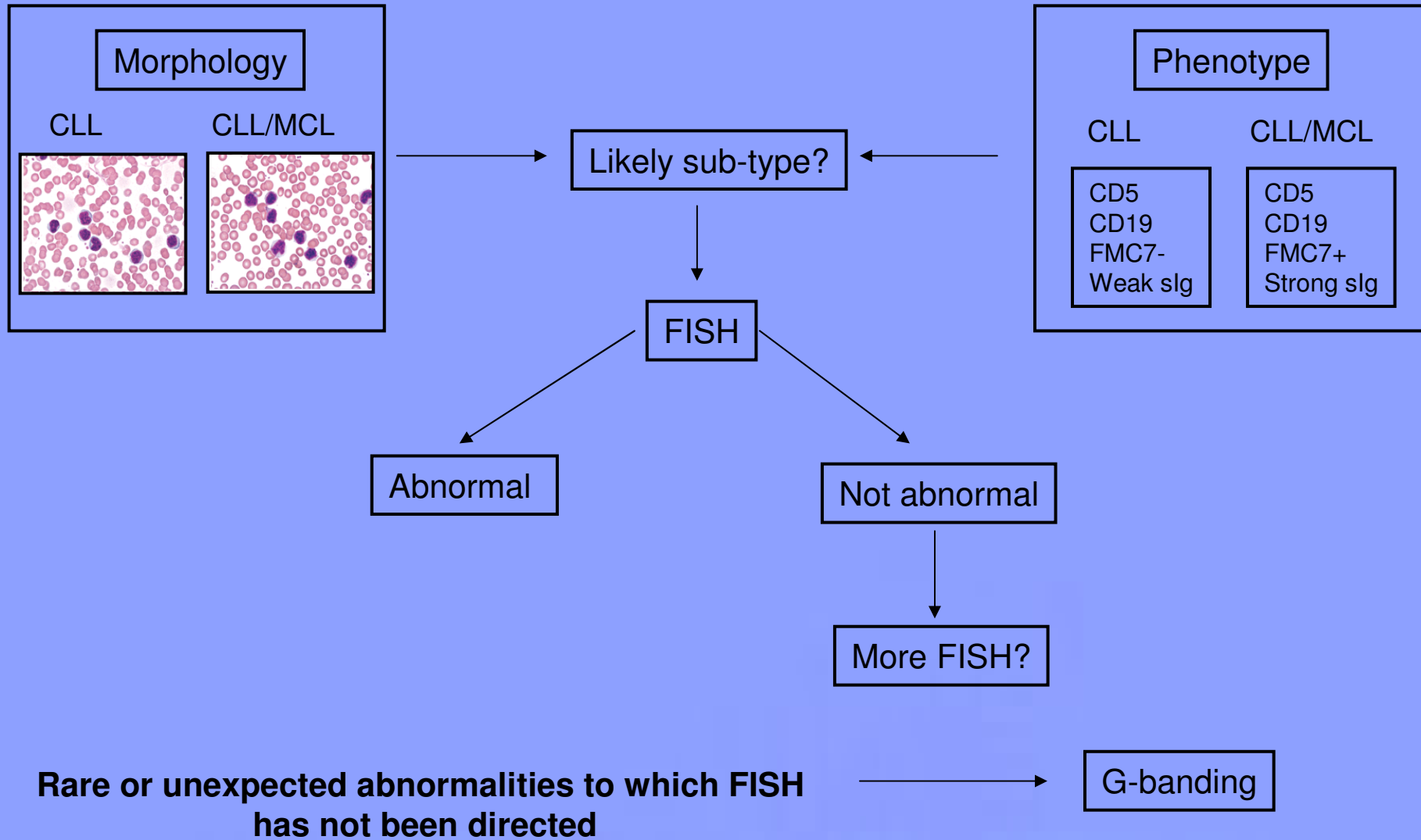


Use of the oligonucleotide DSP30 with IL2 in the investigation of low grade B cell lymphoid neoplasms

Chris Lowe, Newcastle

- Cytogenetic abnormalities of diagnostic and prognostic importance have been identified in some mature B cell neoplasms
- In Newcastle, a targeted FISH approach has been used for detection

FISH/morphology/phenotype strategy for CLL/MCL



The trouble with G-banding.....

- Low success and abnormality rate in mature B cell neoplasms

In CLL, this is due to leukaemic cell arrest in G_0 or early G_1 of the cell cycle

- B cell mitogens: EBV, Pokeweed, Lipopolysaccharide, TNF alpha, SAC, TPA and CD40L stimulation

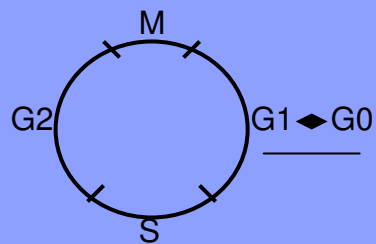
The CpG oligonucleotide DSP30 and cytokine IL2 are established as highly effective in CLL

| Study | Neoplasm | No. of cases | Success Rate | Abnormality Rate |
|----------------------|-------------------------------|--------------|--------------|------------------|
| Dicker et al 2006 | CLL | 132 | 95% | 81% |
| Mayr et al 2006 | CLL | 14 | ? | ~90% |
| Haferlach et al 2007 | CLL | 504 | 99% | 83% |
| Put et al 2009 | CLL | 217 | ? | 51% |
| Wren et al 2010 | CLL | 24 | 75% | 29% |
| Hereema et al 2010 | CLL | 229 | ? | 64% |
| Struski et al 2009 | CLL | 76 | 95% | 98% |
| | Other mature B cell neoplasms | 51 | 79% | 76% |

Summary of use of DSP30 / IL2 as a B cell mitogen for cytogenetic studies

DSP30: single stranded, CpG unmethylated, phosphorothioate oligodeoxynucleotide

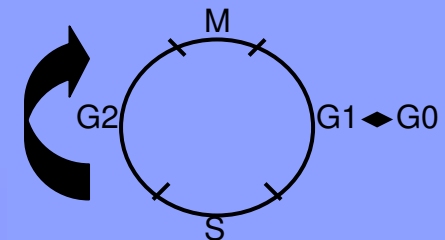
5' TCGTCGCTGTCTCCGCTTCTTCTTGCC



CLL cells arrested in G0 or early G1; unable to apoptose

[Web Version of presentation

DSP30 interacts with B lymphocytes via Toll like receptor 9. In CLL, this induces apoptosis via NF-kB and IL10 expression. For details of pathway, see Liang et al, 2009]



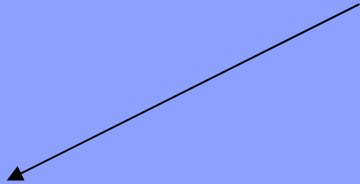
Cell activation results in entry into the cell cycle

DSP30 also induces the IL2 alpha receptor (possibly via CD25) in CLL cells thereby allowing IL2 to generate a co-stimulatory effect

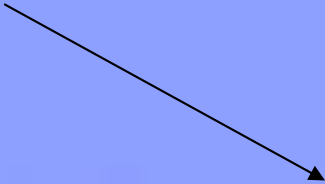
Mature B cell neoplasm
CLL, MCL, WM,
MM, NHL, HCL, lymphocytosis



Unseparated samples of
blood (12), marrow (17)
and lymph node (3)



Stimulated with DSP10 (2uM)
and IL2 (200U/ml) for 72 hrs
Consumable cost ~£6 per culture

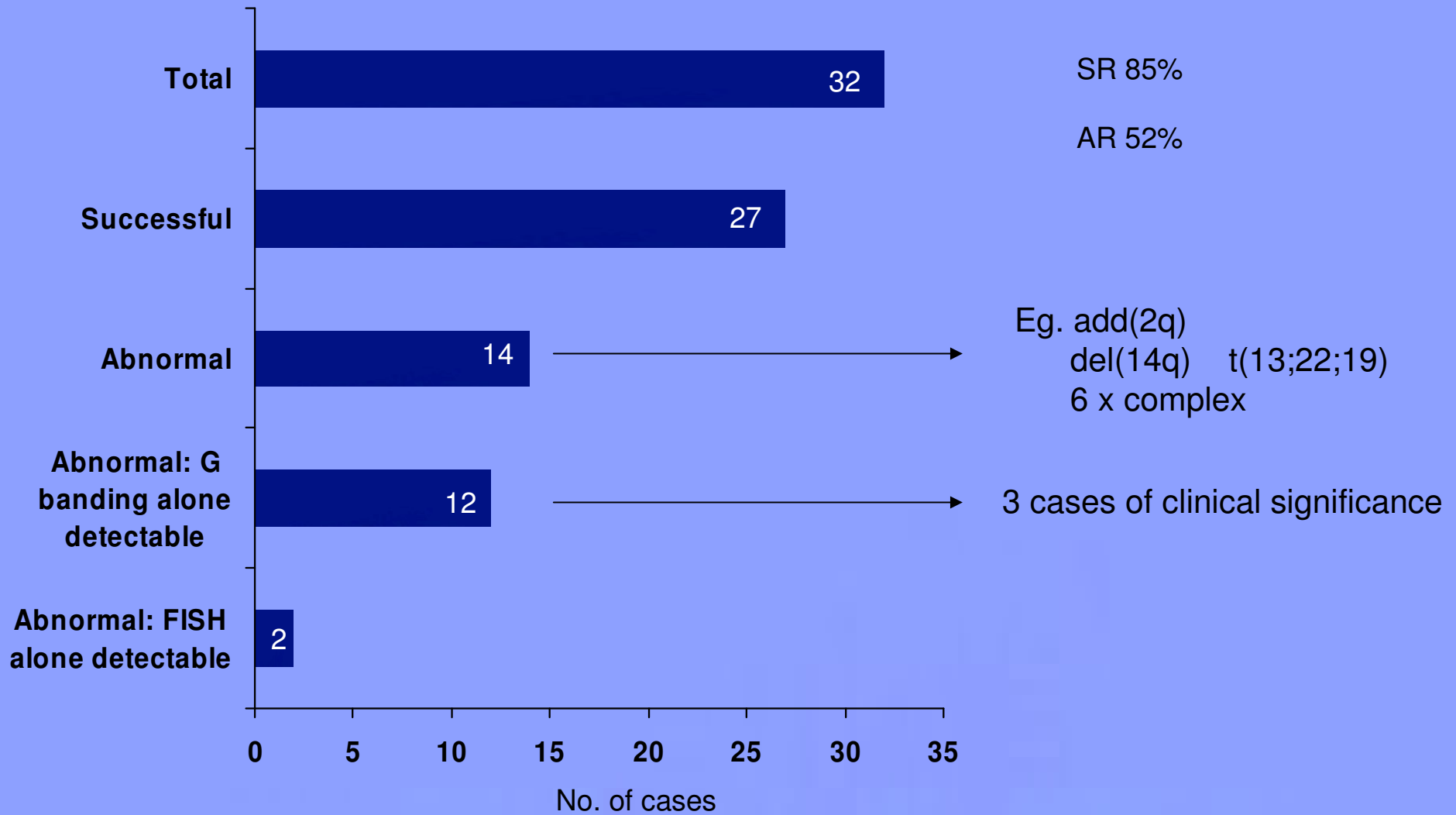


Unstimulated for 24 hr

Incidence of
abnormalities compared

Results

In an initial study (n=19) the abnormality rate was significantly different between stimulated and unstimulated cultures (p=0.033 in a paired t-test)



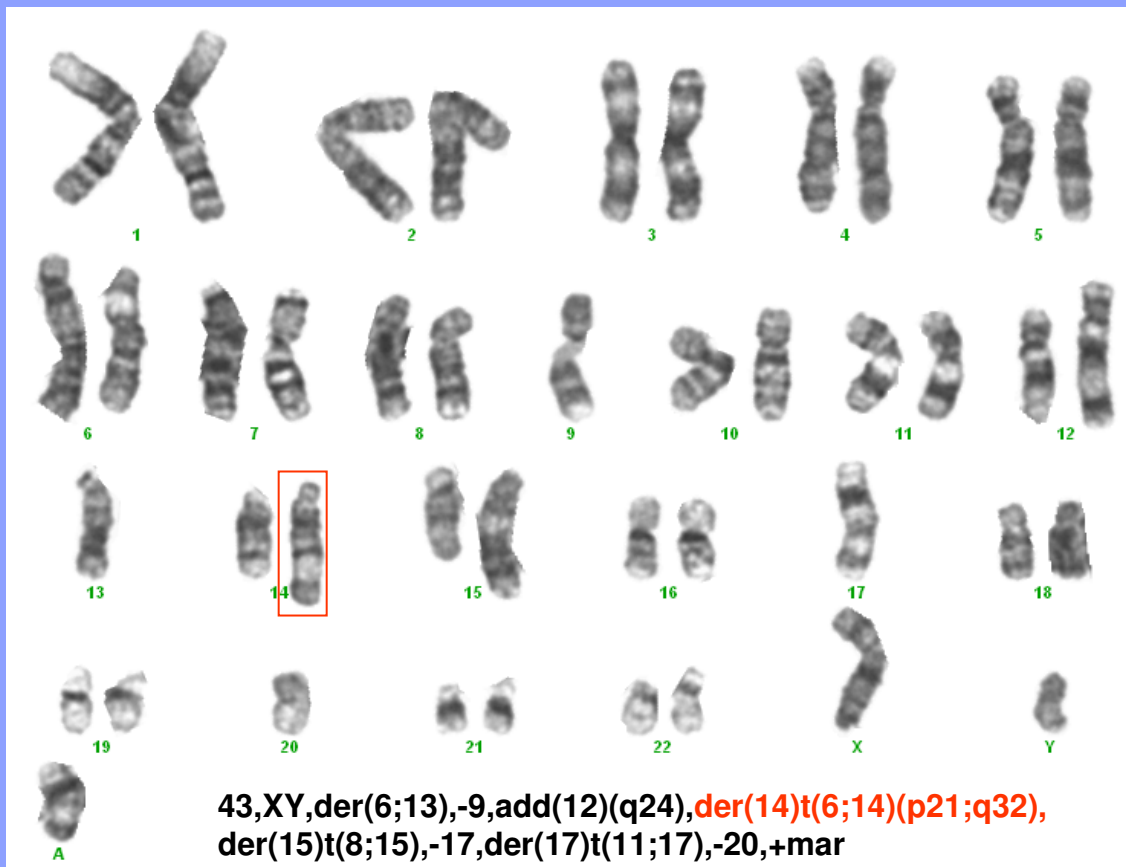
Case 1

75 year old man referred for atypical CLL

Without G-banding

IGH-CCND1 FISH → Negative → FISH/MLPA for abnormalities prognostic in CLL

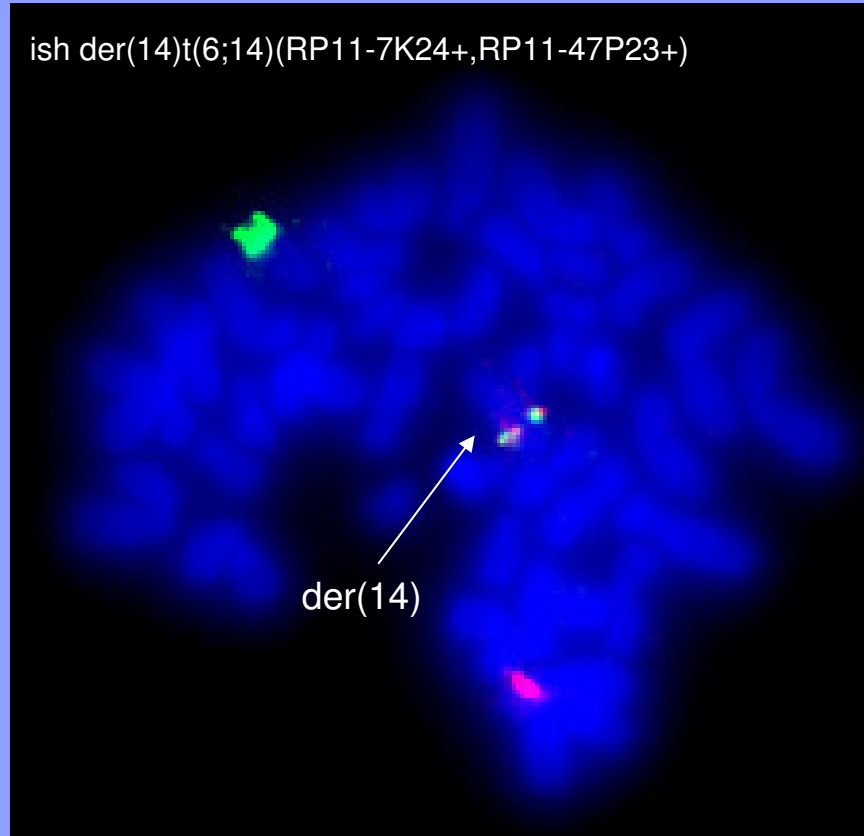
With G-banding



der(14)t(6;14)

Rare abnormality in mature B cell neoplasms including mantle cell lymphoma

ish der(14)t(6;14)(RP11-7K24+,RP11-47P23+)



t(6;14)(p21;q32) results in IGH-CCND3 rearrangement

Supported by FISH using BACs flanking IGH and CCND3

Confirmed IGH-CCND3 fusion positive using a commercial dual fusion probe



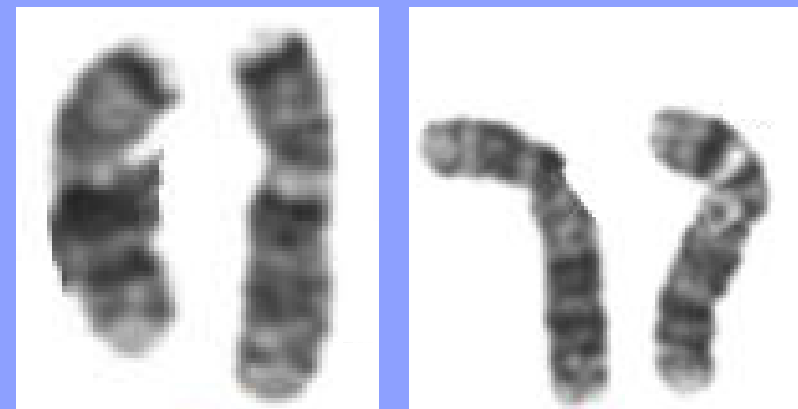
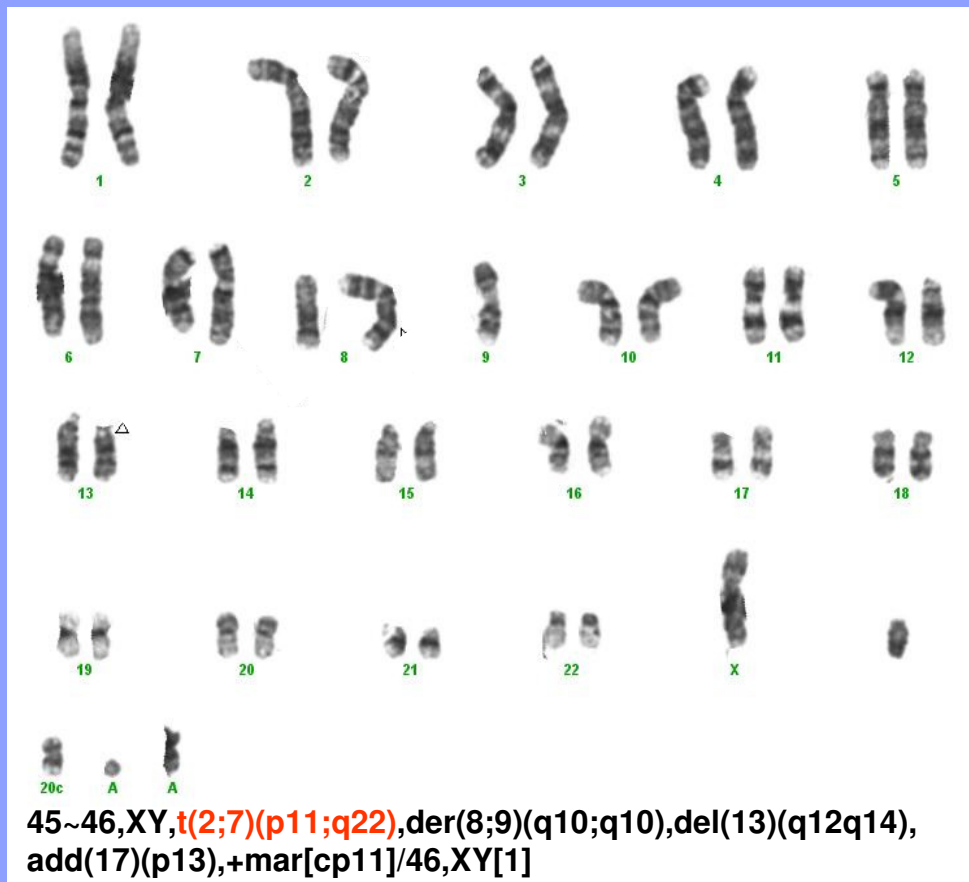
Cases 2 and 3

76 year old man and 77 year old woman both referred for lymphocytosis, ?MCL

Without G-banding

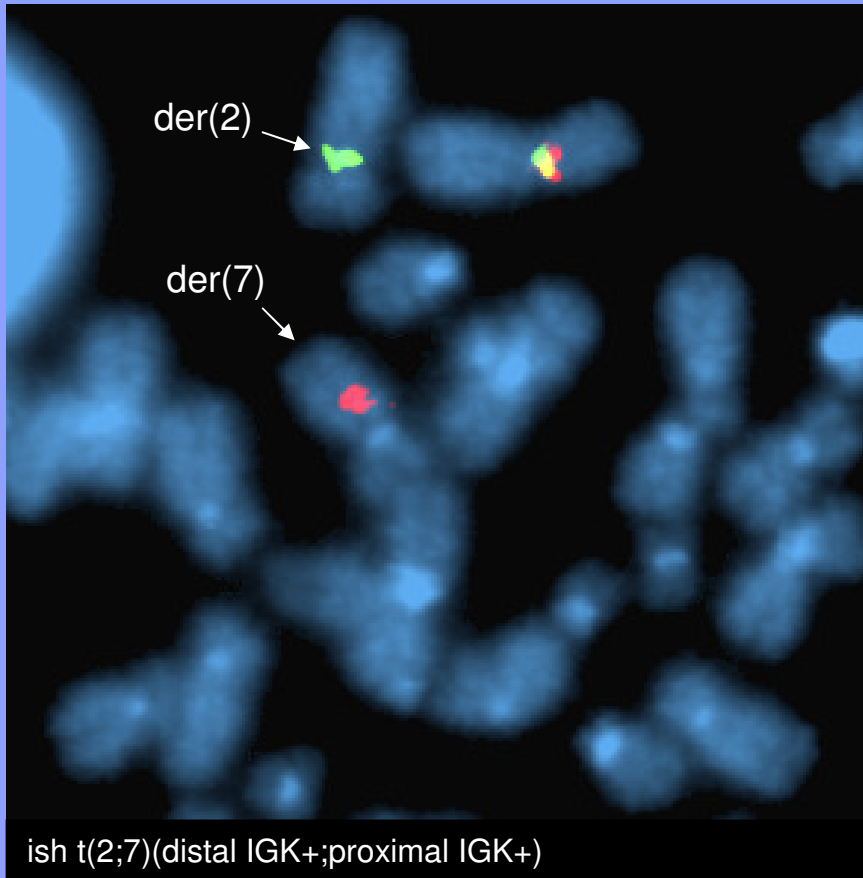
IGH-CCND1 FISH → Negative → Not IGH-CCND1 positive MCL

With G-banding

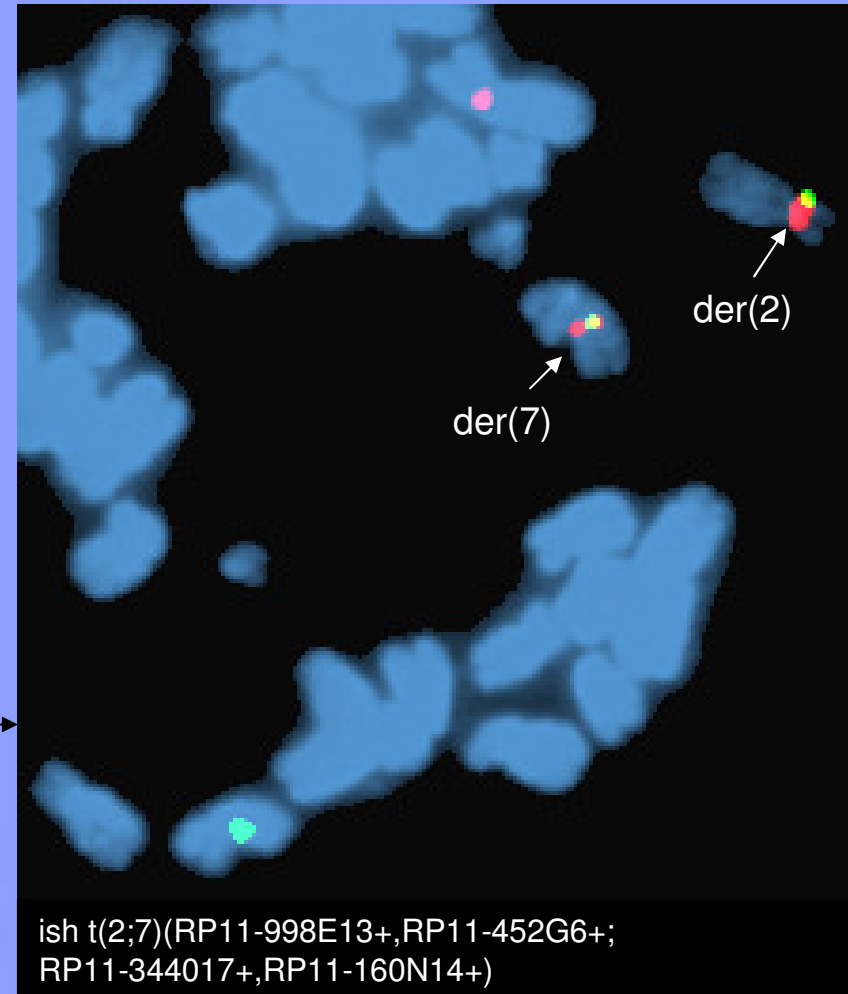


t(2;7)(p11;q22)

Rare abnormality in mature
B cell neoplasms including
splenic marginal zone lymphoma



Involvement of IGK confirmed using an IGK break apart probe



t(2;7) results in IGK-CDK6 rearrangement

Supported by FISH using BACs flanking CDK6 and IGK

Other advantages of G-banding

- Metaphases may help in the evaluation of borderline positivity after interphase FISH
- In **high grade lymphoma** it is important to exclude 'dual hits', necessitating a sequence of interphase FISH

G-banding:

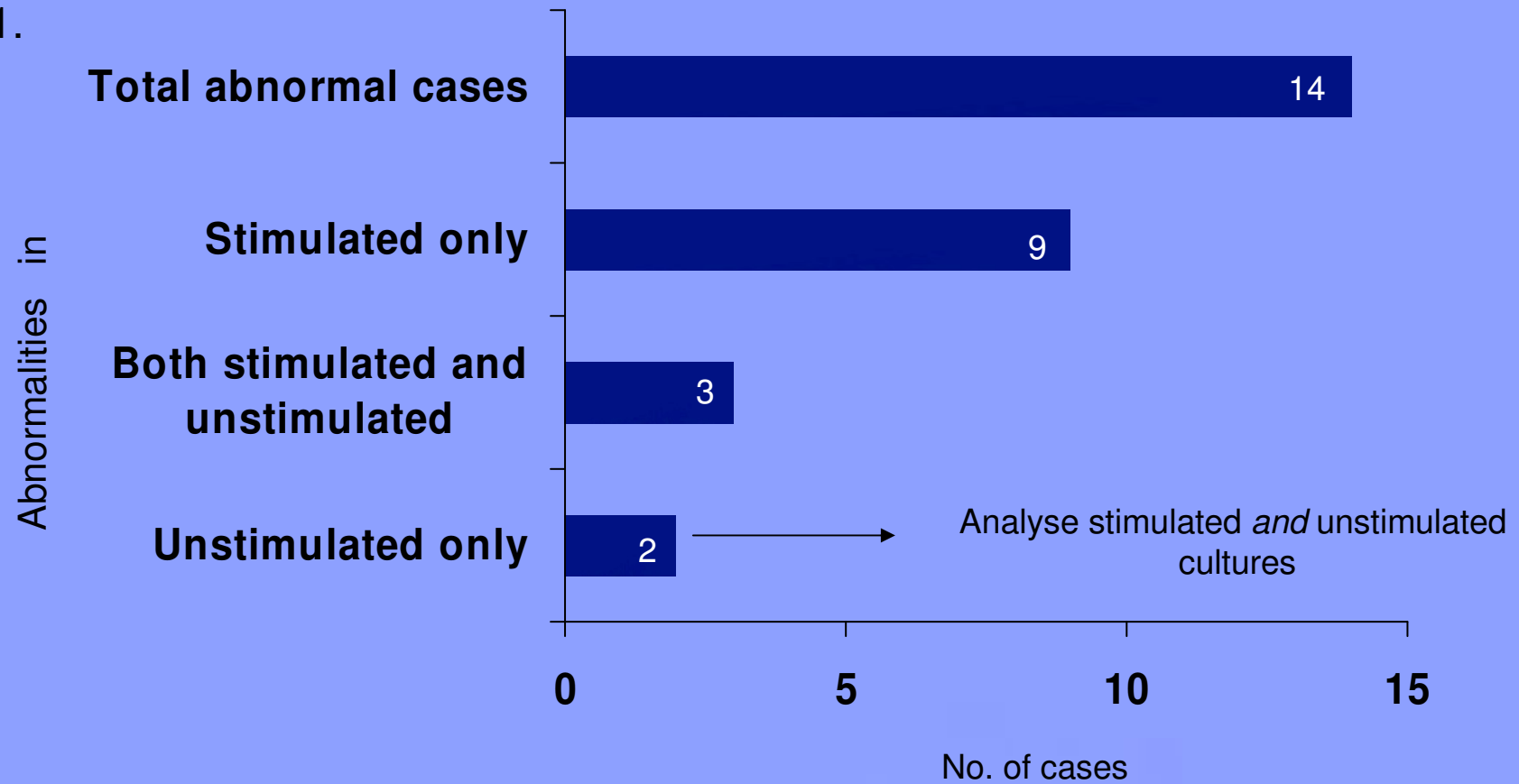
may be a more efficient means of establishing MYC +/- BCL2 and BCL6 rearrangements

may identify the partner chromosome

confirm the 'hits' are in the same cell (not possible with interphase FISH in the case of small clones)

Caveats

1.



2. Normal G banding; Abnormal FISH → Still need FISH

Questions

- Does DSP30/IL2 select for abnormal clones in general or for particular abnormalities?
- Is DSP30 / IL2 applicable to all mature B cell neoplasms?
- Is DSP30 / IL2 applicable to high grade lymphomas?

Possible strategy

?mature B cell neoplasm
Stimulated and unstimulated
cell culture

Abnormalities sometimes restricted to unstimulated cultures
May be needed to establish unselected clone size

Confirmed neoplastic cells in sample

Phenotype and morphology strongly suggestive of a
sub-type with associated abnormality

No Yes

G banding analysis

FISH

-ve

abnormal

normal / fail

+ve

Report +/-
confirmatory FISH

FISH with a large
panel of probes

Report / further FISH to
exclude other abnormalities

May be more time consuming than single G banding analysis
therefore reserve this until G banding option exhausted

Conclusions

- G-banding allows detection of rare abnormalities; some may be of clinical significance
- The usefulness of incorporation of G-banding into a FISH/phenotype/morphology strategy is dependent upon the frequency of these abnormalities
- DSP30/IL2 is an effective, non-toxic and inexpensive adjunct to unstimulated cultures

Acknowledgements

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