Linking cancer genomics with patient management

腫瘤基因組學在癌病治療之應用

Dr Edmond S K Ma

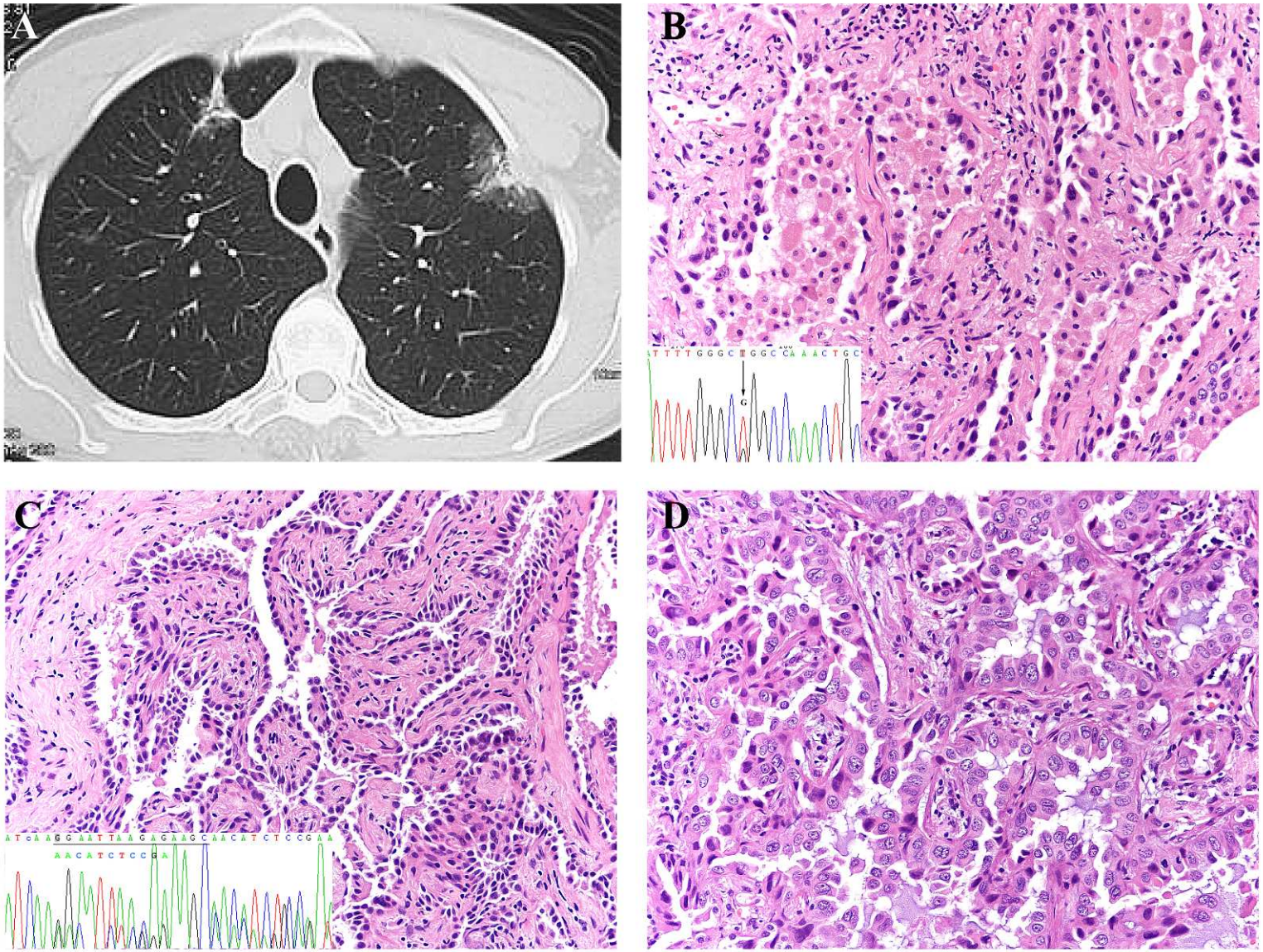
Department of Pathology

Hong Kong Sanatorium & Hospital

10th CLMC Taipei

- Founded in 1922
- Private Hospital
- Wards (450 beds) and Clinics
- Comprehensive oncology centre

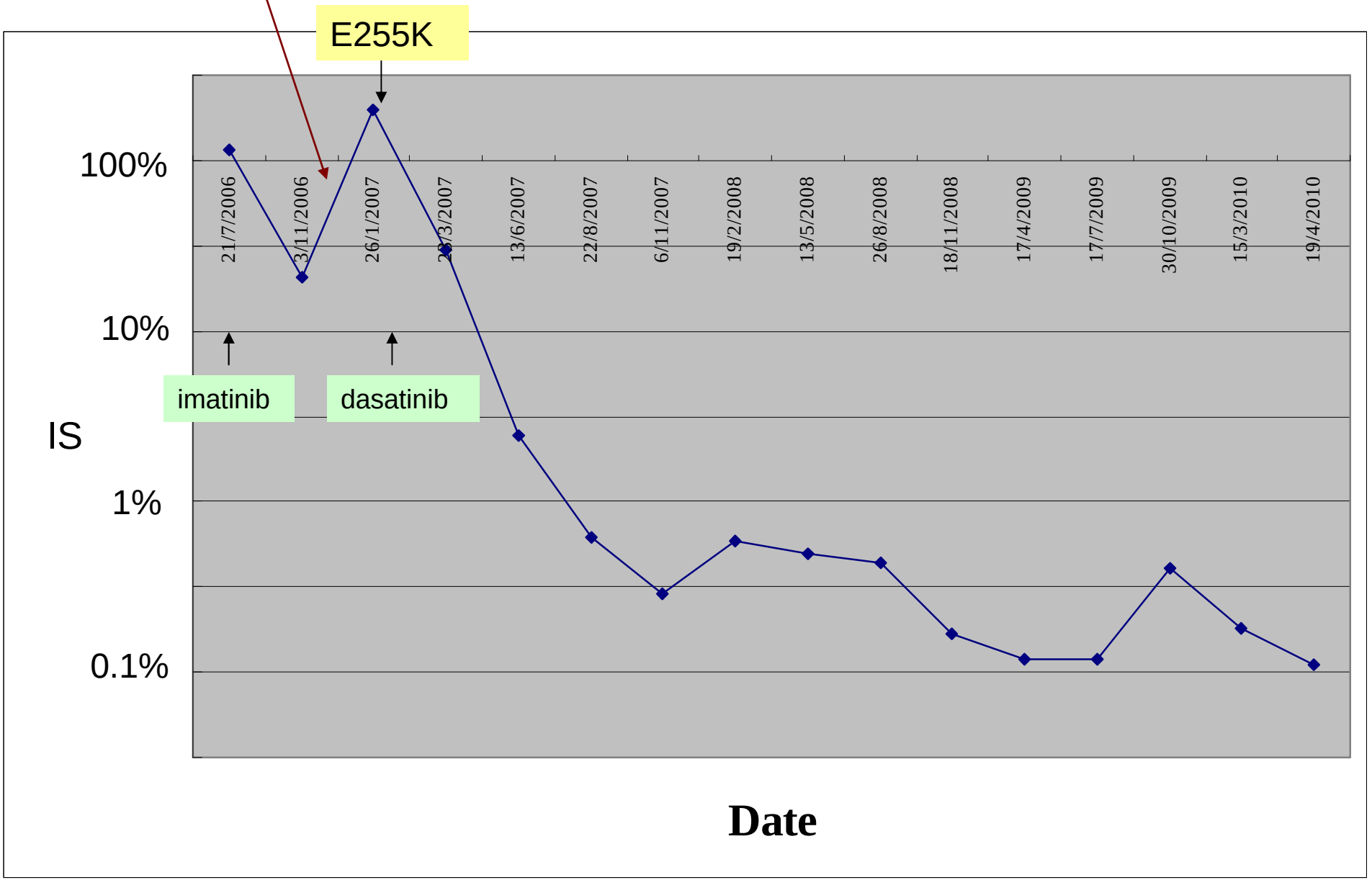
Example 1: Non-small cell lung cancer in a Chinese male, aged 75, ex-smoker



Ma ES *et al*, Ann Thorac Surg 90: e38 – 39, 2010

Example 2: Chronic myeloid leukaemia in a Chinese Male, aged 45

BCR-ABL Transcript level: 0.21 → 1.98 = 9.5 fold increase



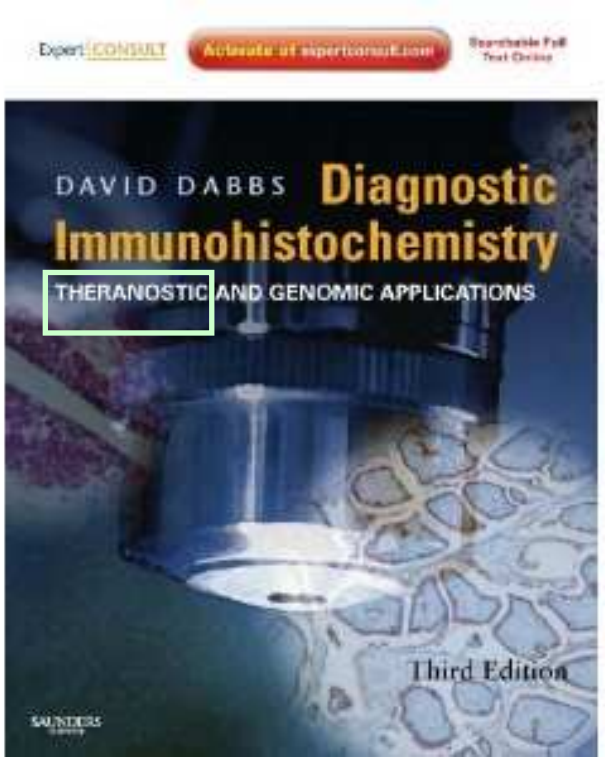
Cancer genomics and patient care

- Prognosis and risk stratification
- Identification of drug target
- Molecular monitoring and detection of resistance
- Pharmacogenomics

Personalized medicine

- Tailor medical care to individual needs based on genetic variation
- Predictive markers in Oncology
  - Outcome prediction
    - Prognosis and Risk stratification
  - Treatment response prediction
    - Drug targets
    - Pharmacogenomics
- New term: Theranostic

  (Diagnostics for direct therapeutic use)



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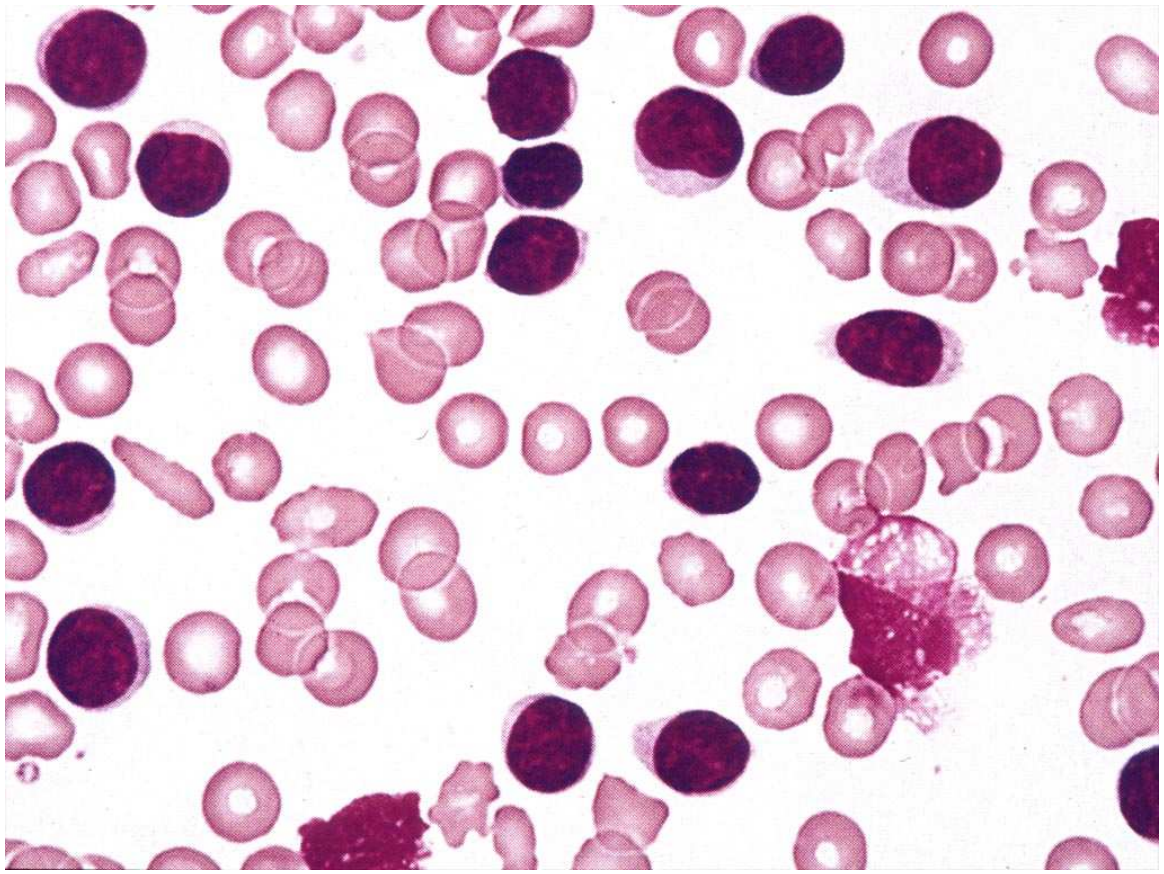
Chronic lymphocytic leukaemia (CLL)
慢性淋巴細胞性白血病／小淋巴細胞性淋巴瘤



Genetic abnormalities in CLL

	Karyotype				
	Normal	13q deletion	Trisomy 12	11q deletion	17p deletion
Total patients (%)	18	55	16	18	7
Binet stage (%)*					
A*	53	72	51	25	23
B*	30	20	34	50	41
C	17	8	15	25	36
Overall survival (months)	120	132	120	84	30

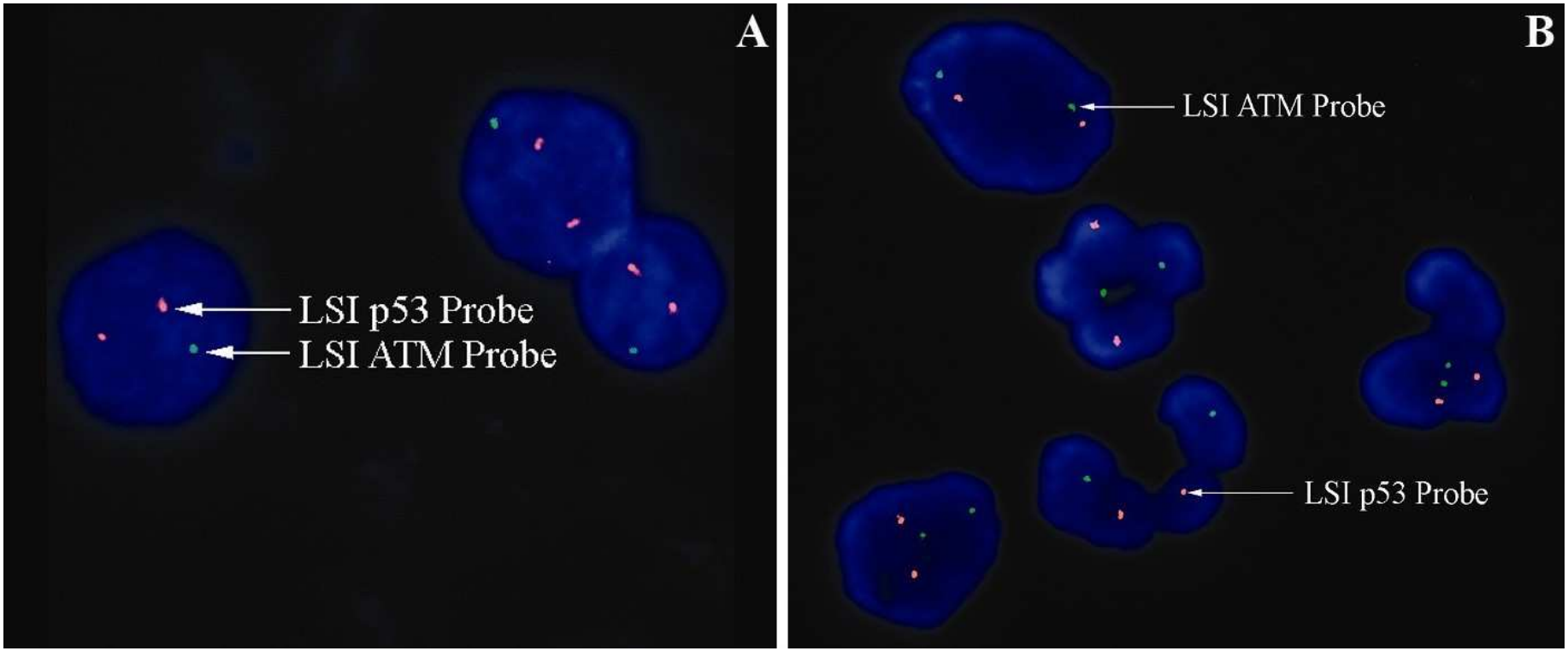
*Data refer to frequency with which every cytogenetic profile is noted in the different Binet stages—ie, 18% of patients have a normal karyotype, of whom 53% are Binet stage A.

Table 1: **Genetic aberrations in chronic lymphocytic leukaemia[18]**

Dighiero & Hamblin, Lancet 371: 1017 – 1029, 2008

Fluorescence *in-situ* hybridization (FISH)

螢光原位雜交

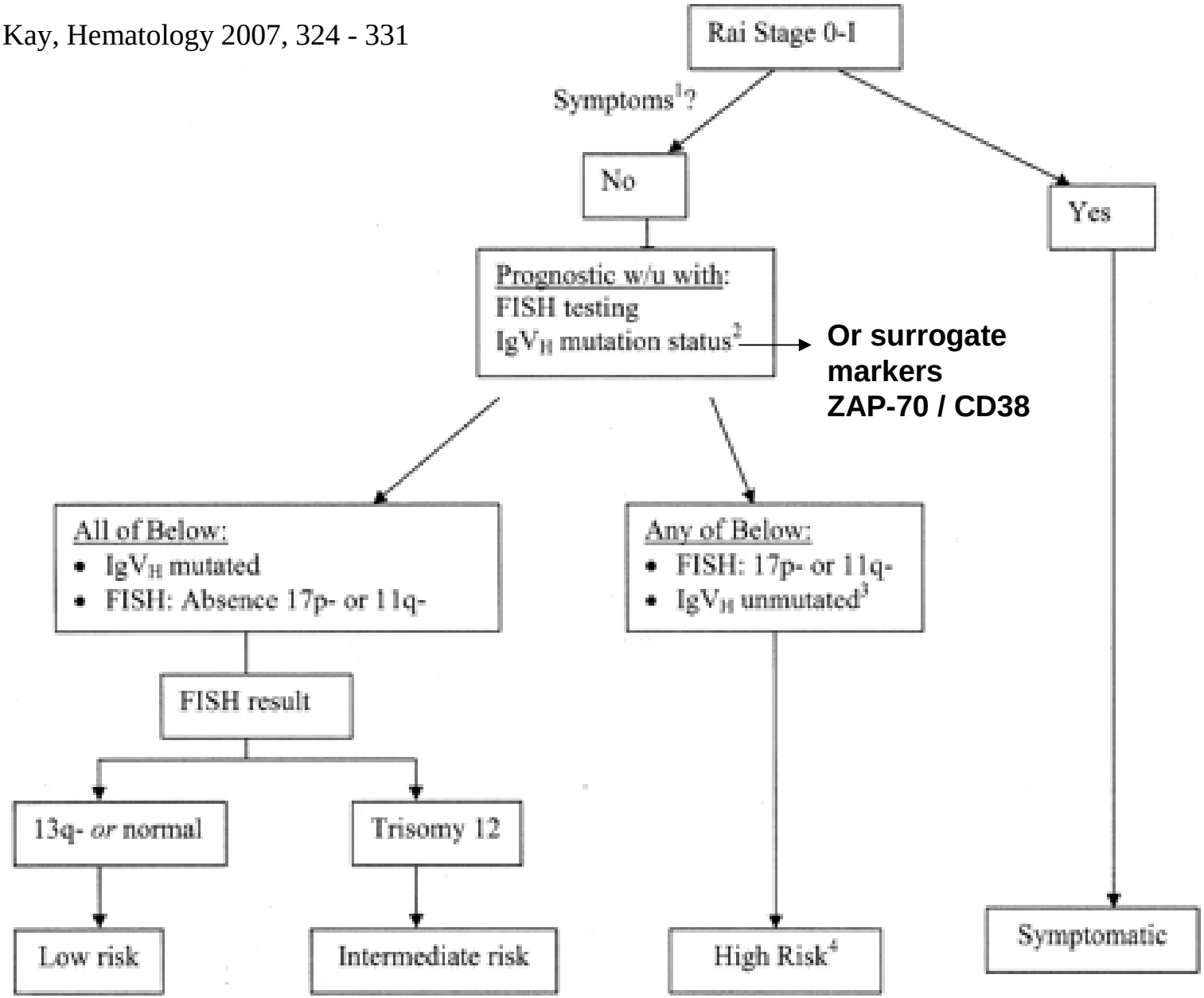


CLL patient with chromosome 11q (ATM) deletion

慢性淋巴細胞性白血病／小淋巴細胞性淋巴瘤

CLL risk stratification

Shanafelt & Kay, Hematology 2007, 324 - 331



Emerging use of interphase FISH in risk stratification

- CLL
  - 13q-, 11q-, 17p-, +12
- Myeloma
  - Favourable
    - Hyperdiploid
  - Unfavourable
    - t(4;14)
    - t(14;16)
    - del(17)p/p53
  - Coupled with immunofluorescence or cell sorting

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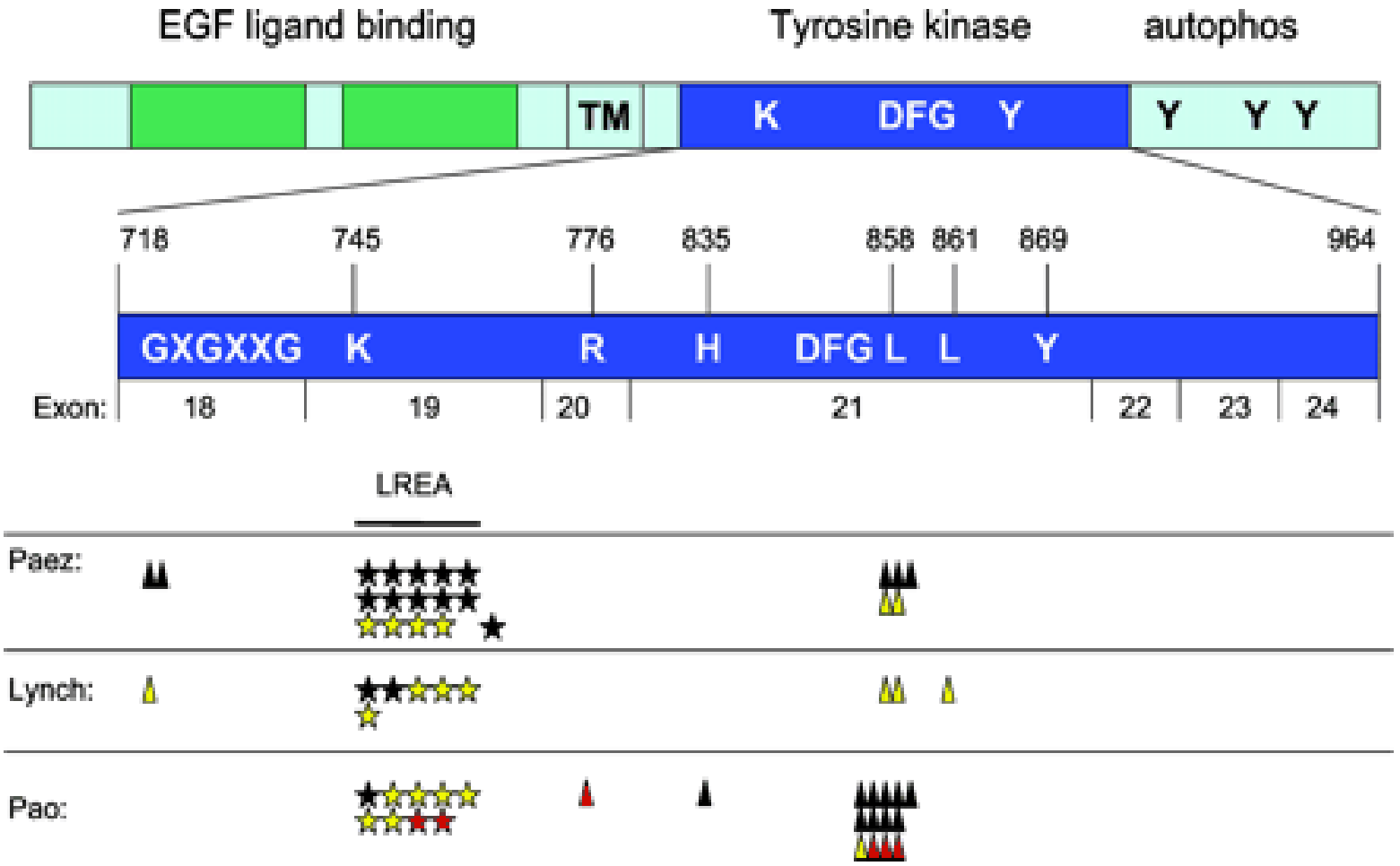
Identification of drug target

- Non-small cell lung cancer
  - EGFR mutation
  - EML4-ALK gene fusion

EGFR testing

- EGFR expression by IHC
- EGFR gene mutation
  - First discovered in 2004
    - Gefitinib: Harvard/DFCI
    - Gefitinib & erlotinib: MSKCC
- EGFR gene amplification

Spectrum of mutations detected in TK domain of EGFR in NSCLC



EGFR gene mutation

- Methods
  - PCR Sequencing
  - Allele specific real-time PCR
  - Others
    - HRM
    - dHPLC
    - Luminex
    - etc

PCR sequencing	Allele specific real-time PCR
Covers all mutations	Covers specific mutations
Less sensitive (15 – 20%)	More sensitive (1%)
Needs microdissection	Can do without microdissection
More tedious	Simpler
Less expensive	More expensive

HKS&H experience

- n = 481 cases
  - accrued from September 2005 to April 2009
- Positive rate 43.7% (210/481)
  - Exon 19 deletion 46% (86/210)
    - ELREA 29.5% (62/210)
  - Exon 21 mutation L858R 39% (82/210)
- Double mutations 10.5% (22/210)
- Rare scenarios
  - Concurrent sensitive and resistant mutants (n = 3)
  - Concurrent EGFR and KRAS (n = 1)

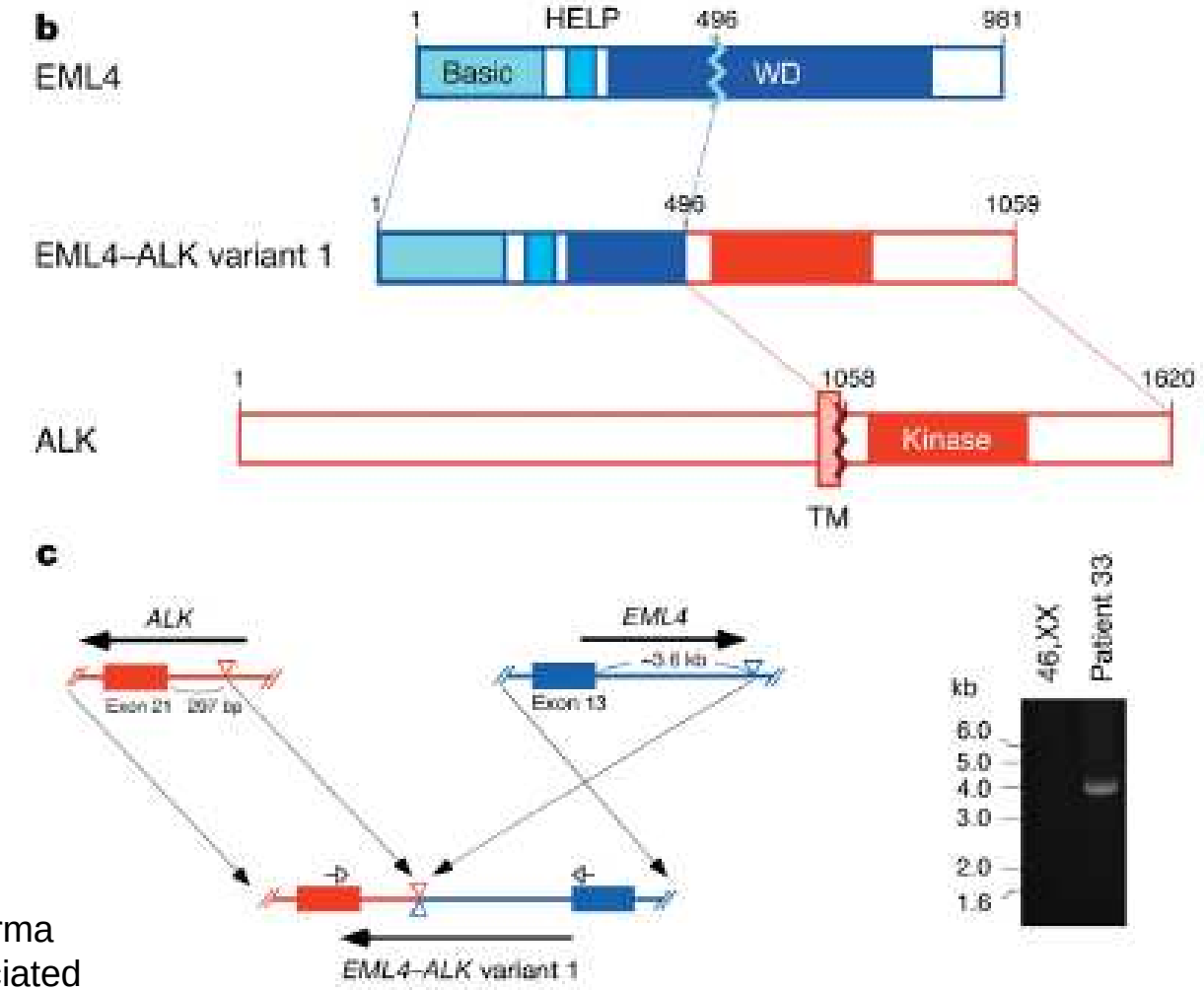
EGFR exon 20 insertion/duplication/deletion mutations

- Associated with primary or *de novo* resistance
  - c.f. secondary or acquired resistance due to T790M
- Positive rate (HKS&H data from 2005 – 2010)
  - 3.6% (29 patients out of 803 tested)
- Treatment outcome
  - Available in 17 patients
  - 8 treated with TKI (gefitinib = 6, erlotinib = 2)
  - Only 1 showed stable disease and alive at 20 months
  - The rest showed progressive disease on treatment from 3 weeks to 4 months

Emerging molecular markers in NSCLC

- MET amplification
- EML4-ALK fusion
  - First identified by Japanese group in 2007 (Nature 448: 561 – 566, 2007)
  - Associated with male patients who are young and never/light smokers
  - Mutually exclusive with EGFR and KRAS
  - Not responsive to EGFR TKI
  - Considered for trial of ALK inhibitors

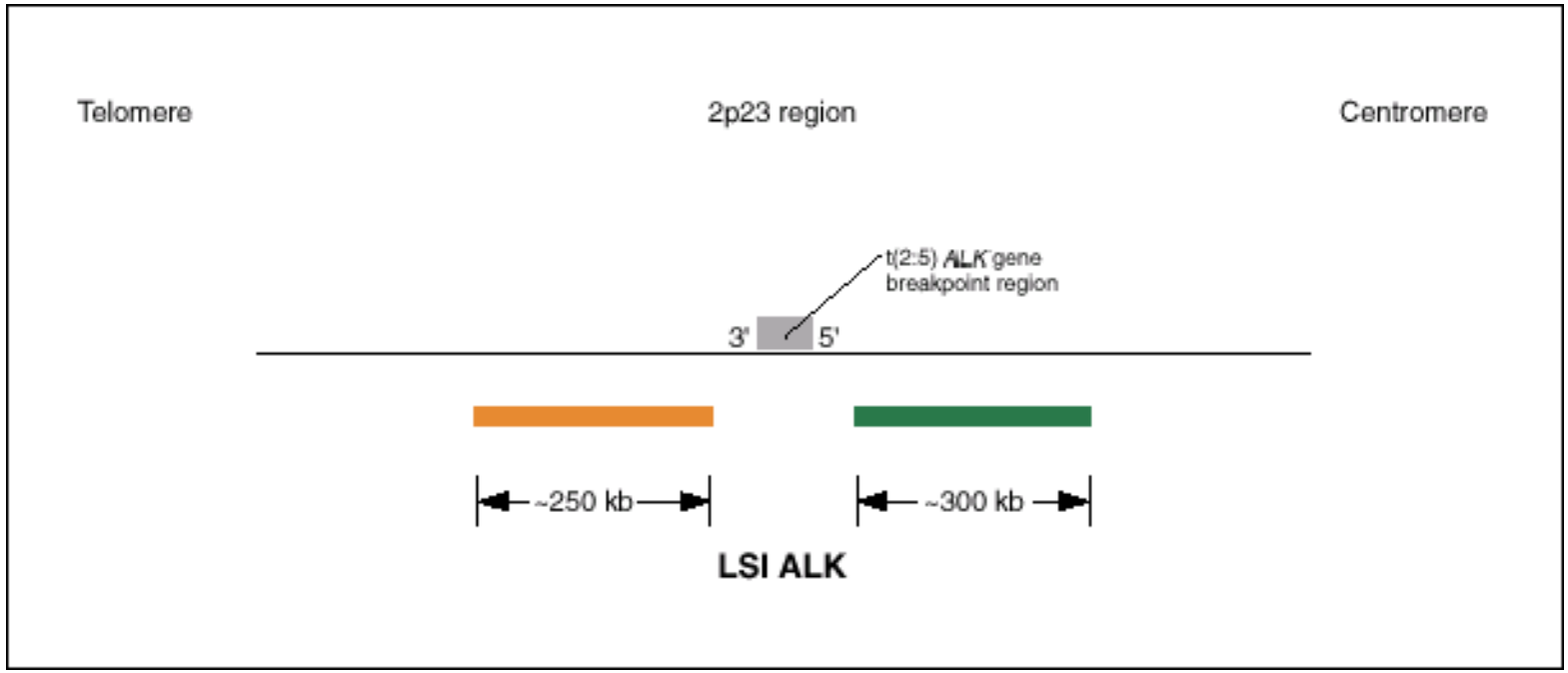
EML4-ALK gene fusion



EML4 = echinoderma microtubule-associated protein-like 4

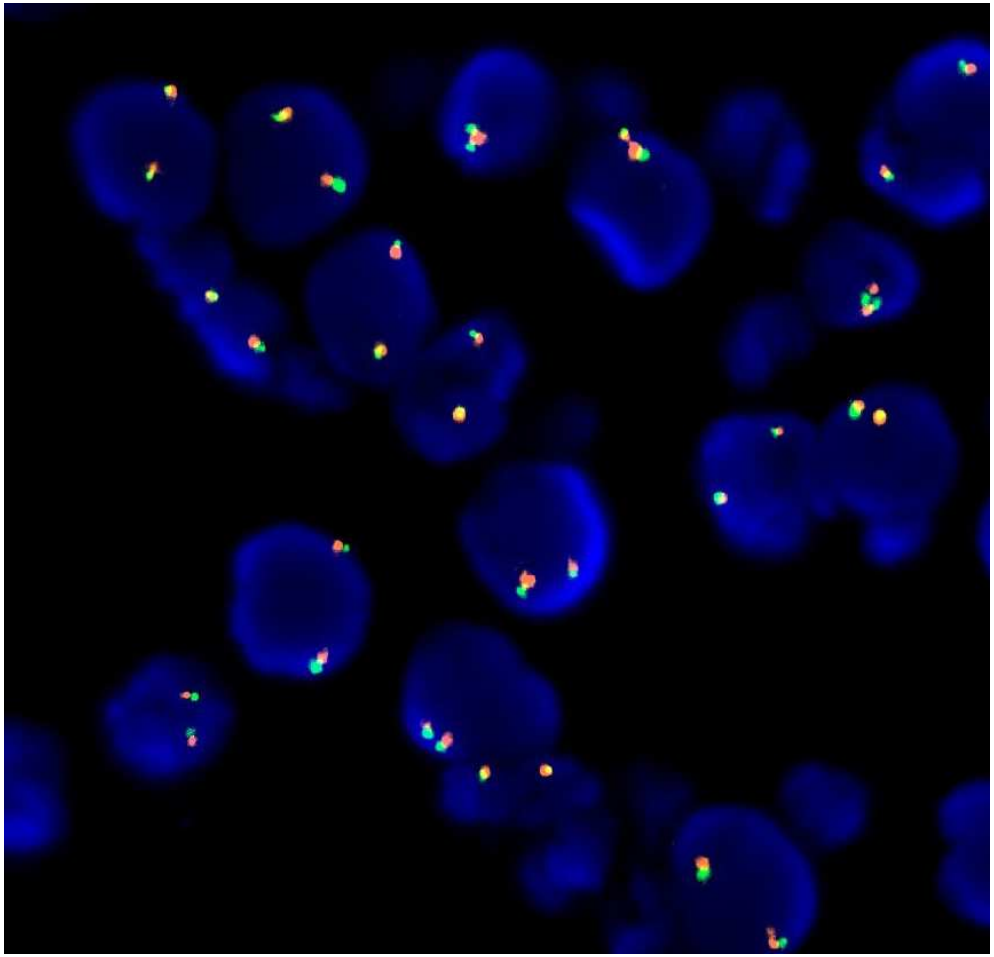
ALK dual-colour split apart FISH probe

Method employed by Shaw AT *et al*, JCO 27: 4247 – 53, 2009

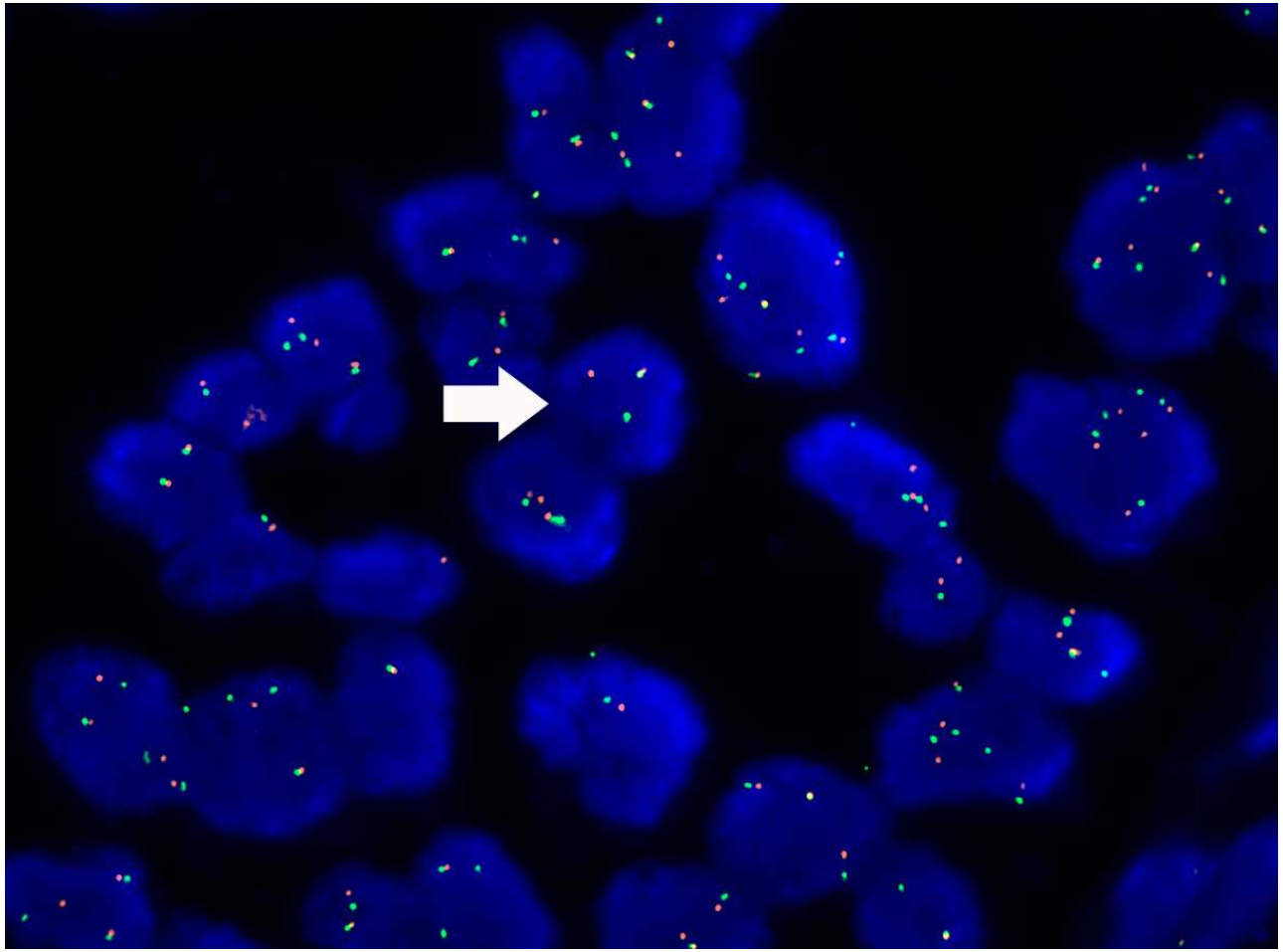


From Abbott Molecular web page

Normal signal pattern



Patient result, F/49, NSCLC

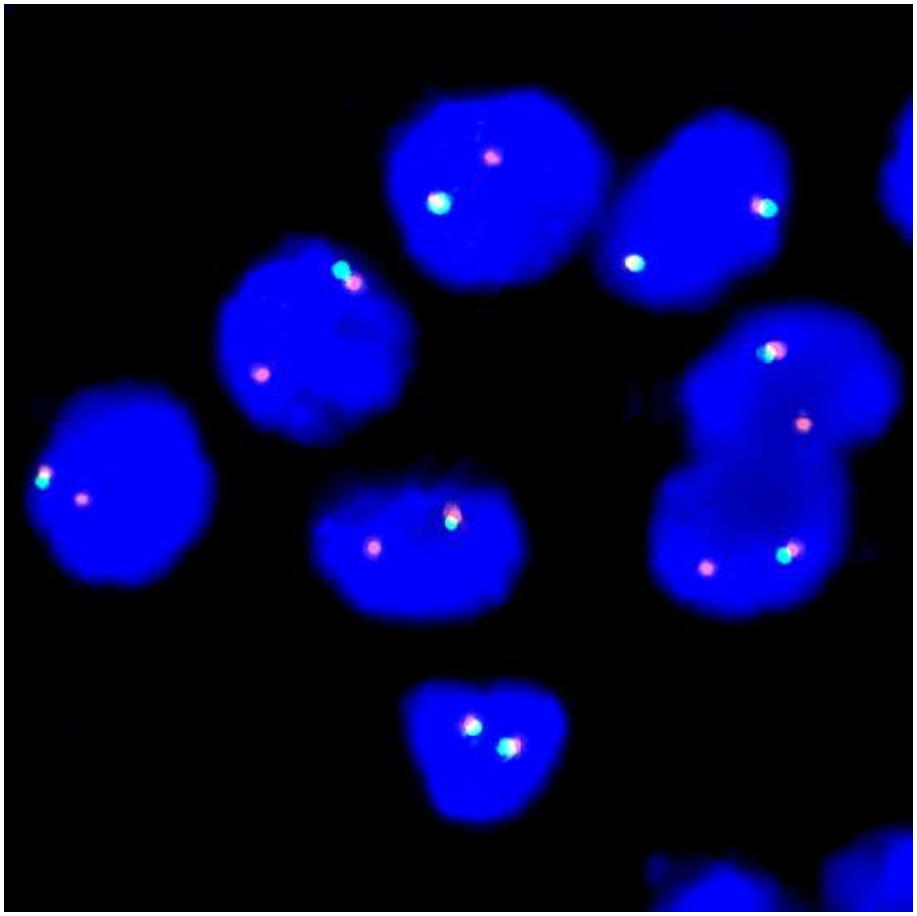


Summary of ALK FISH in NSCLC

- n = 41
- Indeterminate = 2
- Positive = 8 (21%)
  - Typical FISH pattern = 6
  - Atypical FISH pattern (1R1F) = 2
- Concurrent with EGFR mutation = 1
  - EGFR mutation positive = 8
  - KRAS mutation positive = 2

ALK FISH by dual-colour split apart probe

M/58, NSCLC

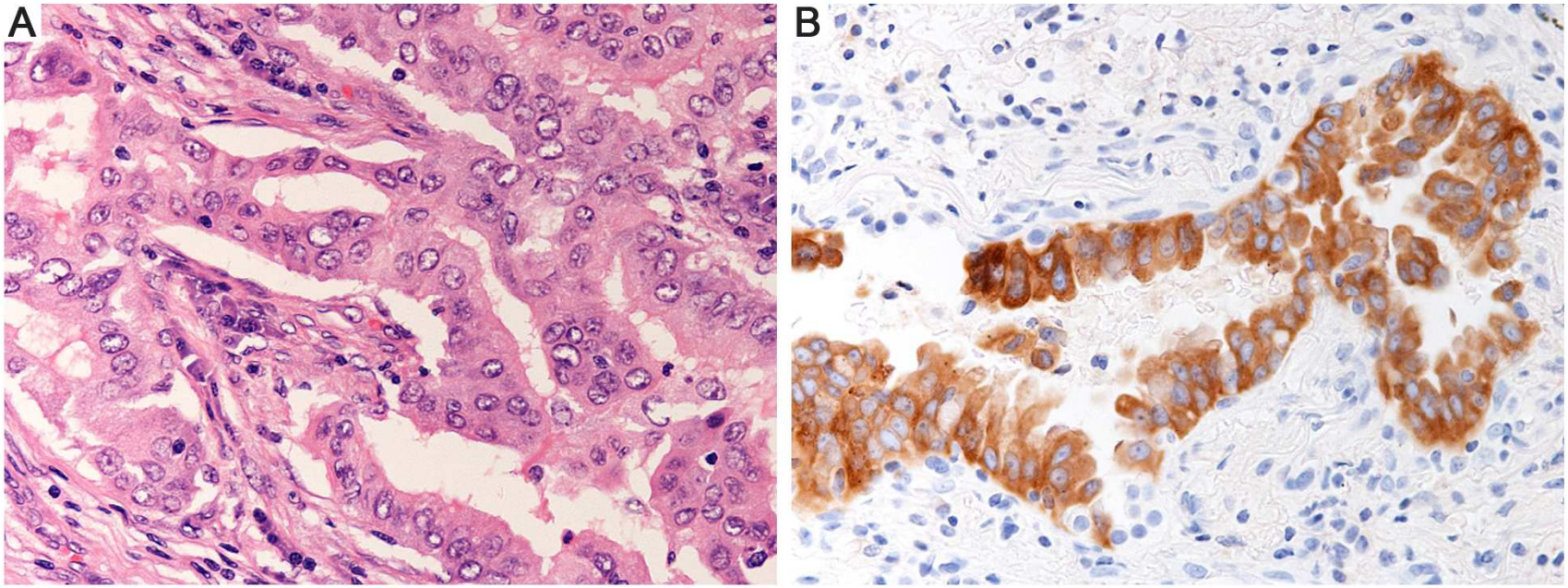


Atypical FISH signal pattern

Laboratory detection of EML4-ALK fusion

- FISH
  - ALK dual colour split apart
  - EML4-ALK dual colour dual fusion
- PCR
  - RT-PCR for the fusion transcript
  - Sequencing
  - Multiplex RT-PCR to cover different isoforms
- IHC with ALK-1 antibody
- Dual ISH (bright field)

ALK immunohistochemistry



Cancer genomics and patient care

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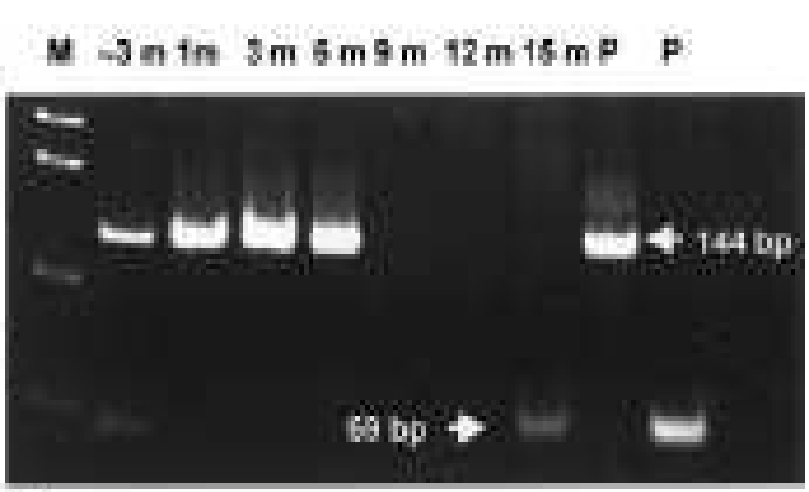
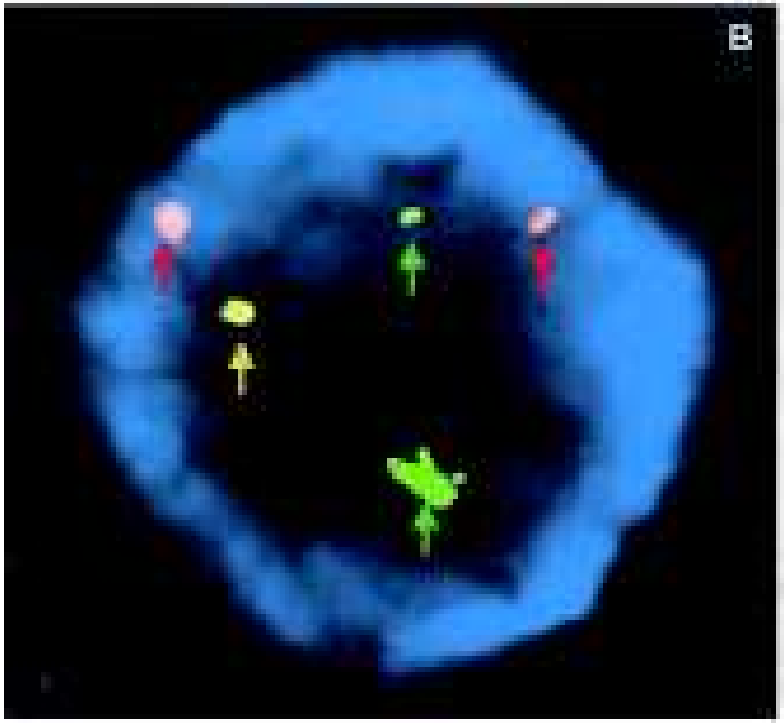
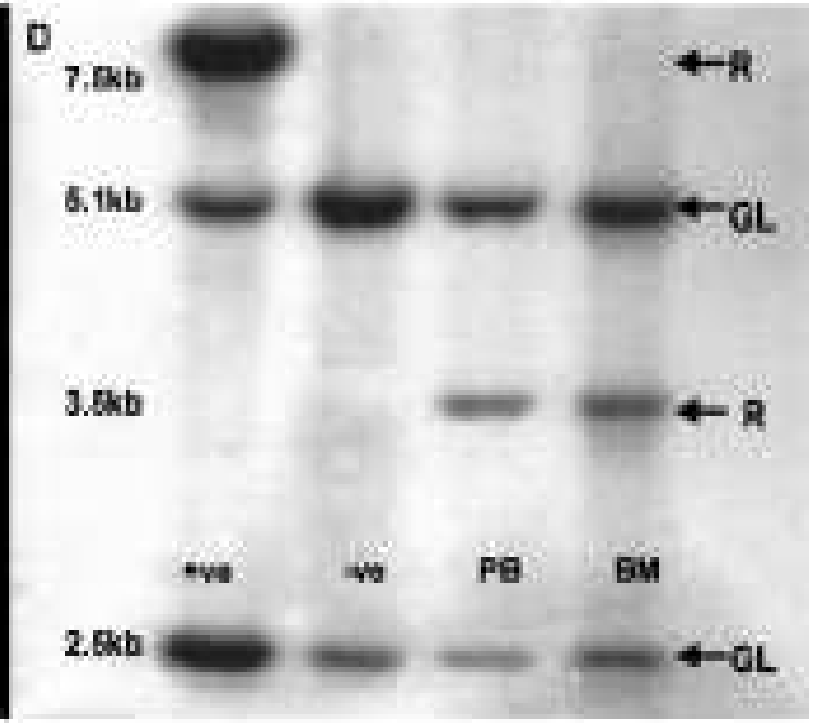
Monitoring treatment response and detection of drug resistance

- Chronic myeloid leukaemia on imatinib therapy

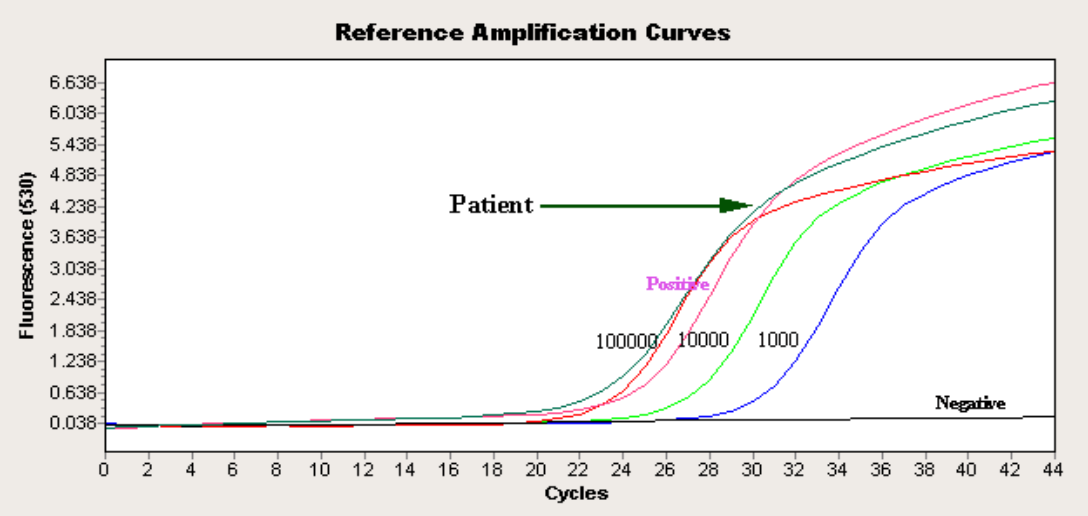

M -3m 1m 3m 6m 9m 12m 15m P P
144 bp
69 bp

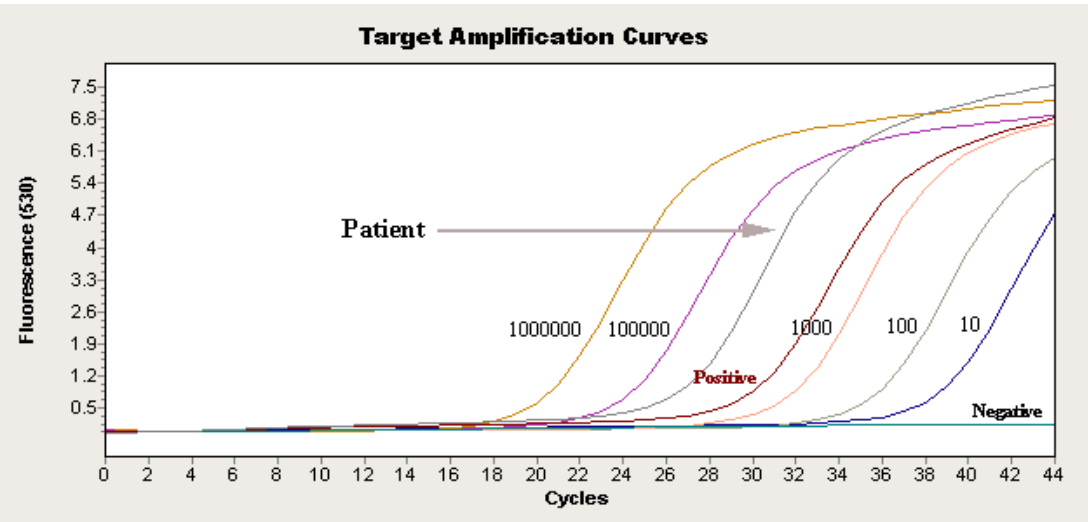
A C


B

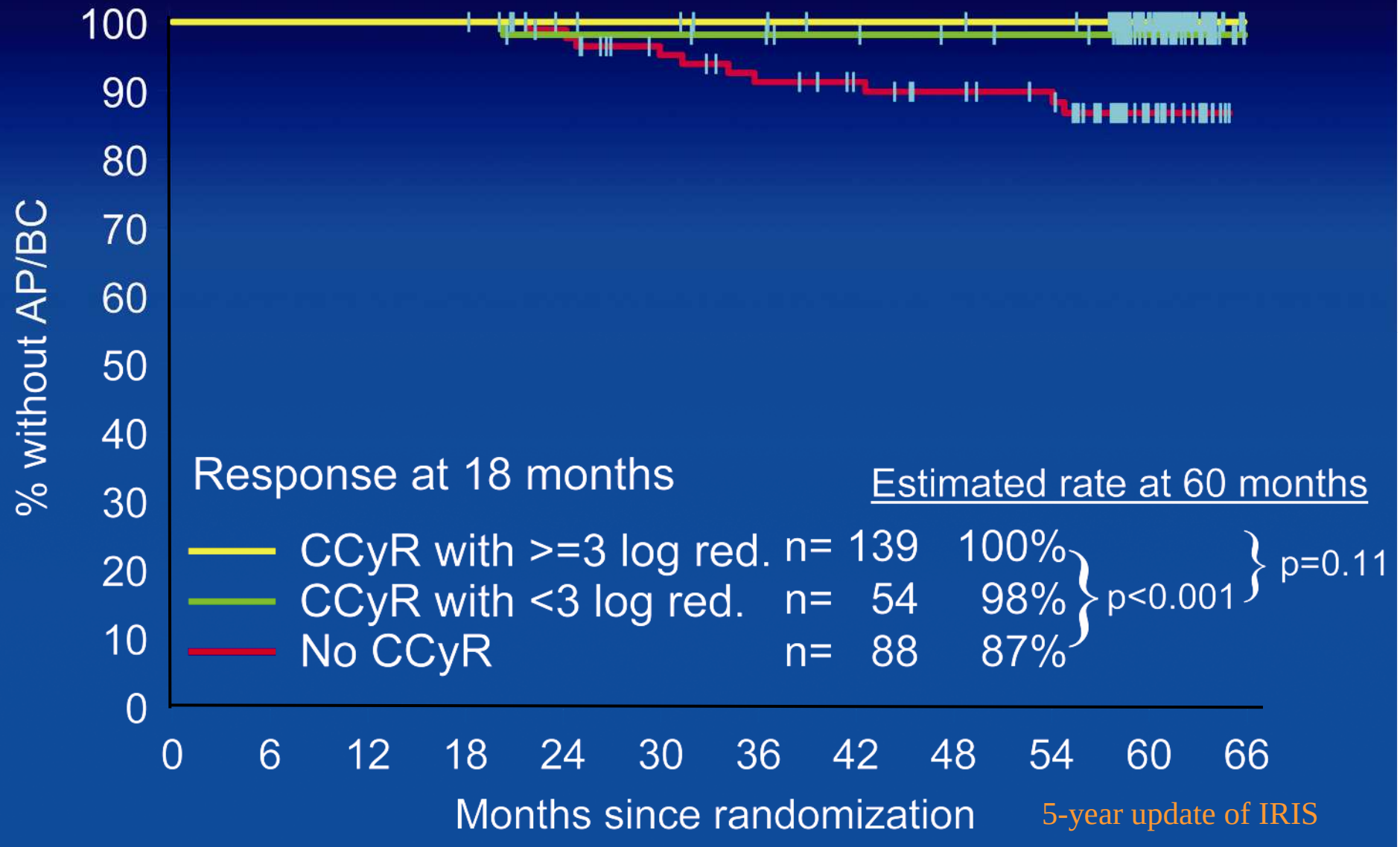

D
7.8kb
5.1kb
3.5kb
2.5kb
R
GL
R
GL
+ve
-ve
PB
BM

BCR-ABL real-time quantitative PCR





Survival Without AP/BC by Molecular Response at 18 months on First-line Imatinib



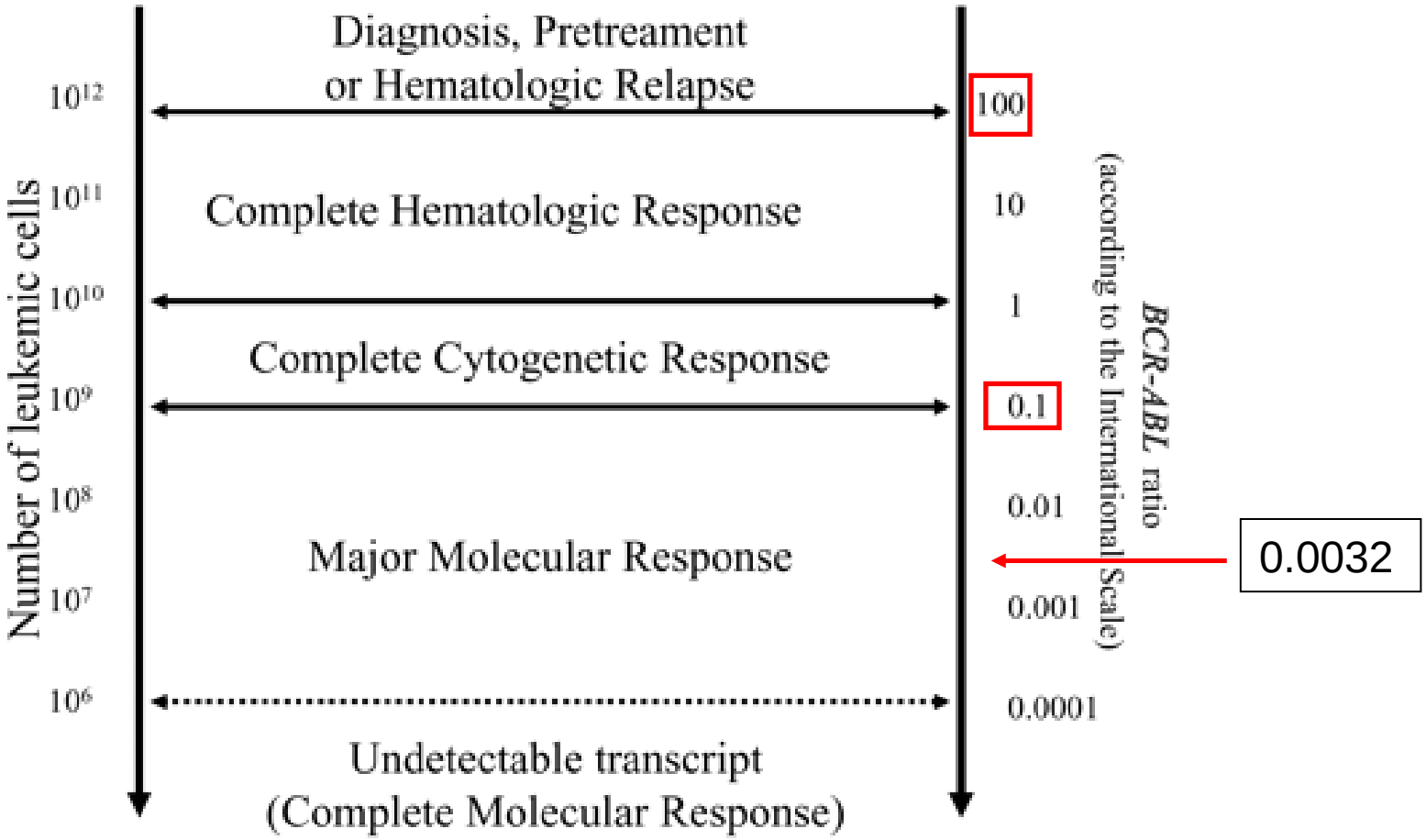
5-year update of IRIS

CML monitoring: clinical significance

- Prognostication
  - Predicts progression-free survival
- Management
  - Detection of acquired imatinib resistance
    - *ABL* kinase domain mutation
  - Treatment decision
    - Dose escalation of imatinib
    - Second generation tyrosine kinase inhibitors
    - Allogeneic HSCT

Standardization of RQ-PCR

International scale based on deriving laboratory specific conversion factors



Baccarani M *et al* for the European LeukemiaNet, Blood 108: 1809-20, 2006

Standardization of RQ-PCR

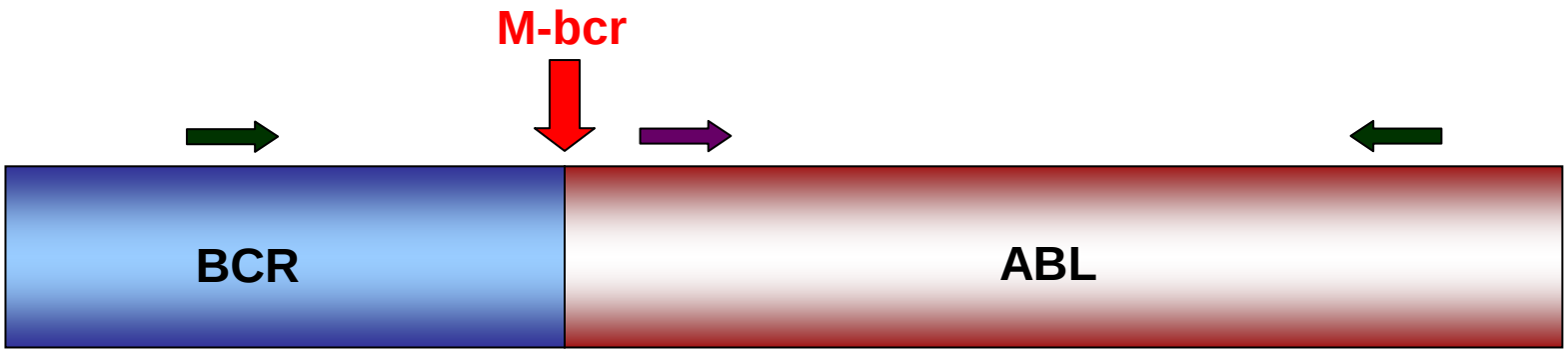
- RQ-PCR
  - *BCR-ABL* 23,500 copies
  - *ABL* 469,000 copies
  - *BCR-ABL/ABL* = 0.05
  - IS = 0.05 x 1.221 = 0.061 (6.1%)
- Laboratory considerations
  - Document the type of transcript
  - Adequate nucleated cells and amplifiable copies of control gene
  - Limit of detection
  - Internal QC
  - Proficiency testing
  - Standardization and reporting of results
  - Reference material

KD mutation detection: indications

- Fail to achieve certain treatment milestone response
- Loss of response
- Progression to accelerated to blastic phase
- Significantly rising *BCR-ABL* transcript level

BCR-ABL Kinase Domain Mutation

- Semi-nested PCR performed to specifically amplify region of kinase domain of BCR-ABL fusion gene transcript
- Direct nucleotide sequencing in both directions was performed using ABI 3130xl genetic analyzer
- Sequence analysis performed with SeqScape software



Methods in Molecular Medicine, vol.125: Myeloid Leukemia: Methods and Protocols

In-vitro activity of tyrosine kinase inhibitors on mutants

Table 1. Sensitivity of Bcr-Abl kinase domain mutants to Abl kinase inhibitors. [a]

	Ba/F3 cellular proliferation IC_{50} values		
	imatinib (nM)	nilotinib (nM)	dasatinib (nM)
Native Bcr-Abl	260	13	0.8
M244V	2000	38	1.3
G250E	1350	48	1.8
Q252H	1325	70	3.4
Y253F	3475	125	1.4
Y253H	>6400	450	1.3
E255K	5200	200	5.6
E255V	>6400	430	11
V299L	540[†]	nd	18[†]
F311L	480	23	1.3
T315A	971	61	125[†]
T315I	>6400	>2000	>200
F317L	1050	50	7.4
F317V	350[†]	nd	53[†]
M351T	880	15	1.1
E355G	2300[‡]	nd	1.8[§]
F359V	1825	175	2.2
V379I	1630	51	0.8
L387M	1000	49	2
H396P	850	41	0.6
H396R	1750	41	1.3

Sensitive
Intermediate sensitivity
Insensitive

Imatinib: sensitive (≤1000 nM), intermediate (≤3000 nM), insensitive (>3000 nM).
Nilotinib: sensitive (≤50 nM), intermediate (≤500 nM), insensitive (>500 nM).
Dasatinib: sensitive (≤3 nM), intermediate (≤60 nM), insensitive (>60 nM).
[a] The IC_{50} value is the concentration of inhibitor resulting in a 50% reduction in cell viability. For experimental details, see [8,12,21,29].
[†] IC_{50} values from Burgess et al., PNAS 2005.[21]
[‡] IC_{50} value from Shah et al., Cancer Cell 2002.[29]
[§] IC_{50} value estimated from Shah et al., Science 2004.[8]

O'Hare T, Eide CA & Deininger MW. Blood, May 11, 2007

		IC_{50} fold increase (WT = 1)			
		Bosutinib	Imatinib	Dasatinib	Nilotinib
	Parental	38.31	10.78	> 50	38.43
	WT	1	1	1	1
P-LOOP	L248V	2.97	3.54	5.11	2.80
	G250E	4.31	6.86	4.45	4.56
	Q252H	0.81	1.39	3.05	2.64
	Y253F	0.96	3.58	1.58	3.23
	E255K	9.47	6.02	5.61	6.69
	E255V	5.53	16.99	3.44	10.31
C-Helix	D276G	0.60	2.18	1.44	2.00
	E279K	0.95	3.55	1.64	2.05
ATP binding region (drug contact sites)	V299L	26.10	1.54	8.65	1.34
	T315I	45.42	17.50	75.03	39.41
	F317L	2.42	2.60	4.46	2.22
SH2-contact	M351T	0.70	1.76	0.88	0.44
Substrate binding region (drug contact sites)	F359V	0.93	2.86	1.49	5.16
A-LOOP	L384M	0.47	1.28	2.21	2.33
	H396P	0.43	2.43	1.07	2.41
	H396R	0.81	3.91	1.63	3.10
	G398R	1.16	0.35	0.69	0.49
C terminal lobe	F486S	2.31	8.10	3.04	1.85

Sensitive	≤ 2
Moderately resistant	2.01-4
Resistant	4.01-10
Highly resistant	> 10

Redaelli S *et al*, J Clin Oncol 27: 469 – 71, 2009

Spectrum of *ABL* Kinase Domain Mutation seen at HKS&H

- Found in 23 out of 60 (38.3%) up to early April 2010
- Double mutation = 5; triple = 1

  (i.e. total 30 mutations)

- P-loop mutations = 14
  - M244V = 4
  - L248V = 2
  - G250E = 1
  - Y253F/H = 2
  - E255K = 4
  - E255V = 1
- E279K = 1
- T315I = 5
- F317L = 6
- M351T = 1
- F359V/C = 3

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  - **E255K = 4**
  - **E255V = 1**
- E279K = 1
- **T315I = 5**
- **F317L = 6**
- M351T = 1
- **F359V/C = 3**

2G-TKI clinically relevant mutations

- Resistant to 2G-TKI
  - T315I
- Less sensitive to nilotinib
  - Y253H, E255K/V, F359V/C
- Less sensitive to dasatinib
  - F317L, V299L

Branford S, Melo JV, Hughes TP. Blood 2009; 114: 5426 - 35

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Pharmacogenomics in oncology

Genetic target	Anti-cancer drug
CYP2D6	tamoxifen
TPMT	6-mercaptopurine
UGT1A1	irinotecan
TYMS or TS	5-FU
ERCC1	cisplatin
RRM1	gemcitabine

Which test?

Which method?

Which drug?

DRUG ⇄ TEST

Analytical validity

Clinical validity

Clinical utility

Ethical, legal, social implication