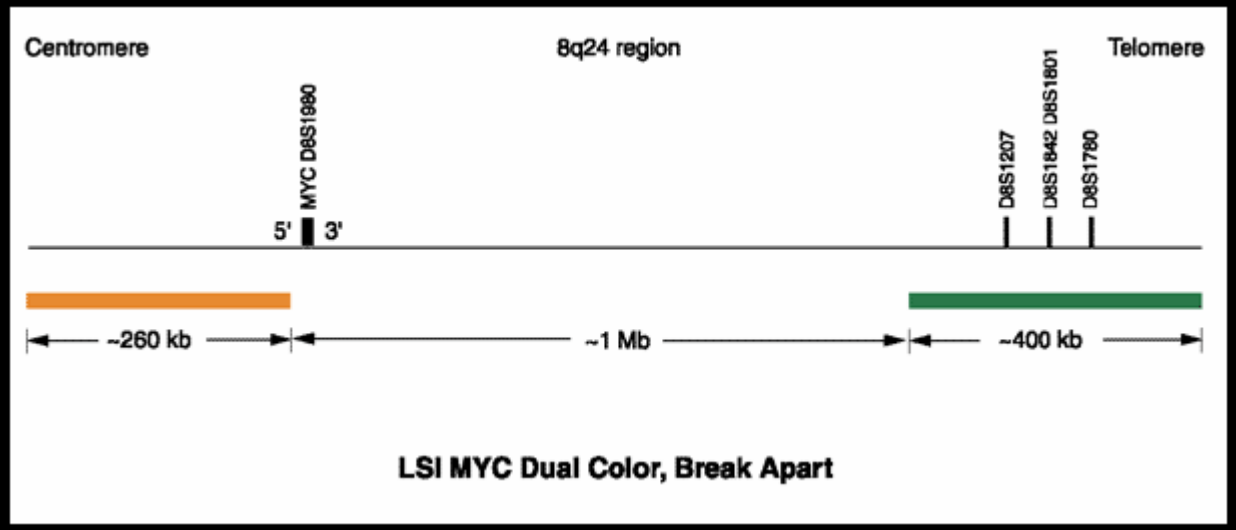
BL ~ *cMYC/IgH* probe set

- Effusion fluid from abdominal mass.
- Cytospin preparation
- <50% cell viability by flow
- no cytogenetics
- Classical t(8;14) detected using FISH.



O'Connor & Barrans, HMDS (2005)

LSI® MYC Dual Color, Break Apart Rearrangement Probe (32-191096)



O'Connor & Barrans, HMDS (2005)

cMYC rearrangement in BL / DLBCL

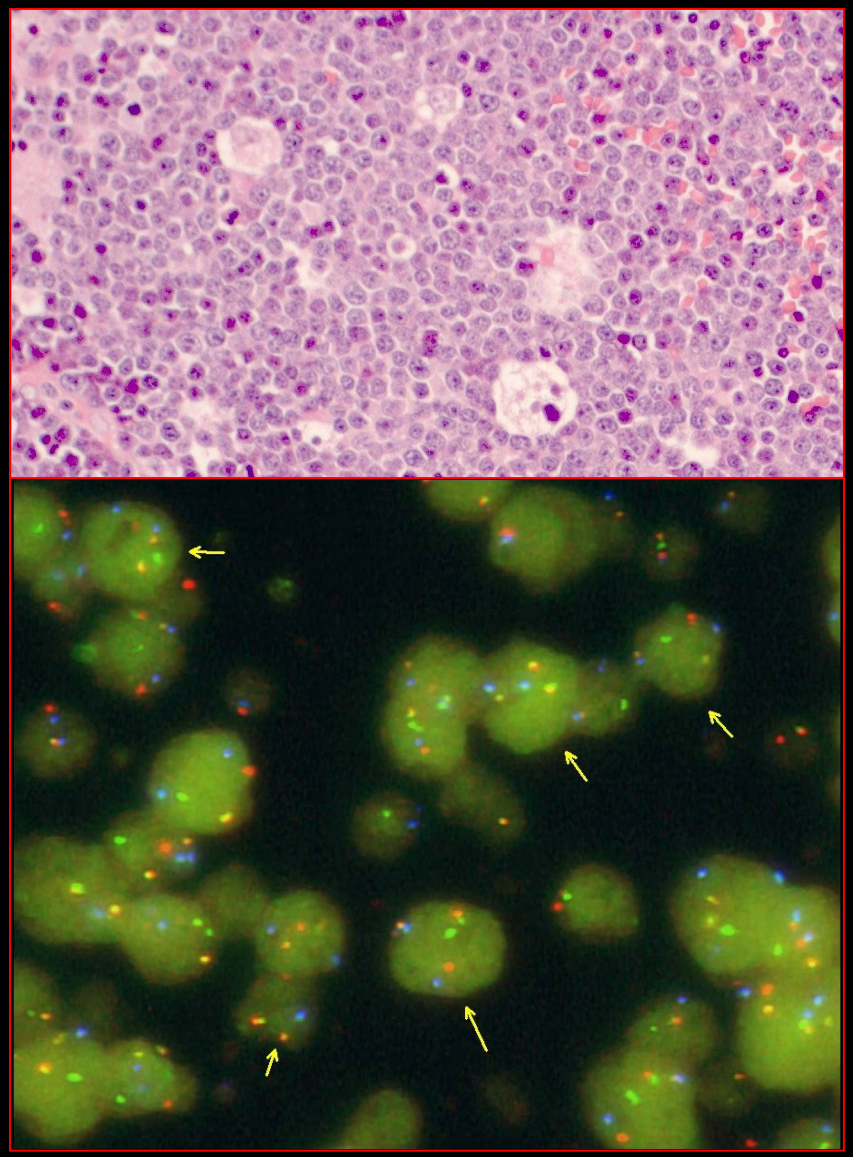
- cMYC rearrangement (most commonly t(8;14)) is the diagnostic feature of Burkitt lymphoma (BL)

- cMYC rearrangement is also detected in a subset of DLBCL
  - ? relationship of cases with these features to BL
  - ? presence of cMYC rearrangement alone sufficient to identify DLBCL with a very aggressive clinical course
  - Morphology unreliable

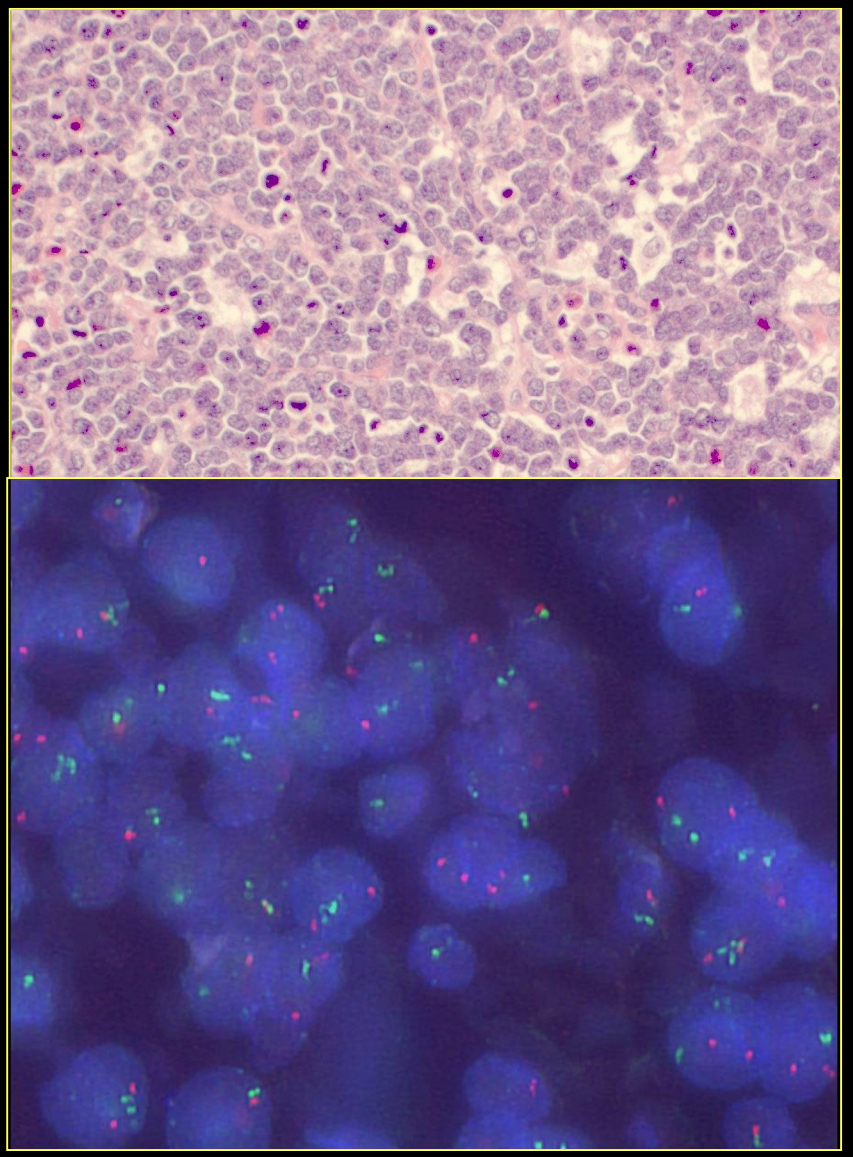
- 82 cases of DLBCL / BL
  - *BL phenotype in 47/82 (57%)* : CD20+,CD3-CD10+,BCL6+,BCL2,P53+P21- and >95% cell cycle fraction, defined by Ki-67 (clone MIB-1).
  - In adults t(8;14) only found in cases with a BL phenotype (in 36/47 (77%))
  - The presence of rearrangement of *cMYC* in cases with *BCL2* abnormalities indicates tFL (3/35 non-BL phenotype cases)

O'Connor & Barrans, HMDS (2005)

t(8;14) Positive
Burkitt Lymphoma



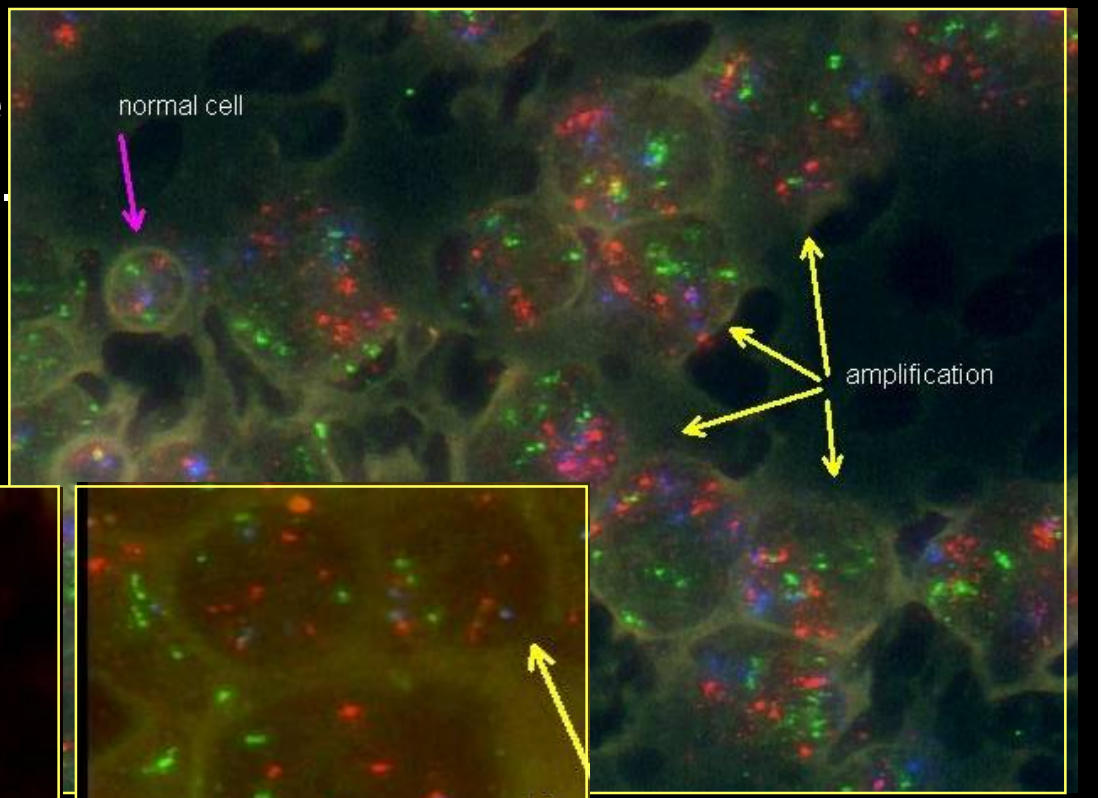
t(8;14) negative,
Typical Phenotype, DLBCL

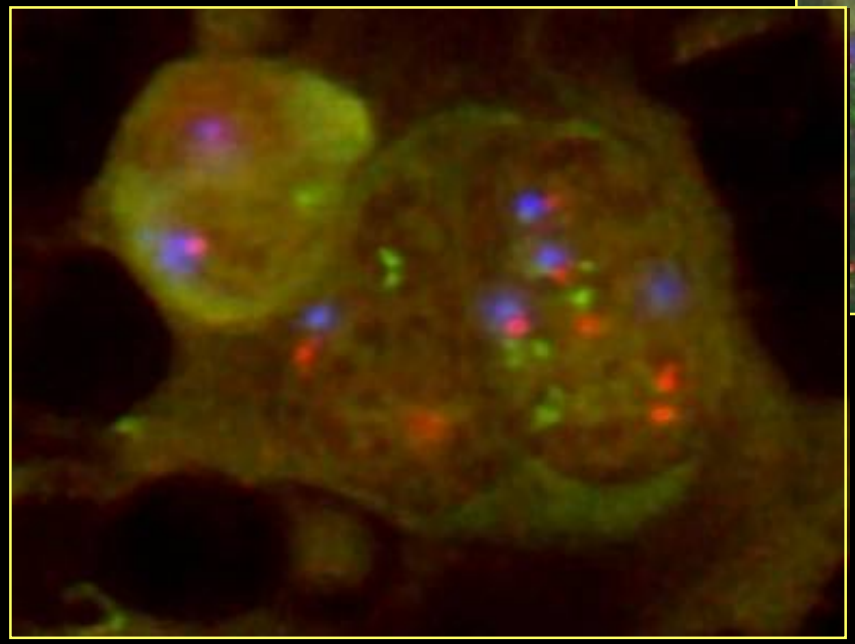


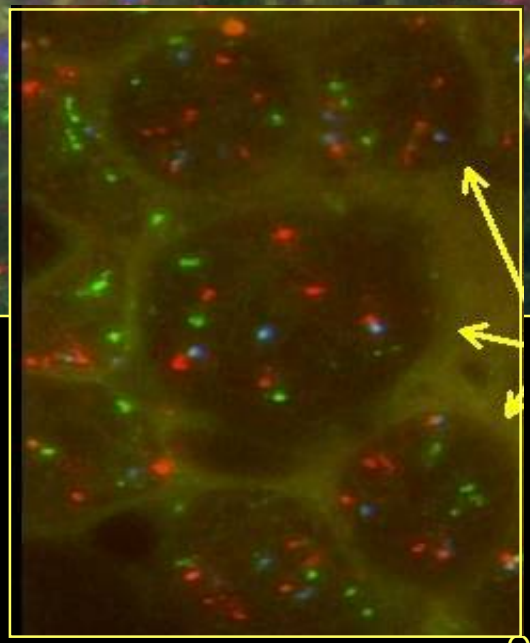
O'Connor & Barrans, HMDS (2005)

DLBCL ~ *c-MYC/IgH* probe set

- Amplification of *c-MYC* (4 copies of chromosome 8 with > 8 copies *c-MYC*).
- Normal neutrophil to left has normal pattern (2B2R2G) lower image.







O'Connor & Barrans, HMDS (2005)

Features of Diffuse large B cell lymphoma

- Diffuse large B cell Lymphoma (DLBCL) is a heterogeneous disease group, varying in clinical presentation, natural history and biology of the cells constituting the tumors
  - DLBCL are thought to be germinal center (GC) or post-GC derived
  - Variable expression of many cellular markers / proteins by immunocytochemistry
  - Many genetic abnormalities are demonstrated, some have prognostic significance
  - Often complex karyotypes.
- One third of all patients die as a result of refractory or early relapsing disease.
- The IPI successfully identifies subgroups of patients with a very poor or a good outcome. However, half of all patients have an intermediate IPI with an indeterminate outcome
- Many studies have investigated whether various biological risk factors can be used to predict outcome in DLBCL.
  - The presence of a GC phenotype / genetic profile is a favourable feature
  - Rearrangement of the *BCL6* gene at 3q27 and the presence of a t(14;18) are adverse prognostic factors

O'Connor & Barrans, HMDS (2005)

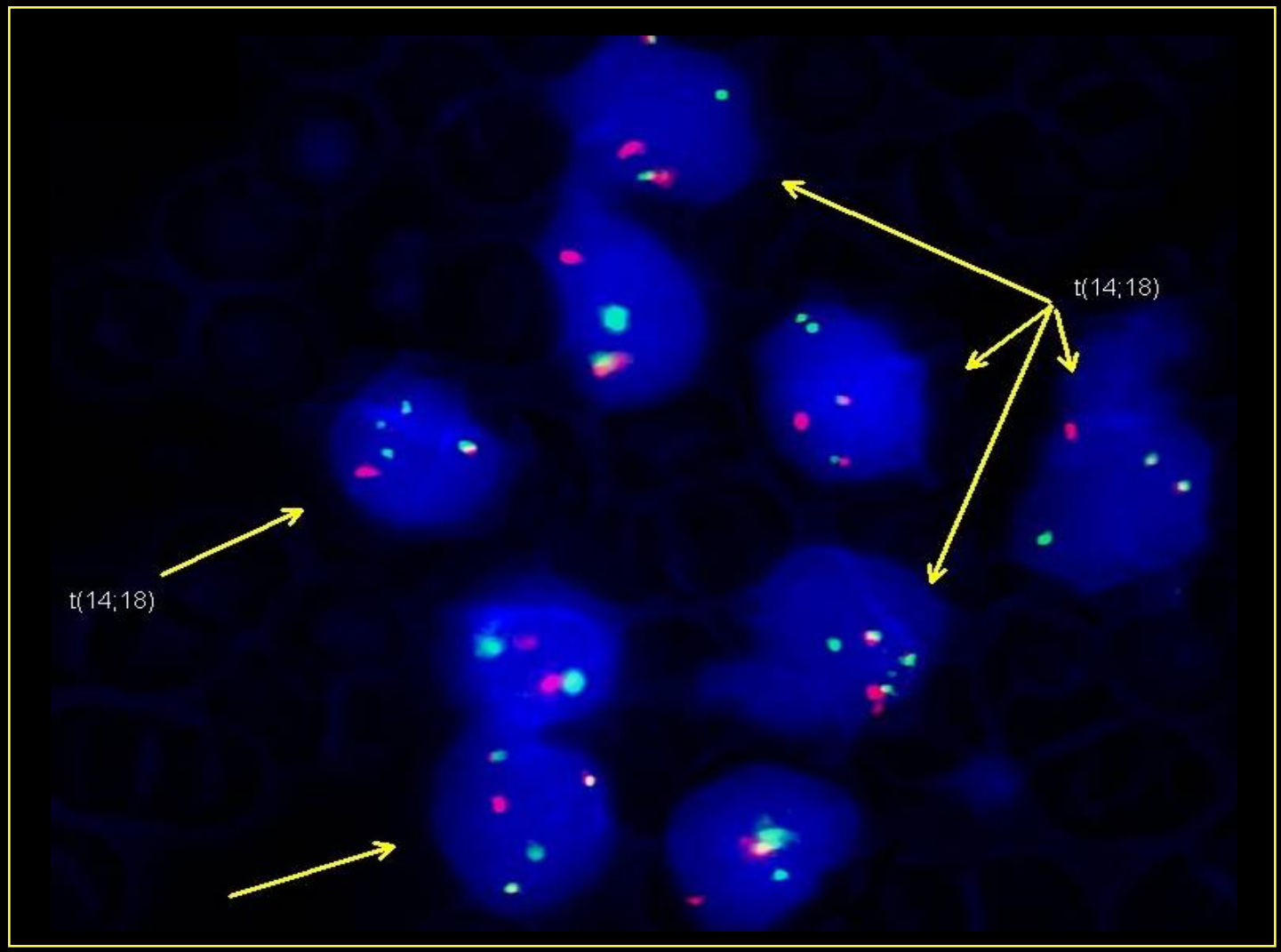
Molecular abnormalities in DLBCL

- 23/246 (9%) (t(14;18) positive) had a previously reported FL at an alternative tissue site (DLBCL with underlying FL)
  - 15/23 had a complex karyotype including *cMYC* rearrangement
- 33/203 (16%) De novo DLBCL had a t(14;18) by FISH
- 42/178 (24%) have rearrangement of *BCL6 (38 abnormal ??)*
- 28% have deletion of *P53* at 17p13

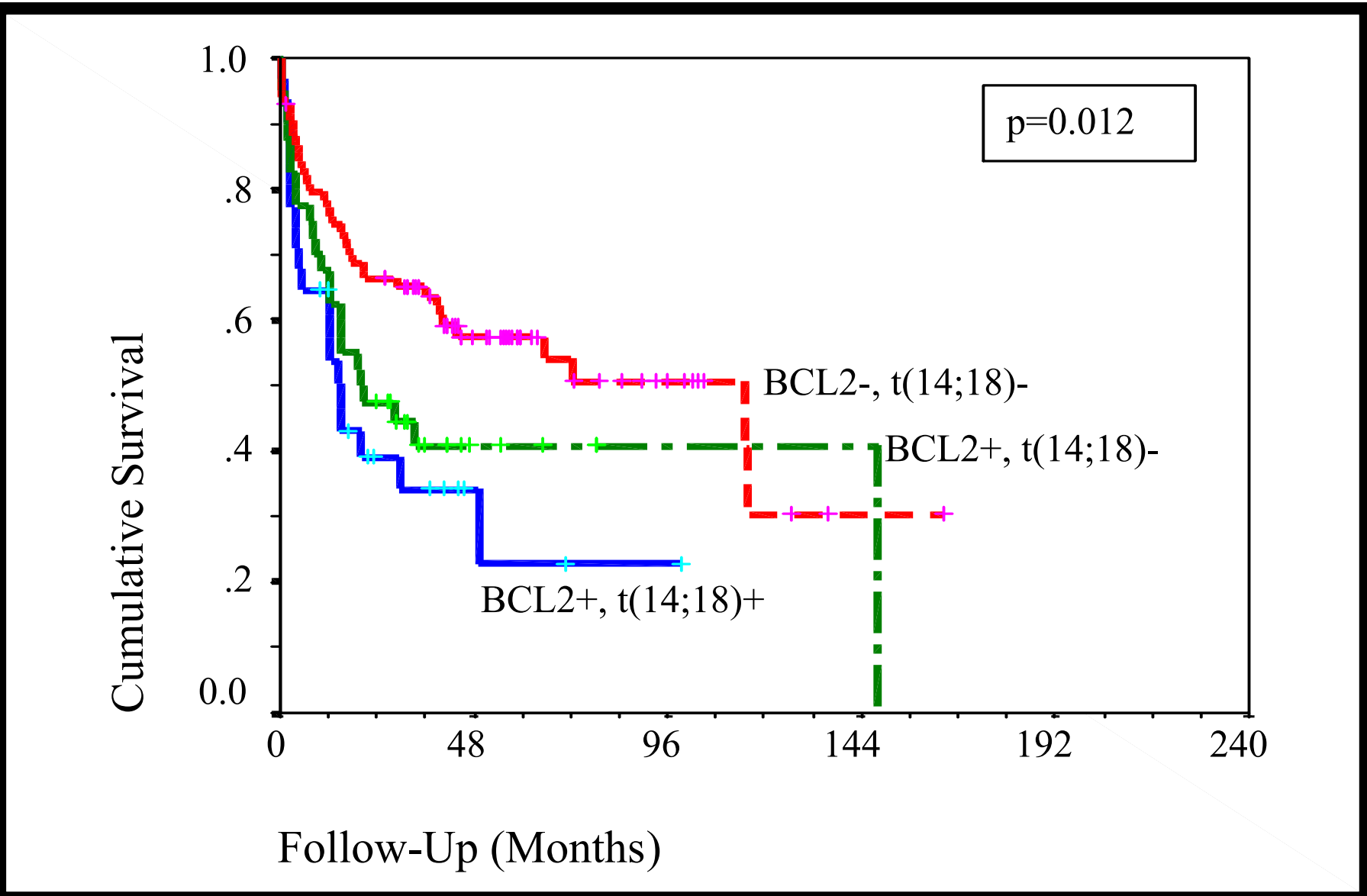
O'Connor & Barrans, HMDS (2005)

BCL2 / IgH probe set in DLBCL

- Standard t(14;18)

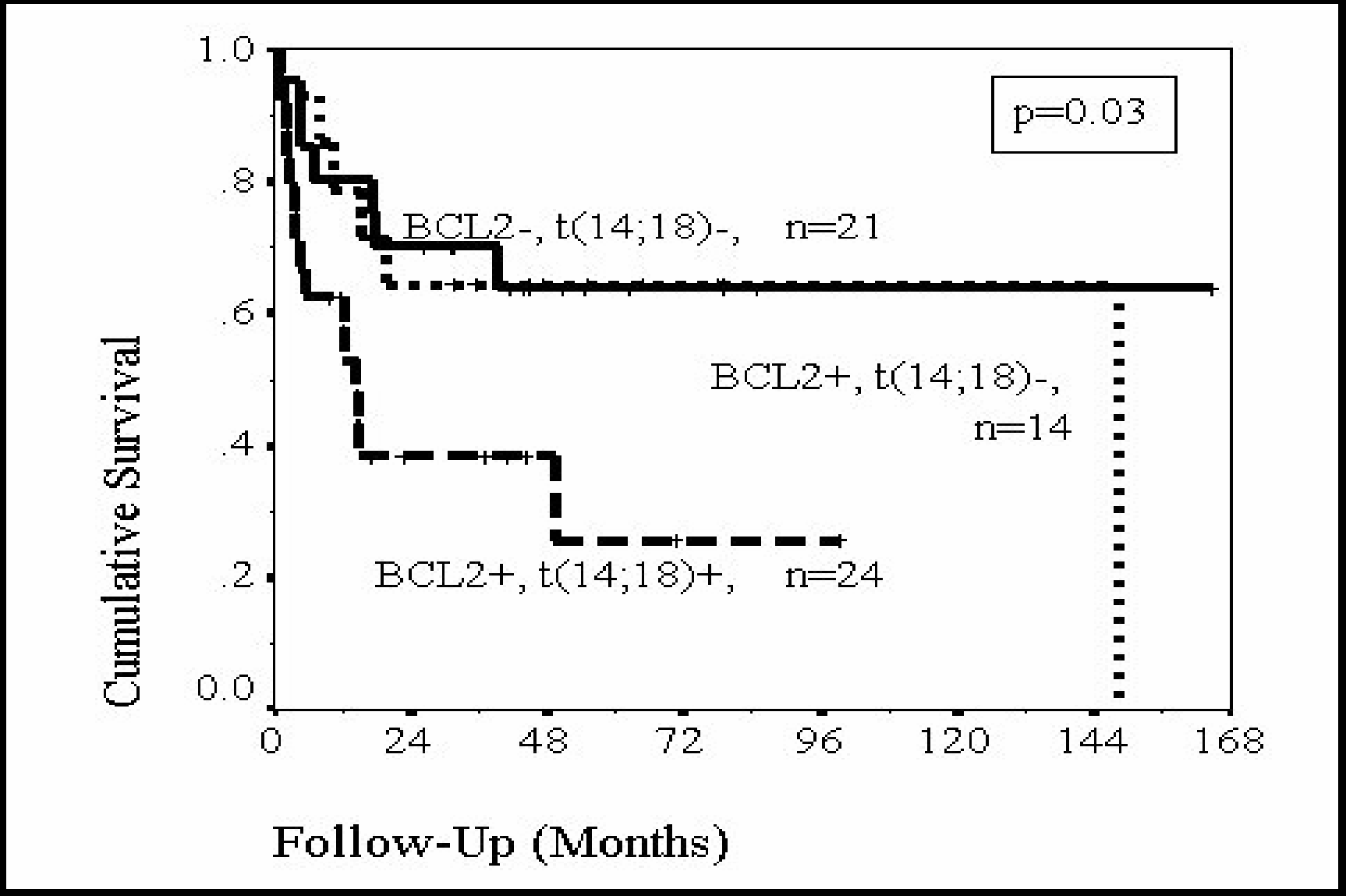


O'Connor & Barrans, HMDS (2005)



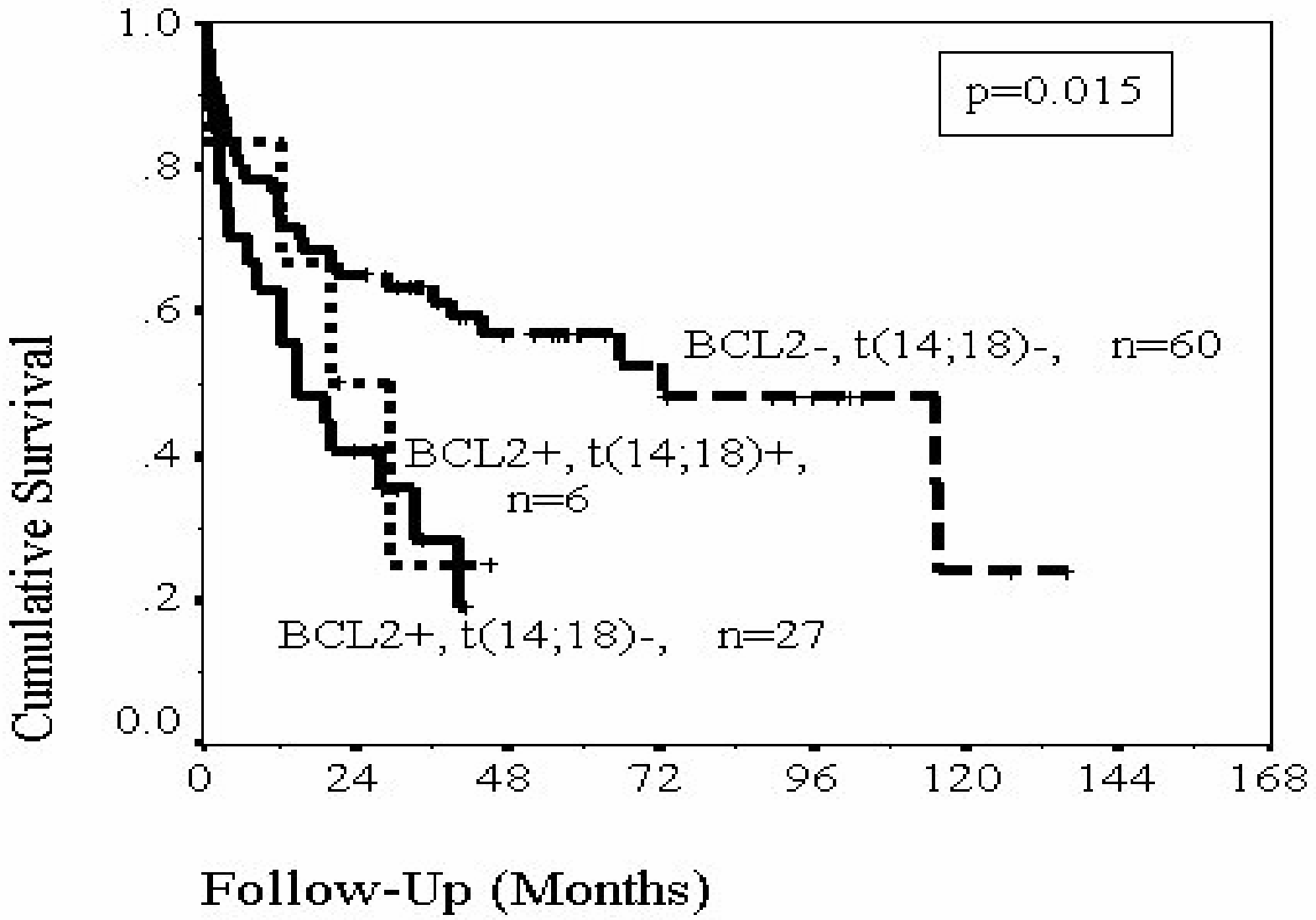
Kaplan-Meier Survival Analysis of Overall Survival in nodal DLBCL, stratified according to the expression of BCL2 and the presence of the t(14;18)

O'Connor & Barrans, HMDS (2005)



Overall Survival of GC-DLBCL classified according to BCL2 and t(14;18) status.

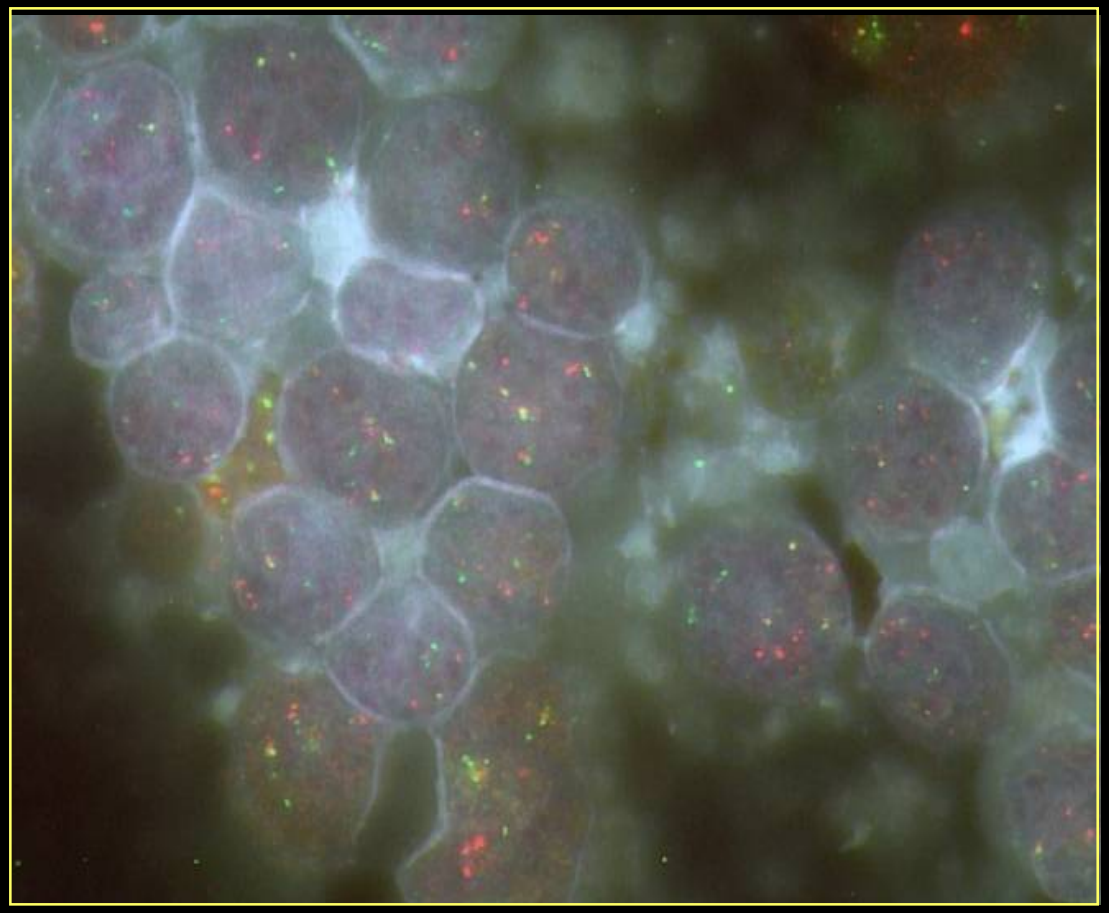
O'Connor & Barrans, HMDS (2005)



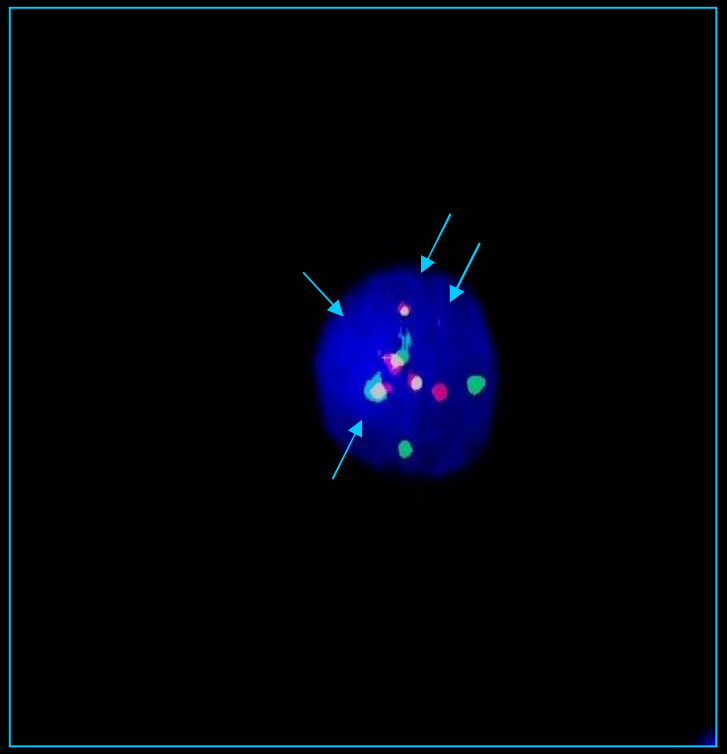
Overall Survival of non-GC DLBCL classified according to BCL2 and t(14;18) status.

O'Connor & Barrans, HMDS (2005)

IgH/BCL2 Probe Set
Alternative signals patterns - common in DLBCL ~ t(14;18) with multiple fusions



(Lymph node dab)

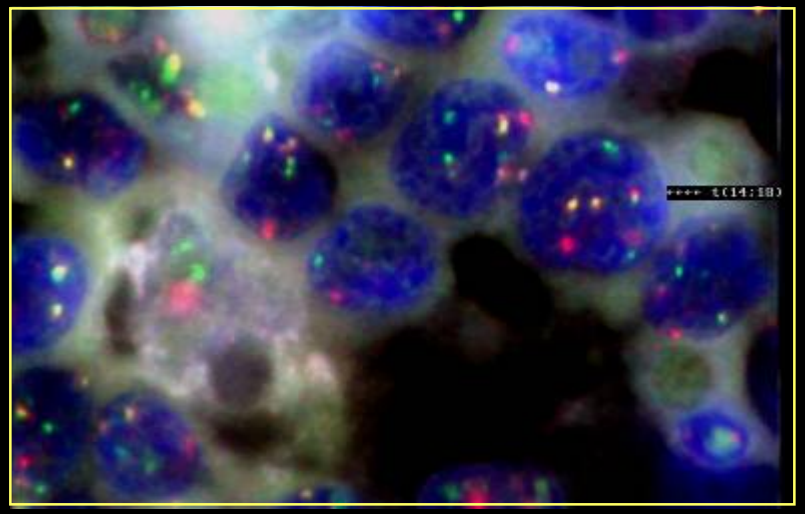


(Paraffin Nuclei)

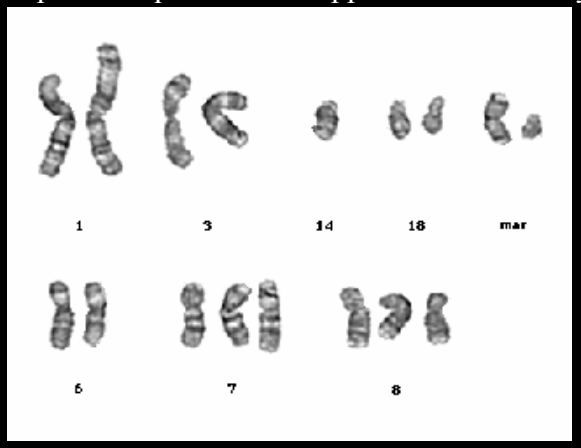
O'Connor & Barrans, HMDS (2005)

tFL - t(14;18) FISH Images Pre- & Post-Transformation And Post-Transformation Karyotype From The Same Patient

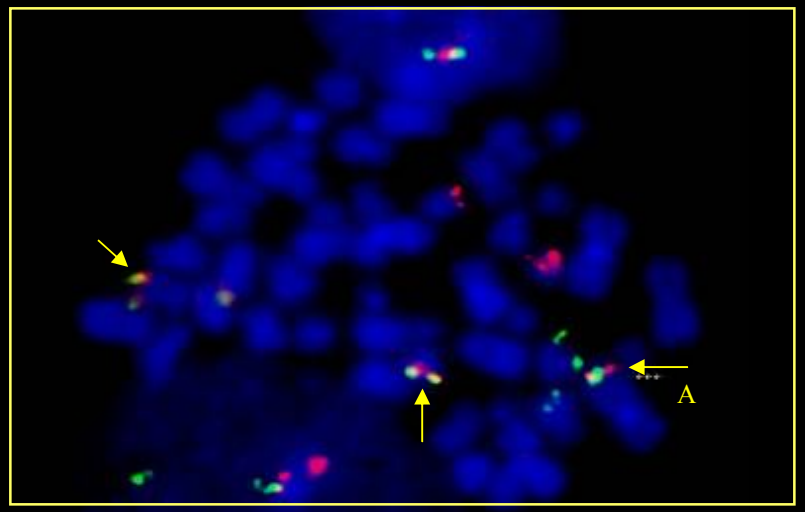
Post transformation sample - touch preparation.
Multiple fusion signals, extra copies also of *IgH* and *BCL2*



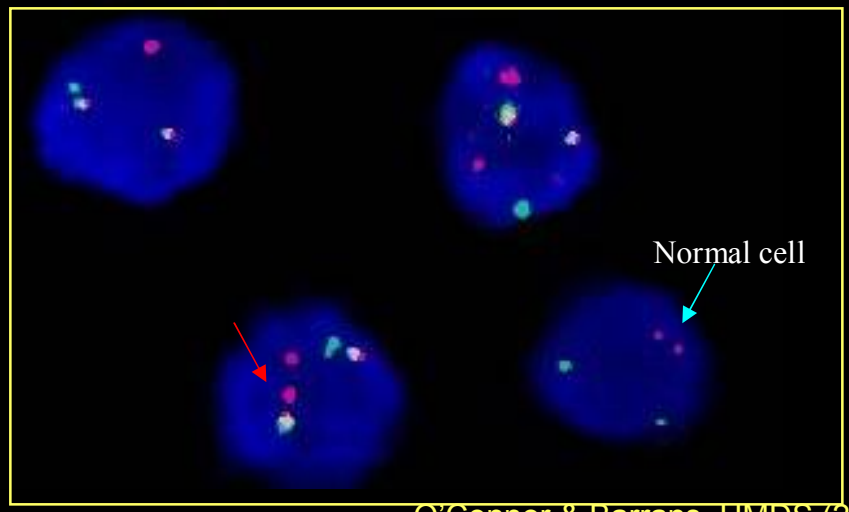
Post transformation karyotype;
Extra material on 1p and material missing from 18 and 14 (??markers).
+7 & +8 and part of 6q is deleted. Apparent abnormality at 3q



Post transformation sample - cytogenetics metaphase preparation.
A is the der(1)t(1;14;18), the fusion signal is on Chromosome 1.

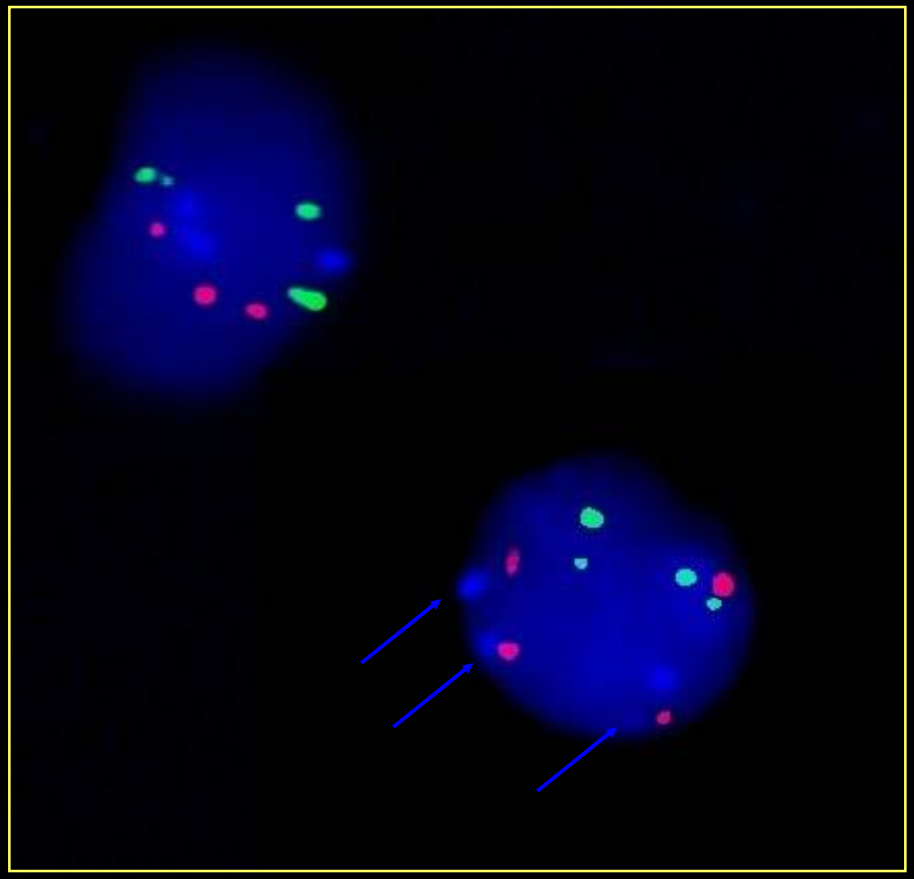


Pre-transformation sample - paraffin extracted nuclei.
Standard t(14;18) double fusions & an extra *BCL2* in one cell



O'Connor & Barrans, HMDS (2005)

IgH/BCL2 Probe Set
Alternative signals patterns - common in DLBCL ~ Trisomy 18



(Paraffin nuclei)

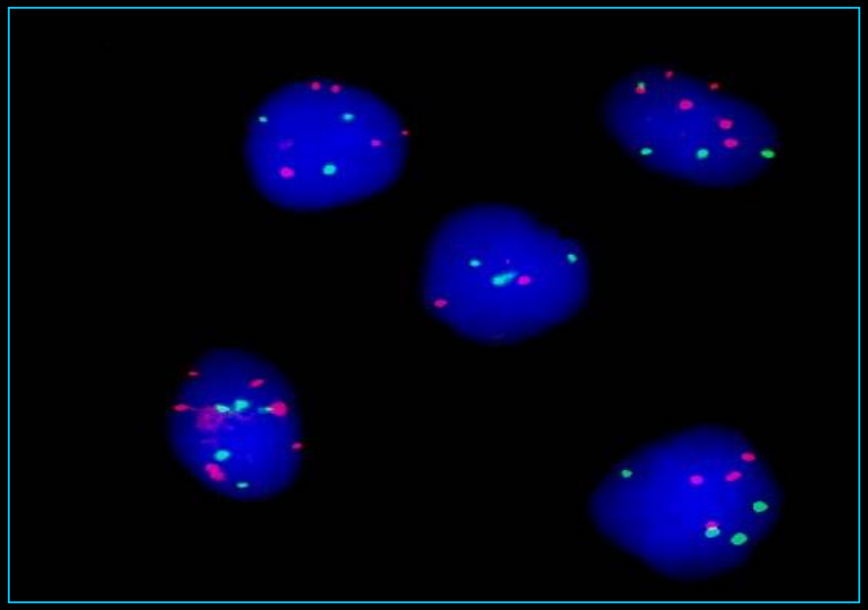
- 3 red signals (*BCL2*)

- 3 blue arrows indicate 3 signals with CEP 18 aqua probe

- 3 green signals also (*IgH*)

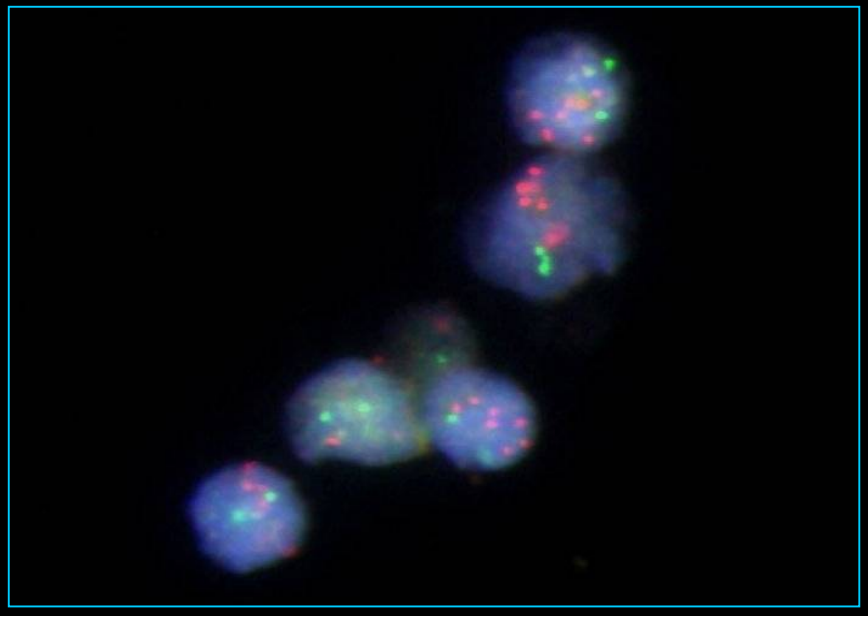
O'Connor & Barrans, HMDS (2005)

IgH/BCL2 Probe Alternative signals patterns - common in DLBCL ~ extra signals



Extra Signals of Both *BCL2* and *IgH*

- 3 and 5 copies of both *IgH* and *BCL2*,
- no fusions,
- aneuploidy - extra copies of chromosomes 14 and 18.



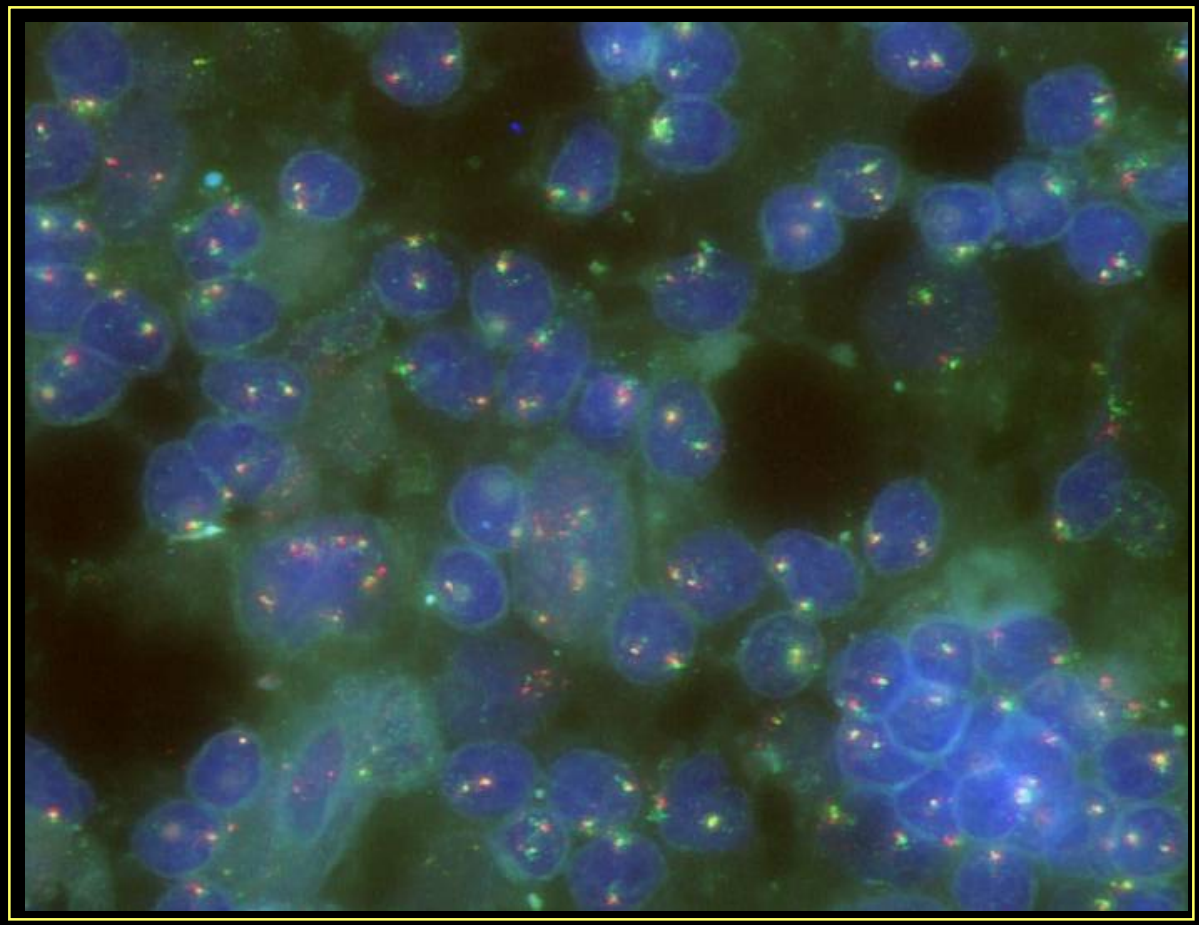
***BCL2* amplification**

- multiple clustered signals of the red (*BCL2*) probe
- no fusions with *IgH*
- 2 *IgH* signals in each cell.

O'Connor & Barrans, HMDS (2005)

DLBCL ~ 3q27 probe set

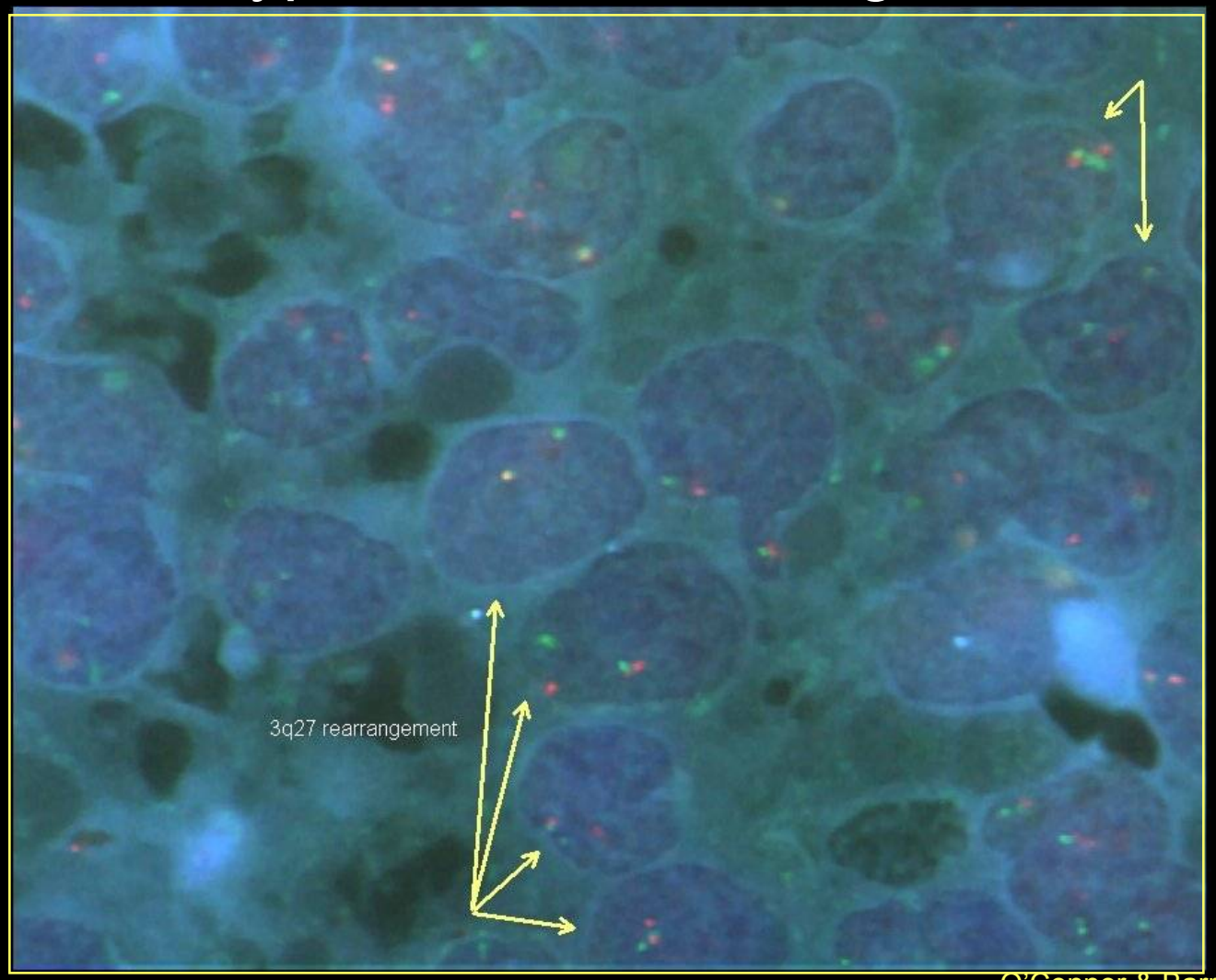
- Normal *BCL6*



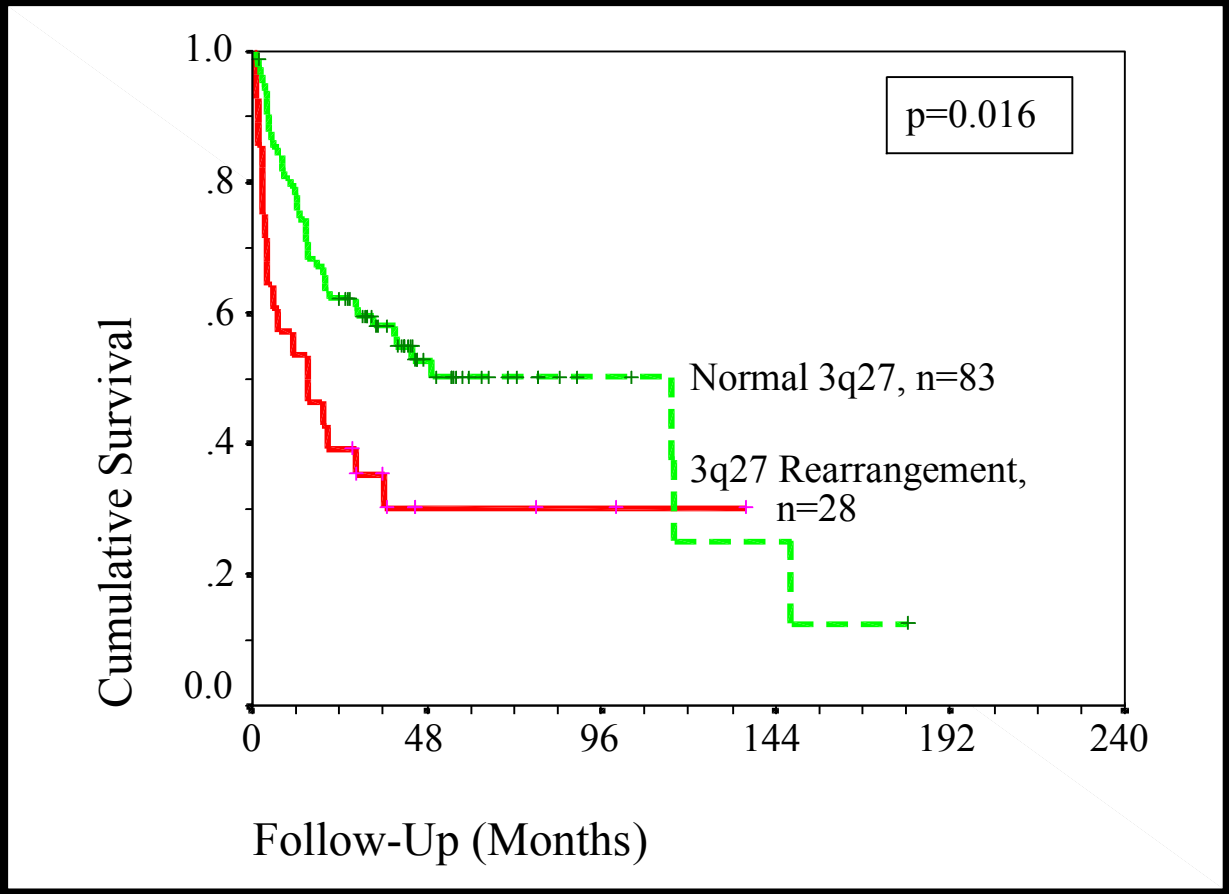
O'Connor & Barrans, HMDS (2005)

DLBCL ~ 3q27 probe set

- Typical *BCL6* rearrangement



O'Connor & Barrans, HMDS (2005)



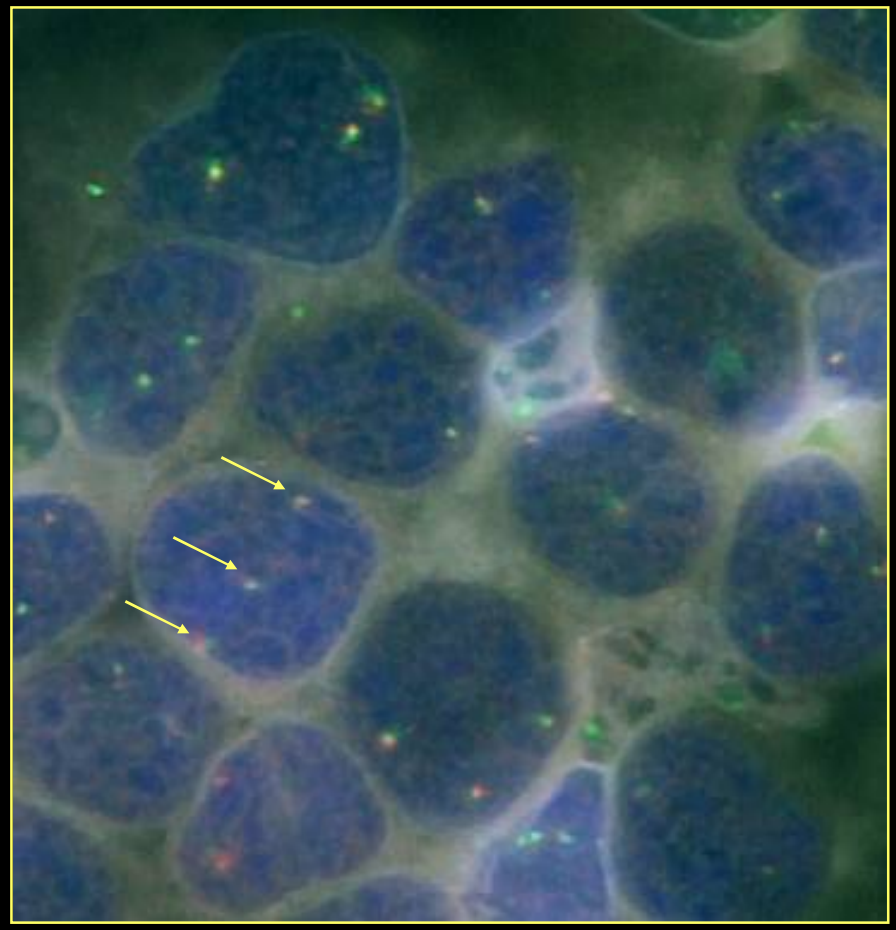
- BCL6 Rearrangement is associated with a poor prognosis in nodal DLBCL
- Some studies have shown no effect
- Different translocation partners may have different biological and prognostic effect

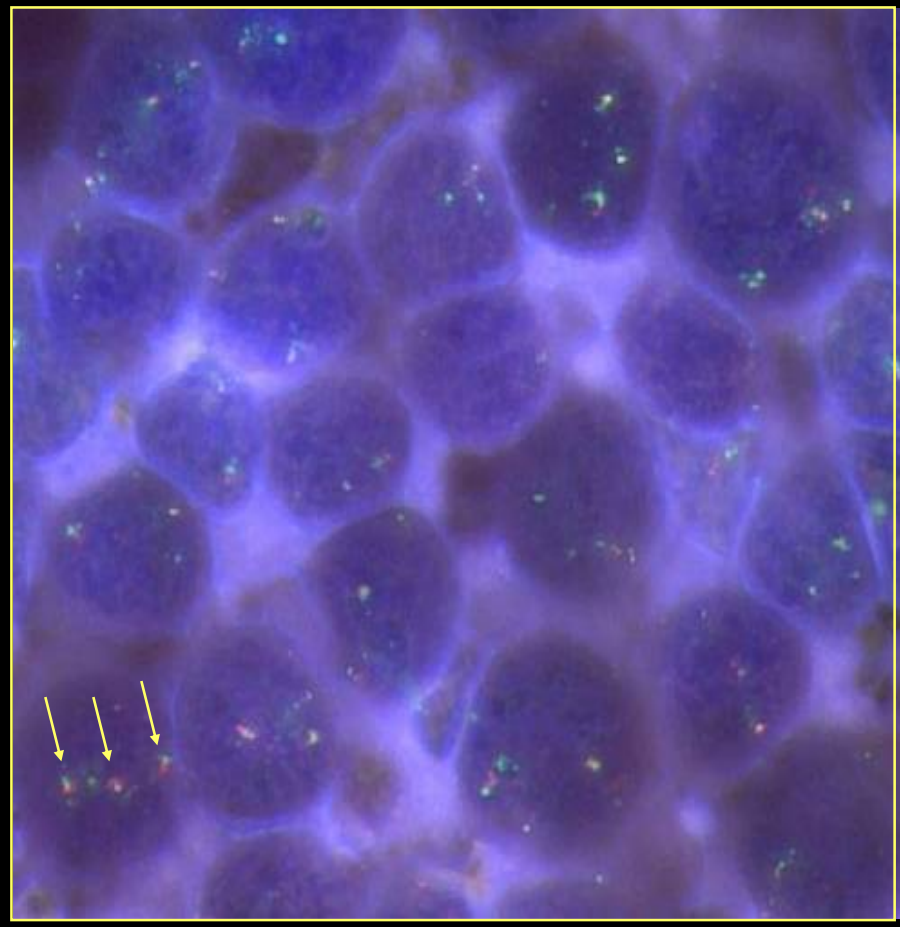
Kaplan-Meier Survival Analysis Of Nodal DLBCL Patients With And Without A 3q27 Rearrangement

O'Connor & Barrans, HMDS (2005)

DLBCL ~ 3q27 probe set

- Trisomy 3 ~ 3 fusion signals



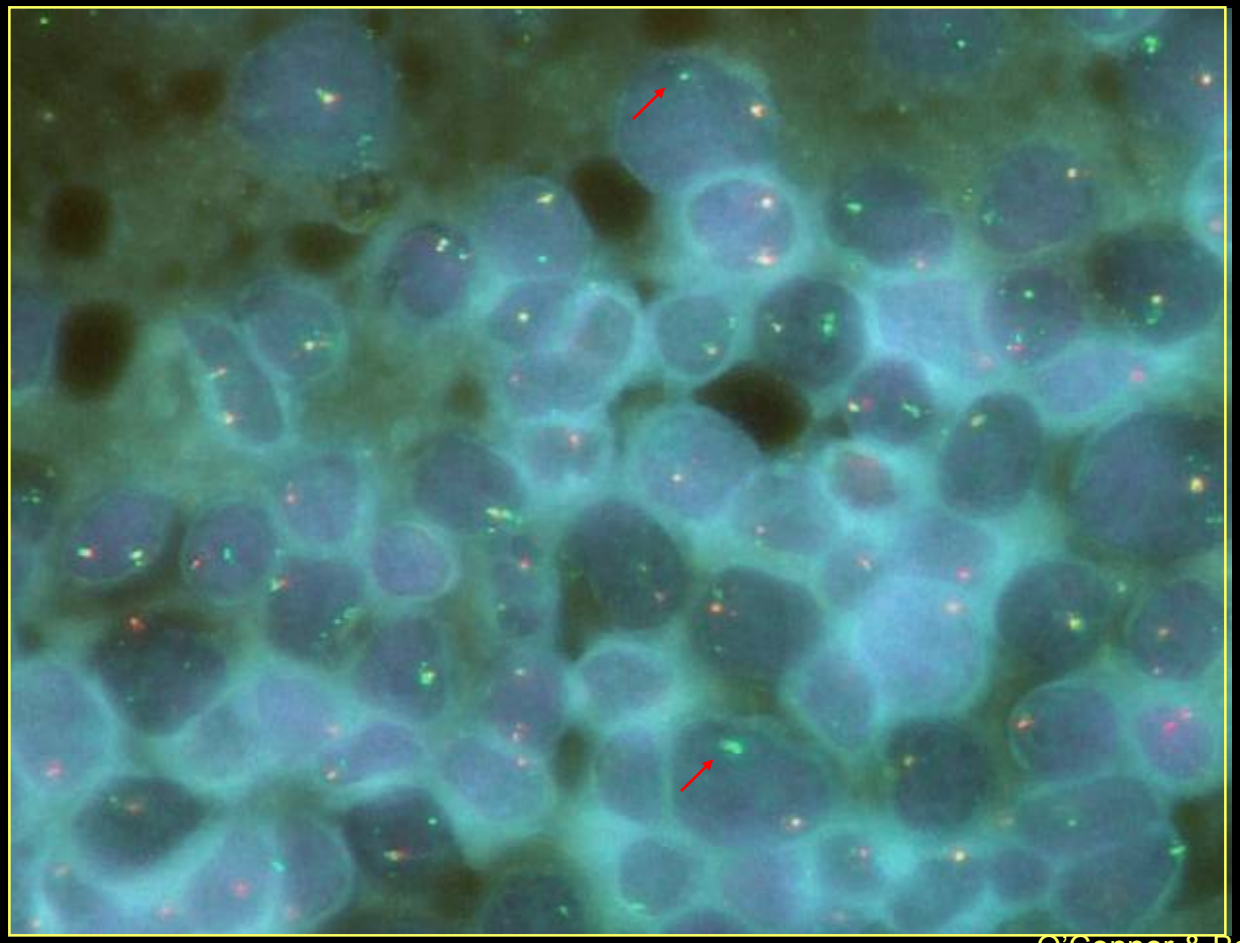


(Lymph node dabs)

O'Connor & Barrans, HMDS (2005)

DLBCL ~ 3q27 probe set

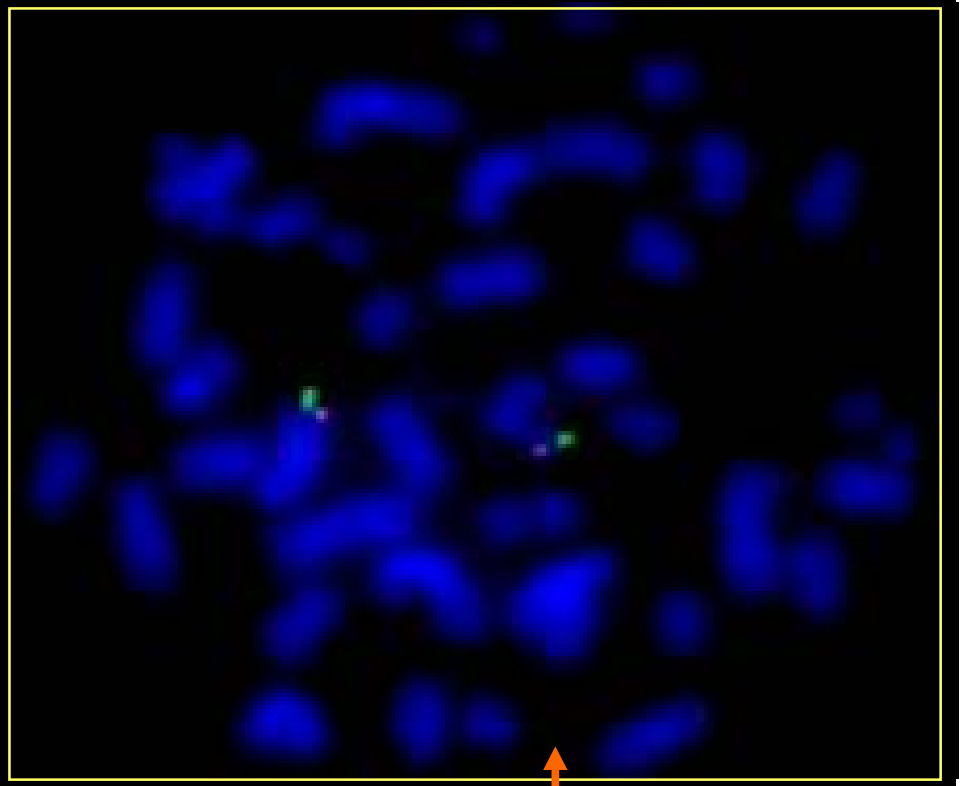
- Rearrangement of 3q27 with loss of the terminal portion of *BCL6* (red signal lost)



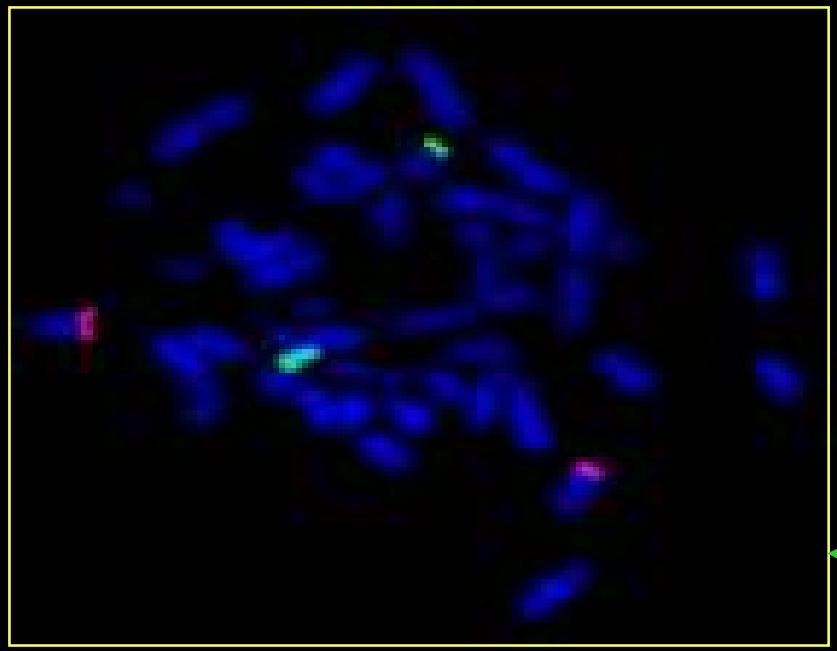
O'Connor & Barrans, HMDS (2005)

DLBCL ~ 3q27 probe set

- Typical BL by WHO classification
- But *cMYC/IgH* probe set showed a normal result.
- 3q27 rearranged by cytogenetics but *BCL6* is not rearranged but is translocated to 22.



BCL-6 breakapart probe set

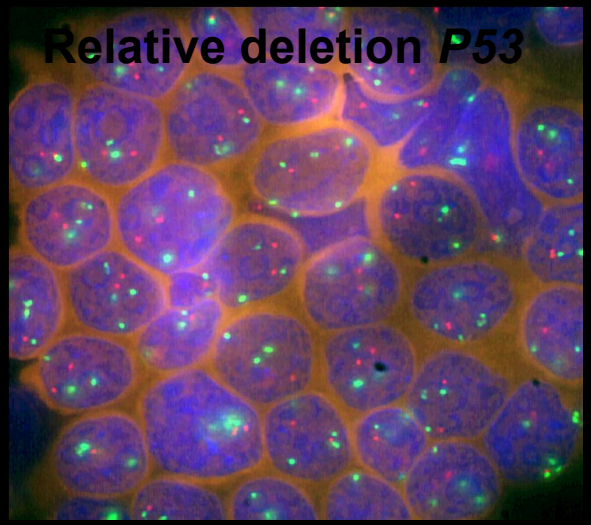


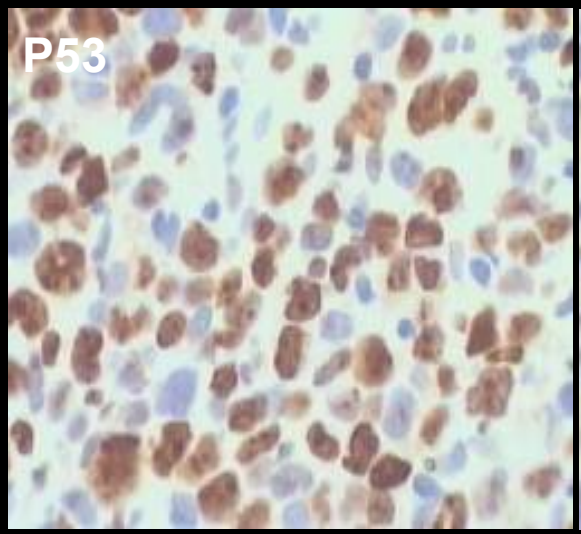
cMYC/IgH translocation probe set

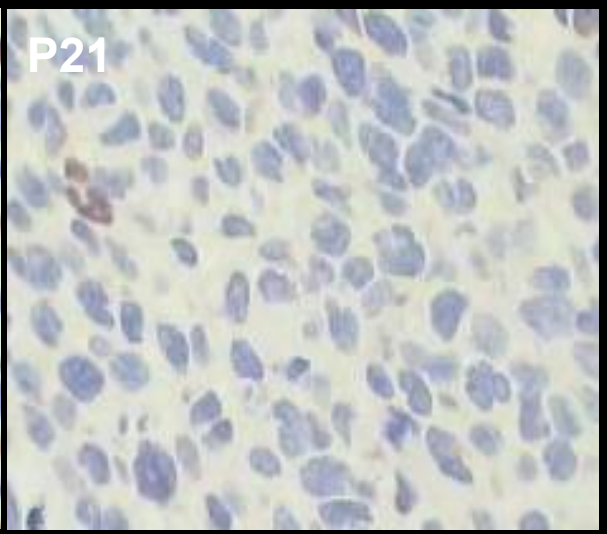
O'Connor & Barrans, HMDS (2005)

Molecular abnormalities in DLBCL

- 28% have deletion of *P53* at 17p13
- Not an independent prognostic factor
- Tumour supressor – mutation 2nd hit

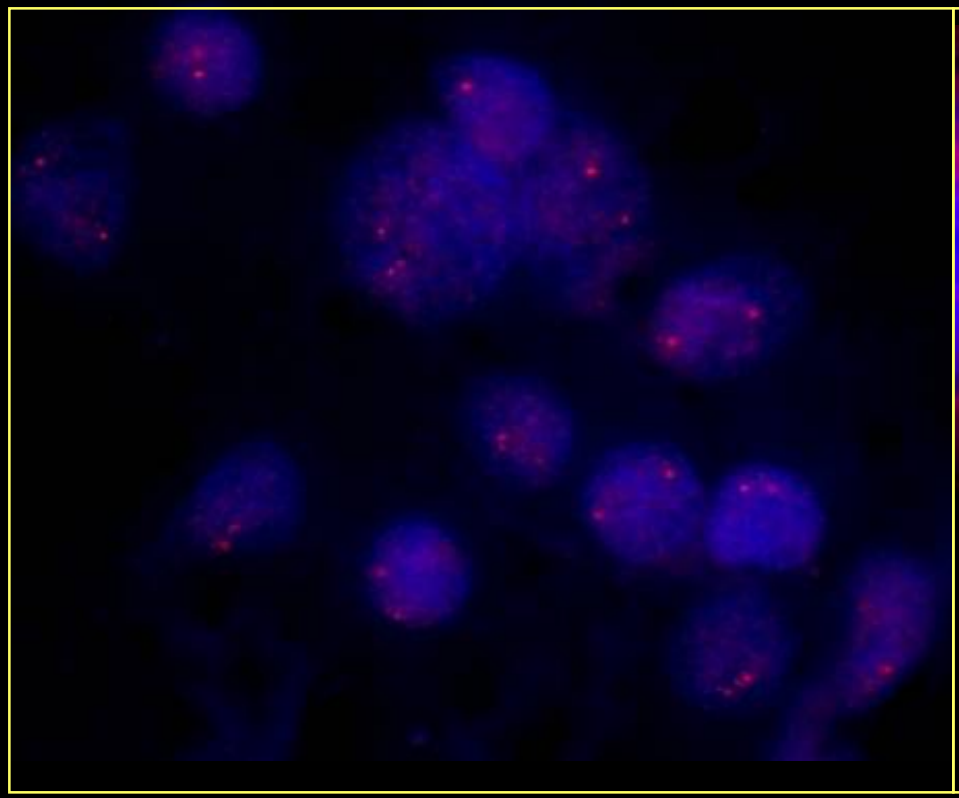


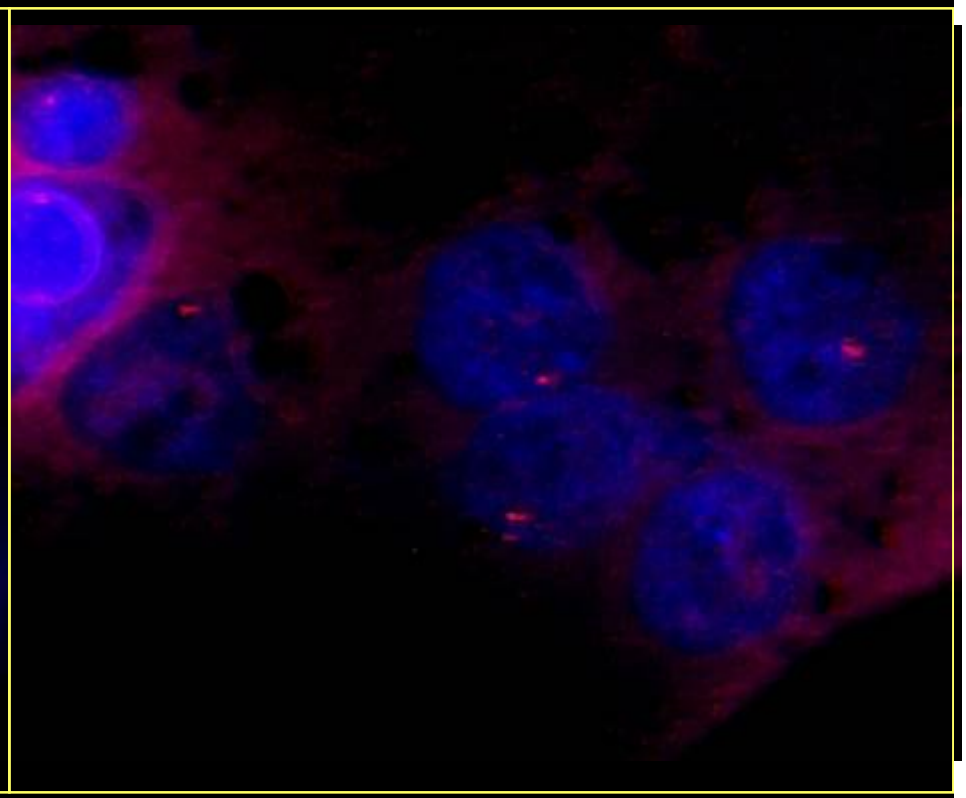




O'Connor & Barrans, HMDS (2005)

p53 deletions - DLBCL





Normal *P53* - 2 copies per cell

P53 deletion - 1 copy per cell

? Association with mutation status

O'Connor & Barrans, HMDS (2005)

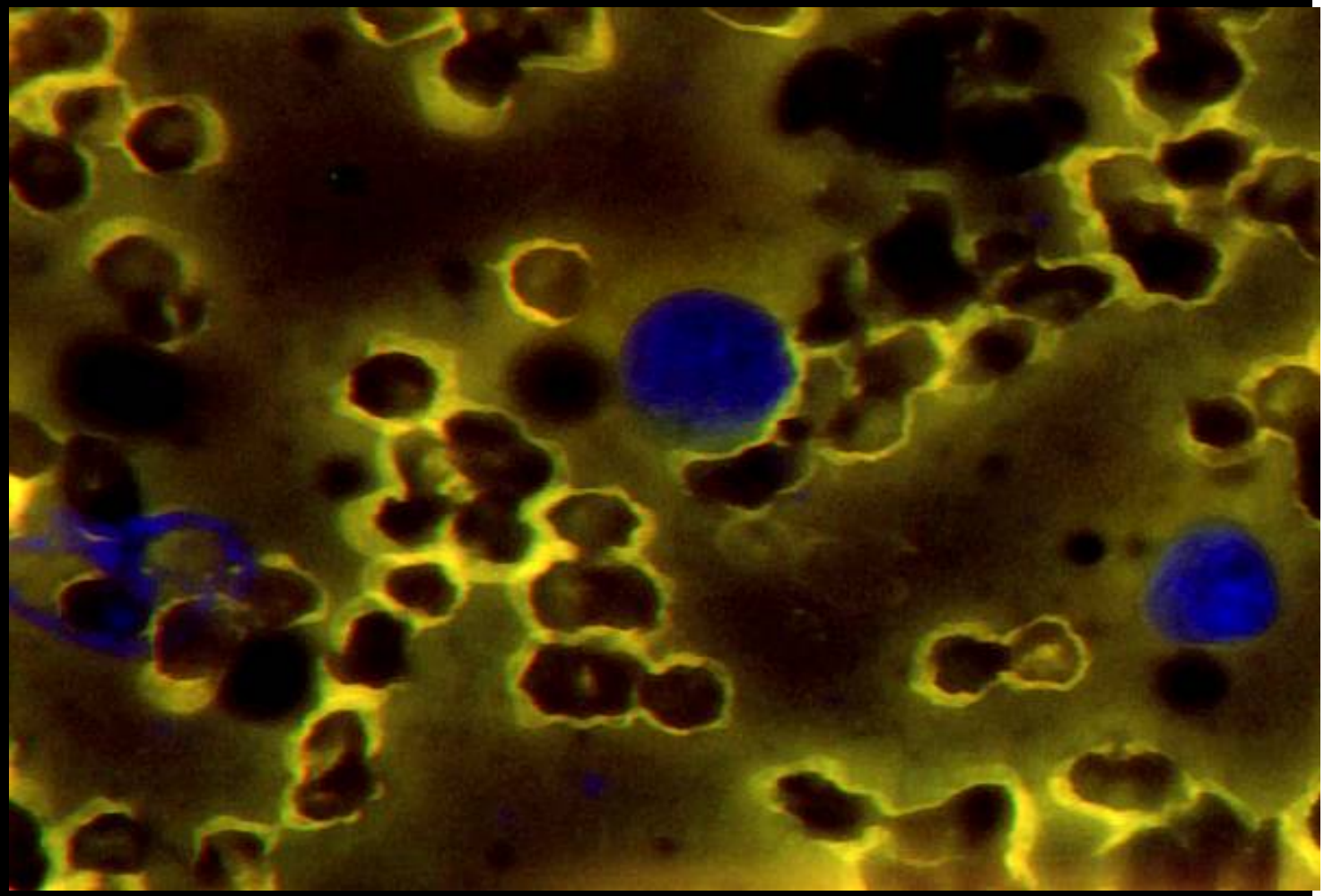
ARTEFACTS

O’Connor & Barrans, HMDS (2005)

Interpretation of FISH: Artefacts

Old sample ~
high background

May work with long fixation



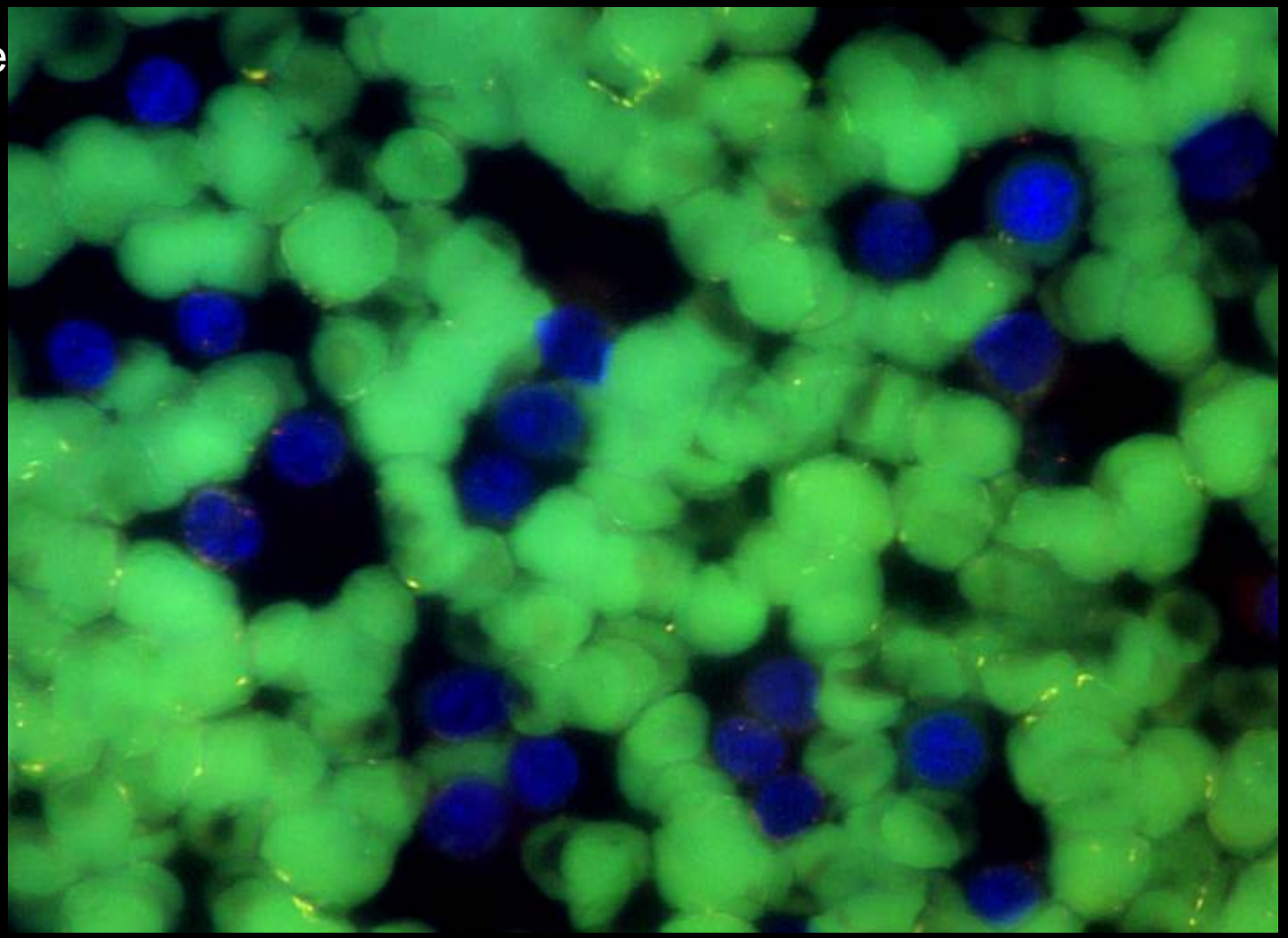
O'Connor & Barrans, HMDS (2005)

Interpretation of FISH: Artefacts

Formalin exposure in transit

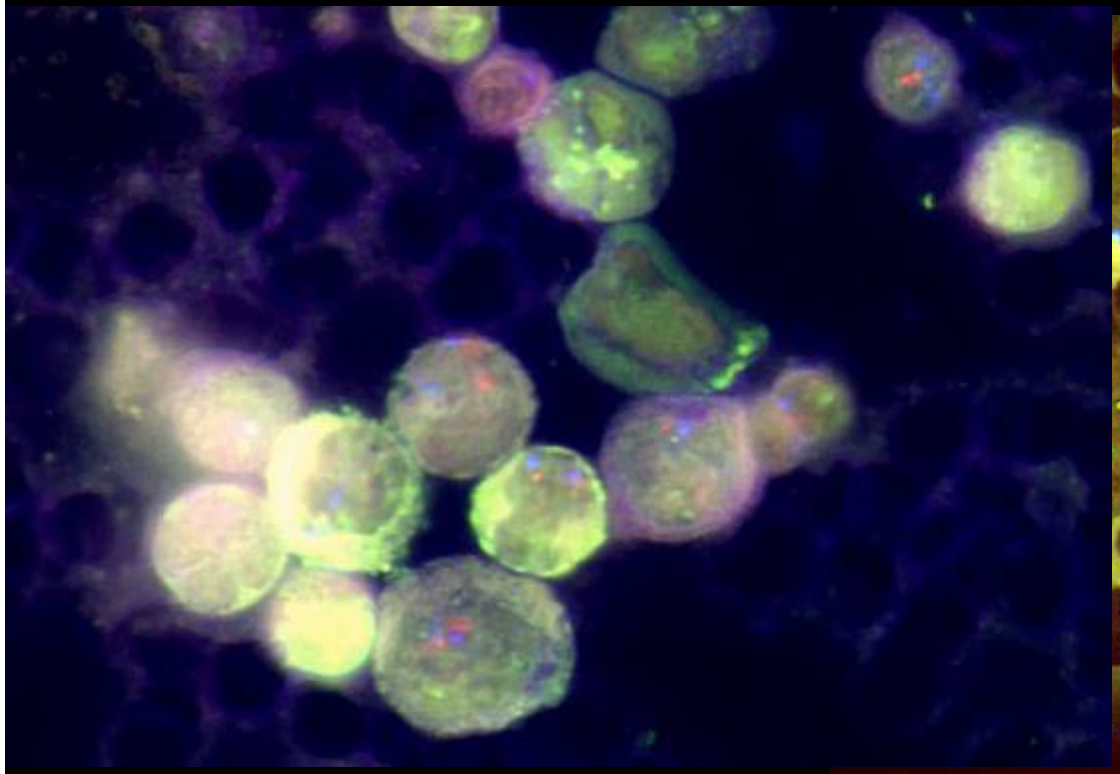
(trephine in same bag)

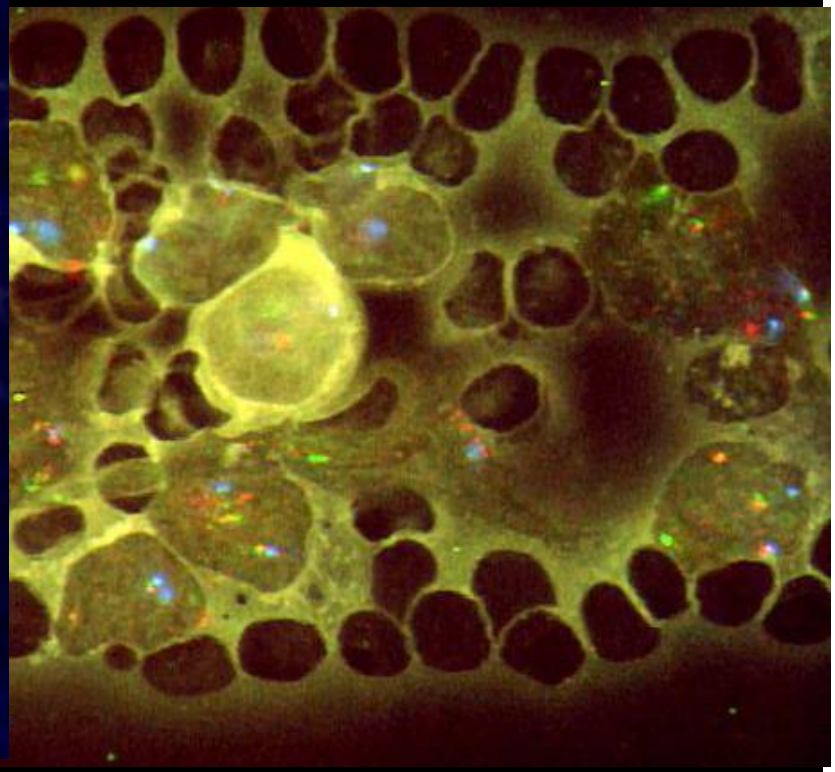
Need repeat sample



O'Connor & Barrans, HMDS (2005)

Interpretation of FISH: Artefacts





Both images are from the same patient:

Left is EDTA BMA: all tumour cells degraded

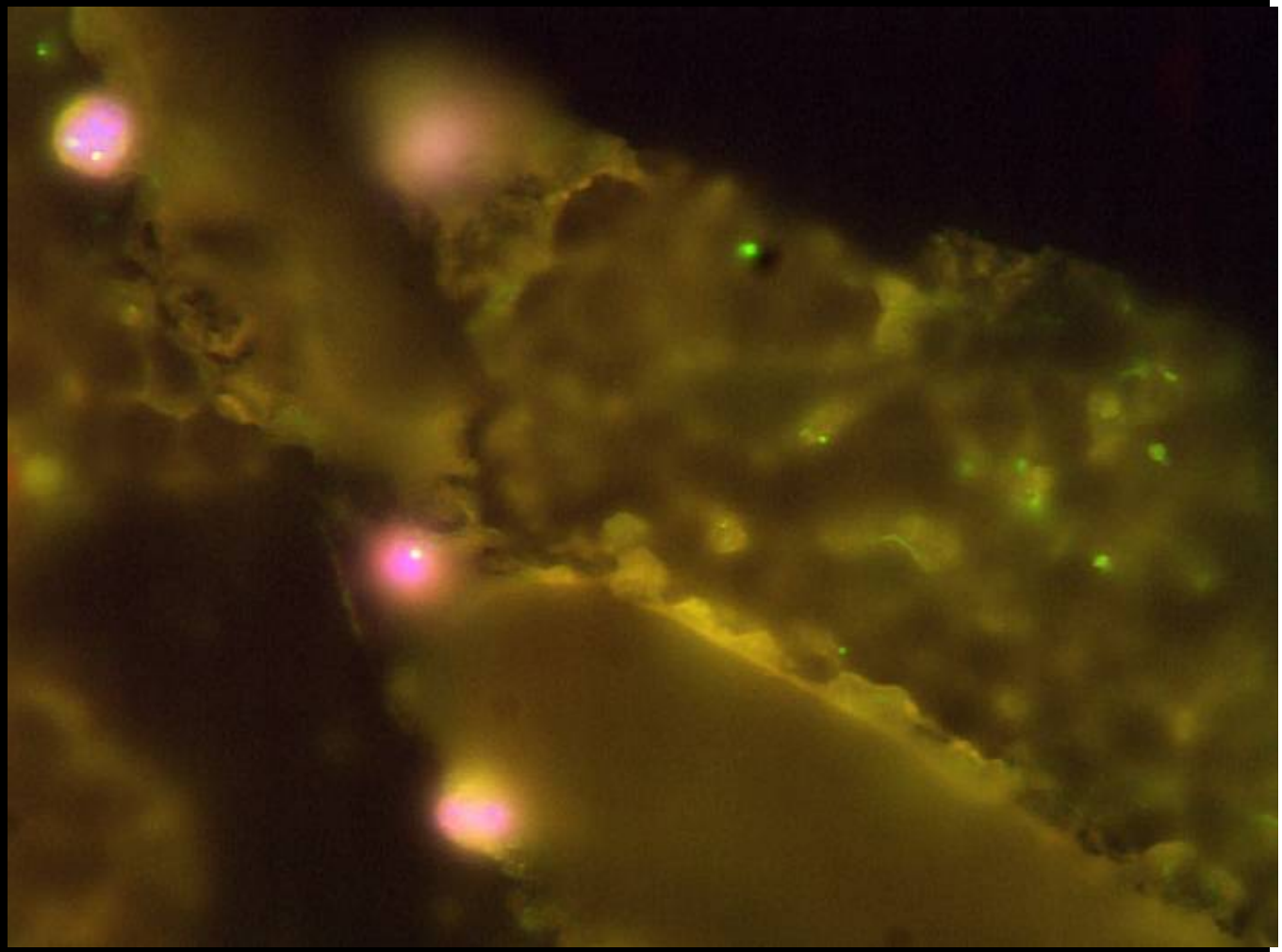
Right is fresh BMA smear: t(8;14) detected ~ Burkitt lymphoma

O'Connor & Barrans, HMDS (2005)

Interpretation of FISH: Artefacts

- Smear floating off
- Bad batch of slides usual cause
- Old smears
- Very thick smears

Can usually find an area to analyse



O'Connor & Barrans, HMDS (2005)

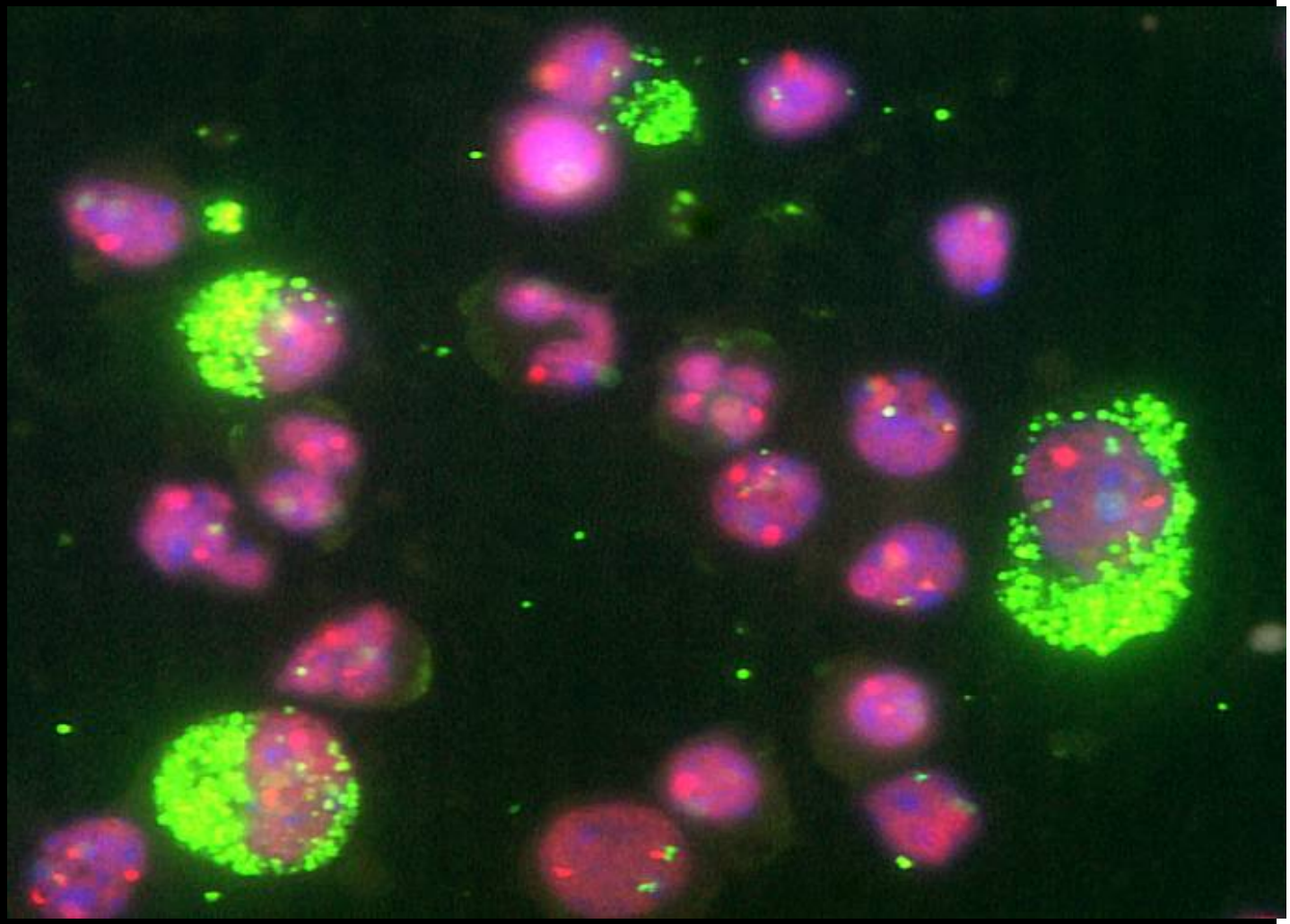
Interpretation of FISH: Artefacts

Eosinophilia:

Common in plasma cell disorders and FL

Cells degranulate and the granules may obscure FISH signals

Image capture can be difficult

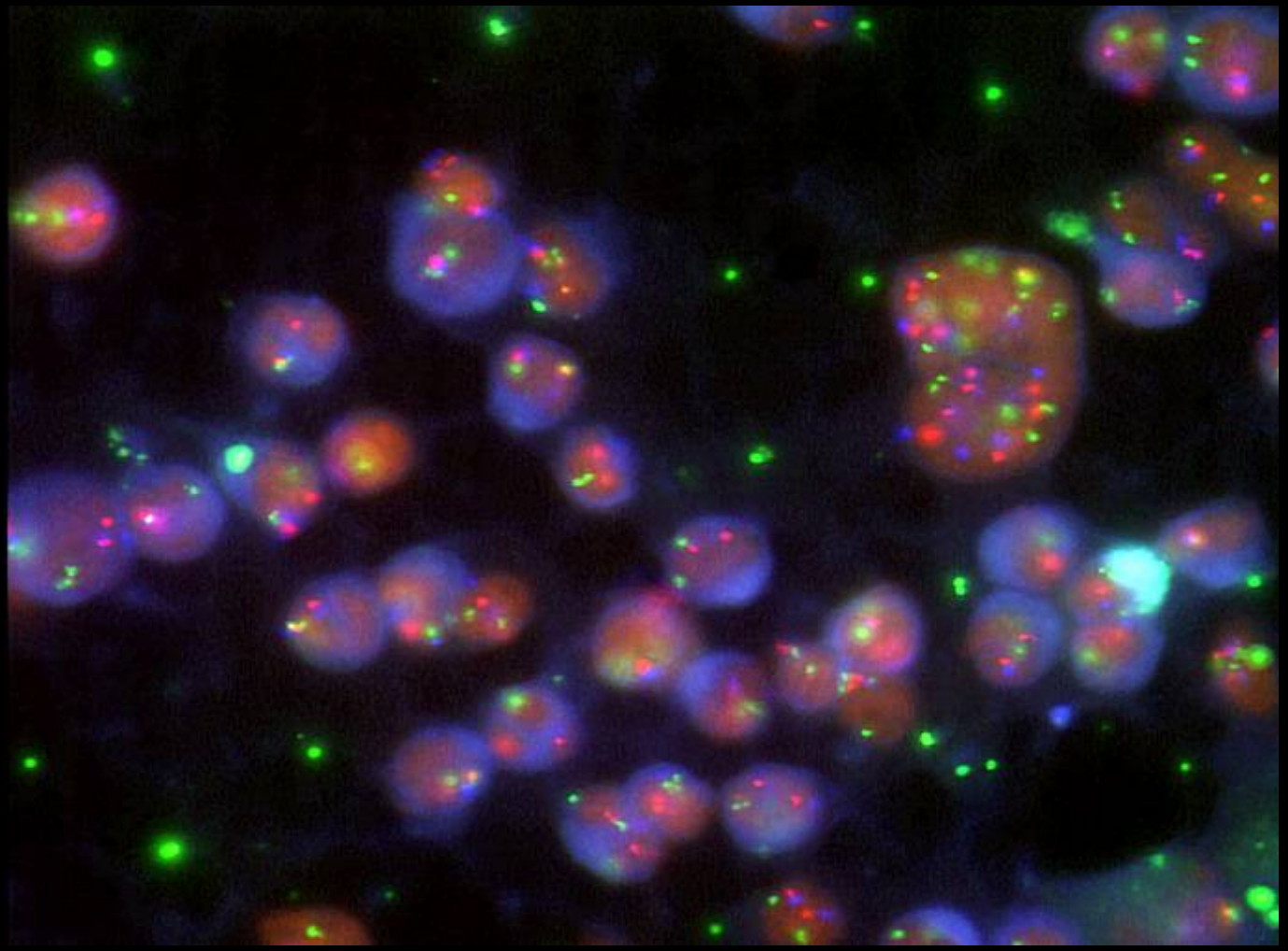


O'Connor & Barrans, HMDS (2005)

Interpretation of FISH: Artefacts

Not an artefact:

Megakaryocytes in BMA smears do not confuse with tumour cells



O'Connor & Barrans, HMDS (2005)

Summary

- FISH techniques are of use in the diagnosis of haematological malignancy.
  - Burkitts, MCL, FL
- Many translocations, and numerical aberrations can be detected by FISH, but at present it is better targeted at those abnormalities that cannot be detected by other methodologies.
  - Especially those associated with B-cell LPDs
- FISH helps us to differentiate those cases that have similar morphology and immunophenotype.
  - MCL v B-CLL; FL v MZL; DLBCL v Burkitts
- Identification of specific aberrations provides prognostic information.
  - Deletion p53 ~ poor risk
  - Deletion 13q14 ~ good risk B-CLL

O'Connor & Barrans, HMDS (2005)

www.hmds.org.uk