Molecular Diagnostics in Gastrointestinal Cancers

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Scope of talk

- Histopathologists with an interest in molecular pathology/genetics.
- Knowledge of molecular genetics.
- Practical and clinically relevant.

Scope of talk

- RAS testing of CRC.
- MMR/MSI testing of CRC.
- HER2 testing of oesophago-gastric carcinoma.
- GIST mutation testing.
- [Melanoma testing]

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- *RAS* testing of CRC.
- MMR/MSI testing of CRC.
- HER2 testing of oesophago-gastric carcinoma.
- GIST mutation testing.
- **[Melanoma testing]**

Scope of talk

- Why test
- How you test
- Issues of testing
- Practical points

RAS testing of CRC

- Referring to ***KRAS*** and ***NRAS*** genes (but not e.g. *GNAS*).

RAS testing of CRC – Why?

- Personalised medicine

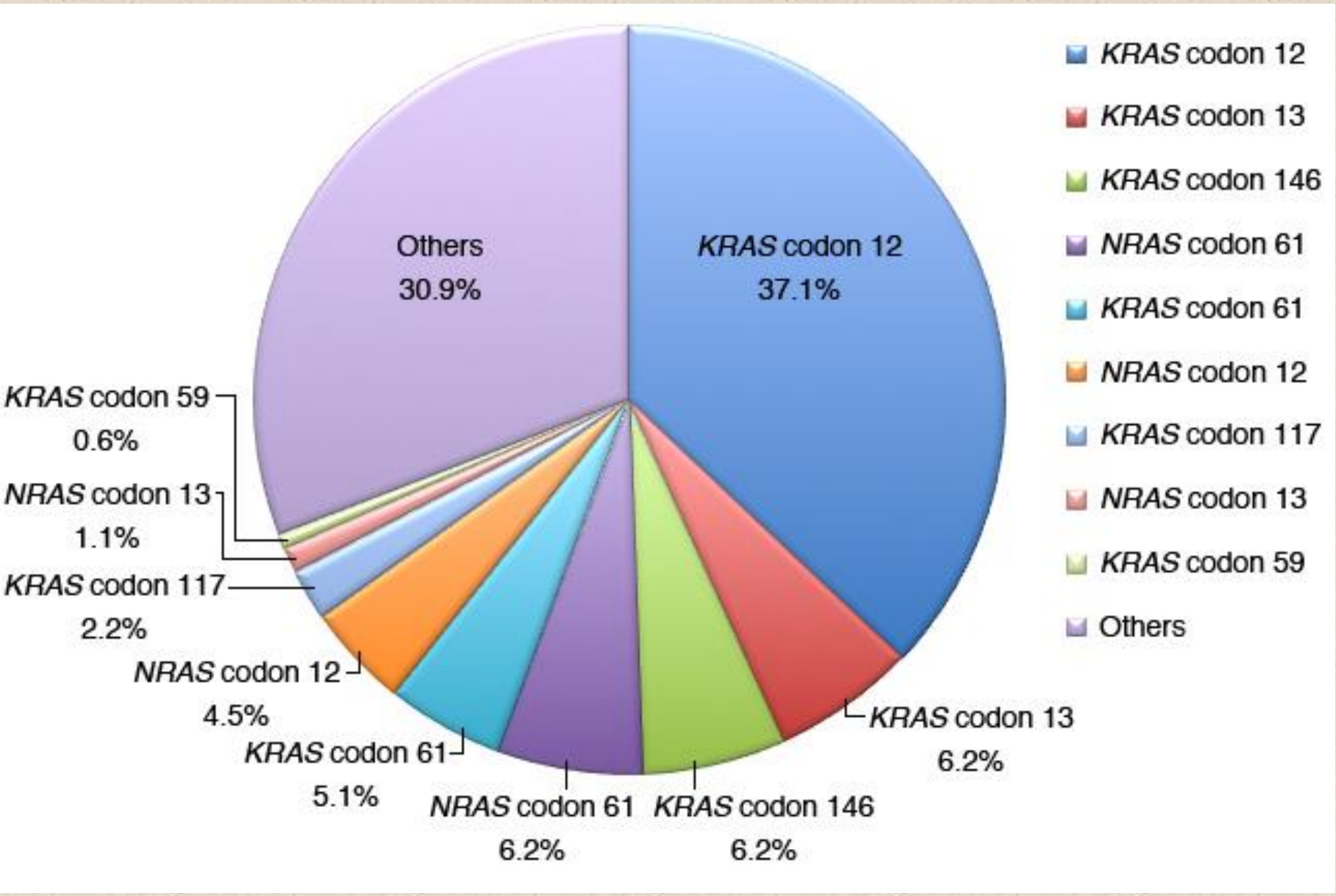
RAS testing of CRC – Why?

- Personalised medicine
- Anti-EGFR therapy (e.g. cetuximab, bevacizumab, and panitumumab) does not work on RAS mutant CRC.

RAS testing of CRC – Why?

- *KRAS* codon 12 and 13 mutants

Bokemeyer C, Bondarenko I, Hartmann JT, *et al*. Efficacy according to biomarker status of cetuximab plus FOLFOX-4 as first-line treatment for metastatic colorectal cancer: the OPUS study. *Ann Oncol* 2011;22:1535–46.
Van Cutsem E, Kohne CH, Hitre E, *et al*. Cetuximab and chemotherapy as initial treatment for metastatic colorectal cancer. *N Engl J Med* 2009;360:1408–17.


Others
30.9%
KRAS codon 12
37.1%
KRAS codon 59
0.6%
NRAS codon 13
1.1%
KRAS codon 117
2.2%
NRAS codon 12
4.5%
KRAS codon 61
5.1%
NRAS codon 61
6.2%
KRAS codon 146
6.2%
KRAS codon 13
6.2%
KRAS codon 12
KRAS codon 13
KRAS codon 146
NRAS codon 61
KRAS codon 61
NRAS codon 12
KRAS codon 117
NRAS codon 13
KRAS codon 59
Others

RAS testing of CRC – Why?

- Cetuximab for the first-line treatment of metastatic colorectal cancer (August 2009) **NICE technology appraisal guidance 176:**
- "Cetuximab is indicated for the treatment of patients with EGFR-expressing, Kirsten rat sarcoma (KRAS) wild-type metastatic colorectal cancer"

RAS testing of CRC – Why?

- FIRE-3, PEAK and PRIME trials

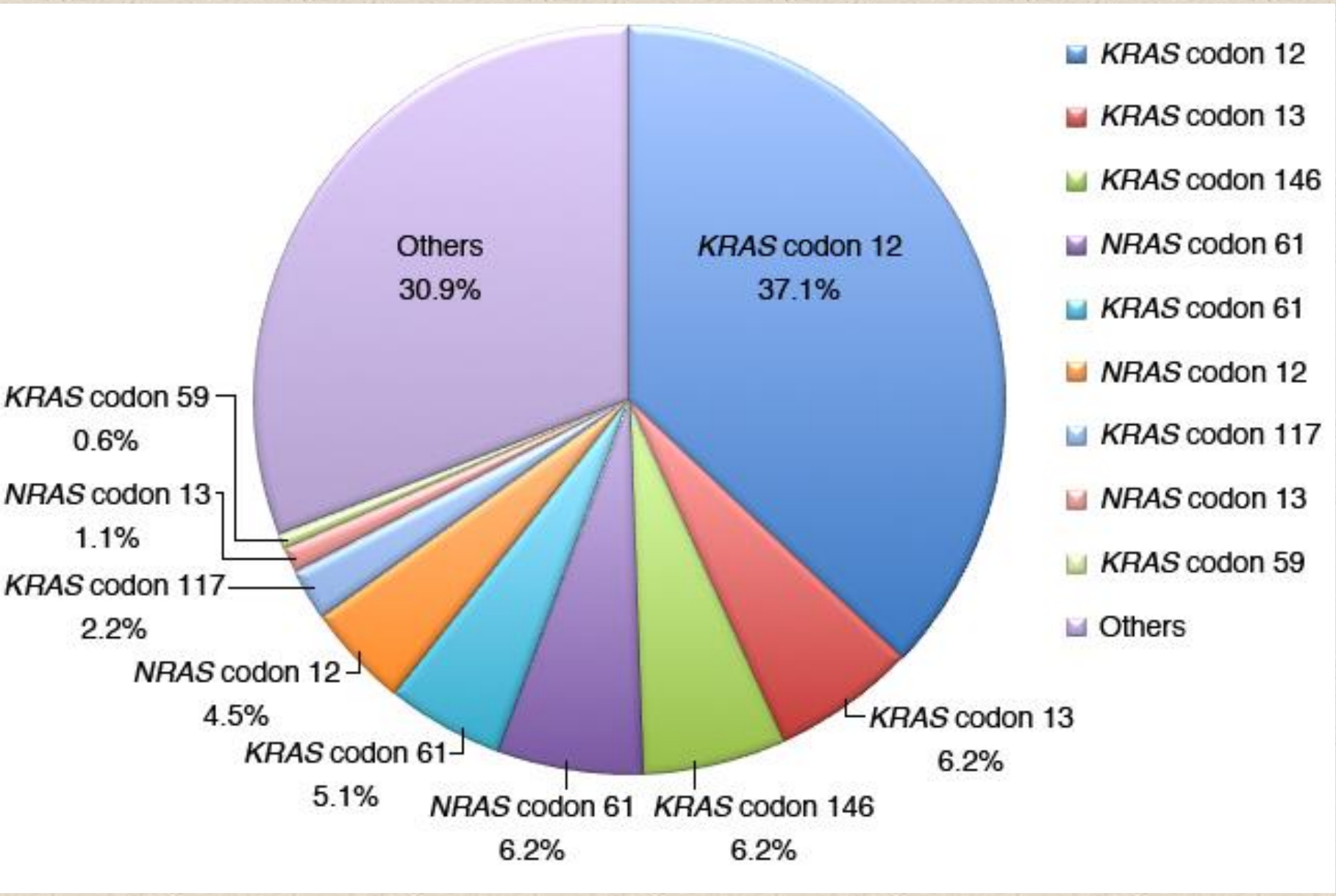
The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Panitumumab–FOLFOX4 Treatment and *RAS* Mutations in Colorectal Cancer

Jean-Yves Douillard, M.D., Ph.D., Kelly S. Oliner, Ph.D., Salvatore Siena, M.D., Josep Tabernero, M.D., Ronald Burkes, M.D., Mario Barugel, M.D., Yves Humblet, M.D., Ph.D., Gyorgy Bodoky, M.D., Ph.D., David Cunningham, M.D., Jacek Jassem, M.D., Ph.D., Fernando Rivera, M.D., Ph.D., Ilona Kocákova, M.D., Ph.D., Paul Ruff, M.D., Maria Błasińska-Morawiec, M.D., Martin Šmakal, M.D., Jean Luc Canon, M.D., Mark Rother, M.D., Richard Williams, M.B., B.S., Ph.D., Alan Rong, Ph.D., Jeffrey Wiezorek, M.D., Roger Sidhu, M.D., and Scott D. Patterson, Ph.D.

N Engl J Med 2013;369:1023-34.
DOI: 10.1056/NEJMoa1305275


Others
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KRAS codon 12
KRAS codon 13
KRAS codon 146
NRAS codon 61
KRAS codon 61
NRAS codon 12
KRAS codon 117
NRAS codon 13
KRAS codon 59
Others

RAS testing of CRC – Why?

- Anti-EGFR drug resistance is predicted by mutations:
  - *KRAS* codons 12, 13, 59, 61, 117 and 146
  - *NRAS* codons 12, 13, 59 and 61

RAS testing of CRC – Why?

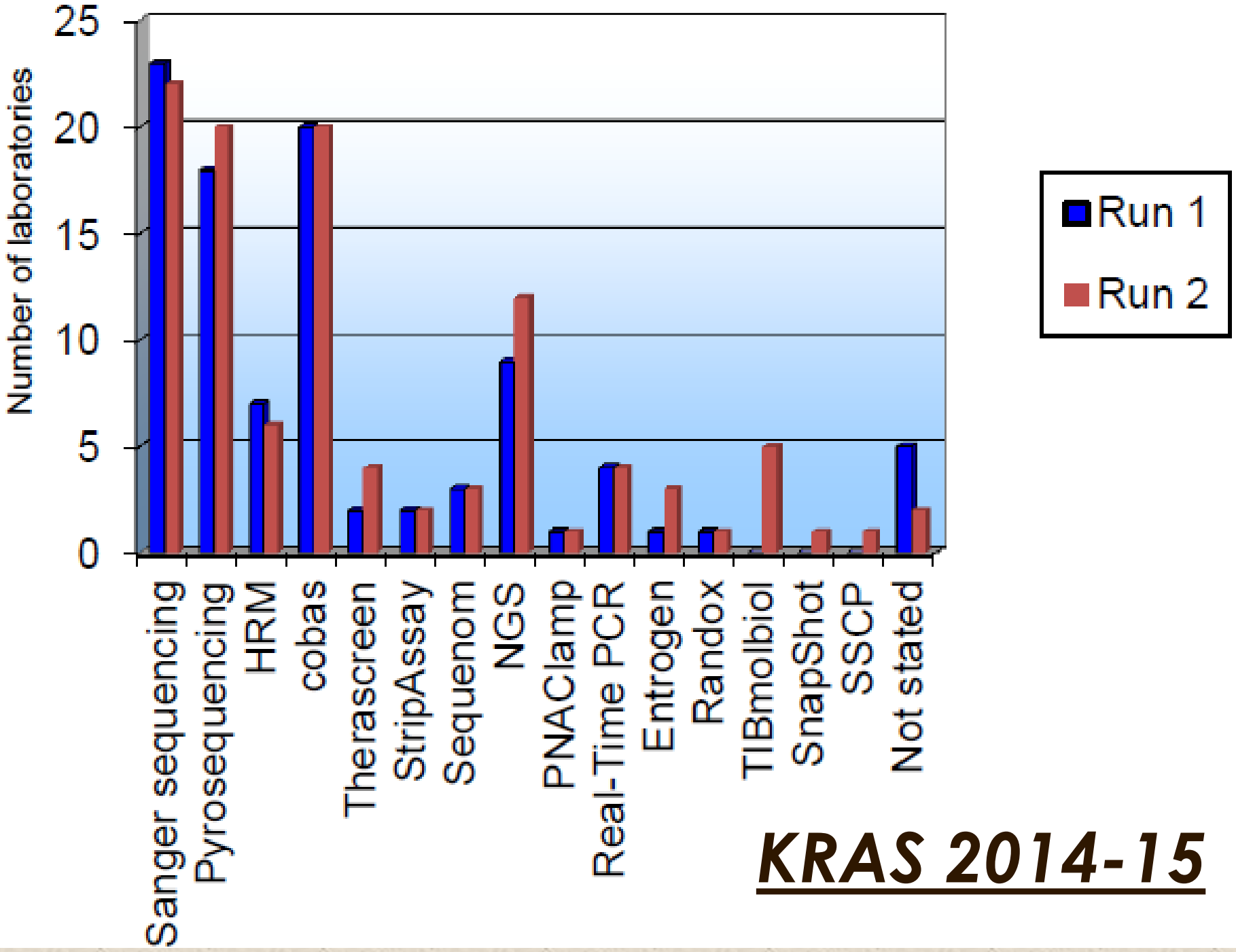
- Current funding:
  - NICE TA176
  - CDF

RAS testing of CRC – Why?

- Current funding:
  - ~~NICE TA176~~
  - ~~CDF~~
  - All funded by NHS England (direct invoicing)

RAS testing of CRC – How?

- UK NEQAS Molecular Pathology CRC schemes:
  - Not all labs are UK based.


Number of laboratories
25
20
15
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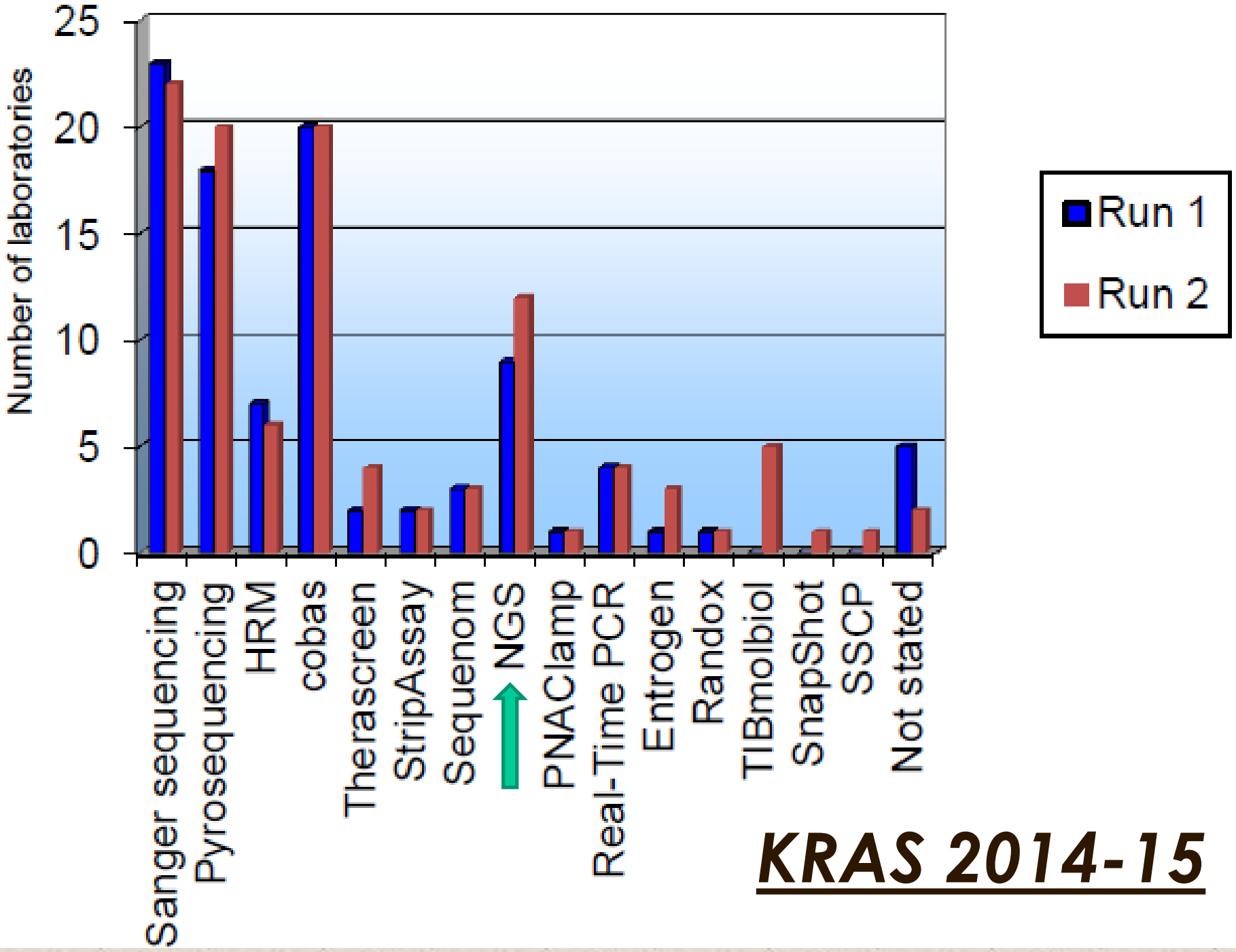
5
0
Sanger sequencing
Pyrosequencing
HRM
cobas
Therascreen
StripAssay
Sequenom
NGS
PNAClamp
Real-Time PCR
Entrogen
Randox
TIBmolbiol
SnapShot
SSCP
Not stated
Run 1
Run 2

KRAS 2014-15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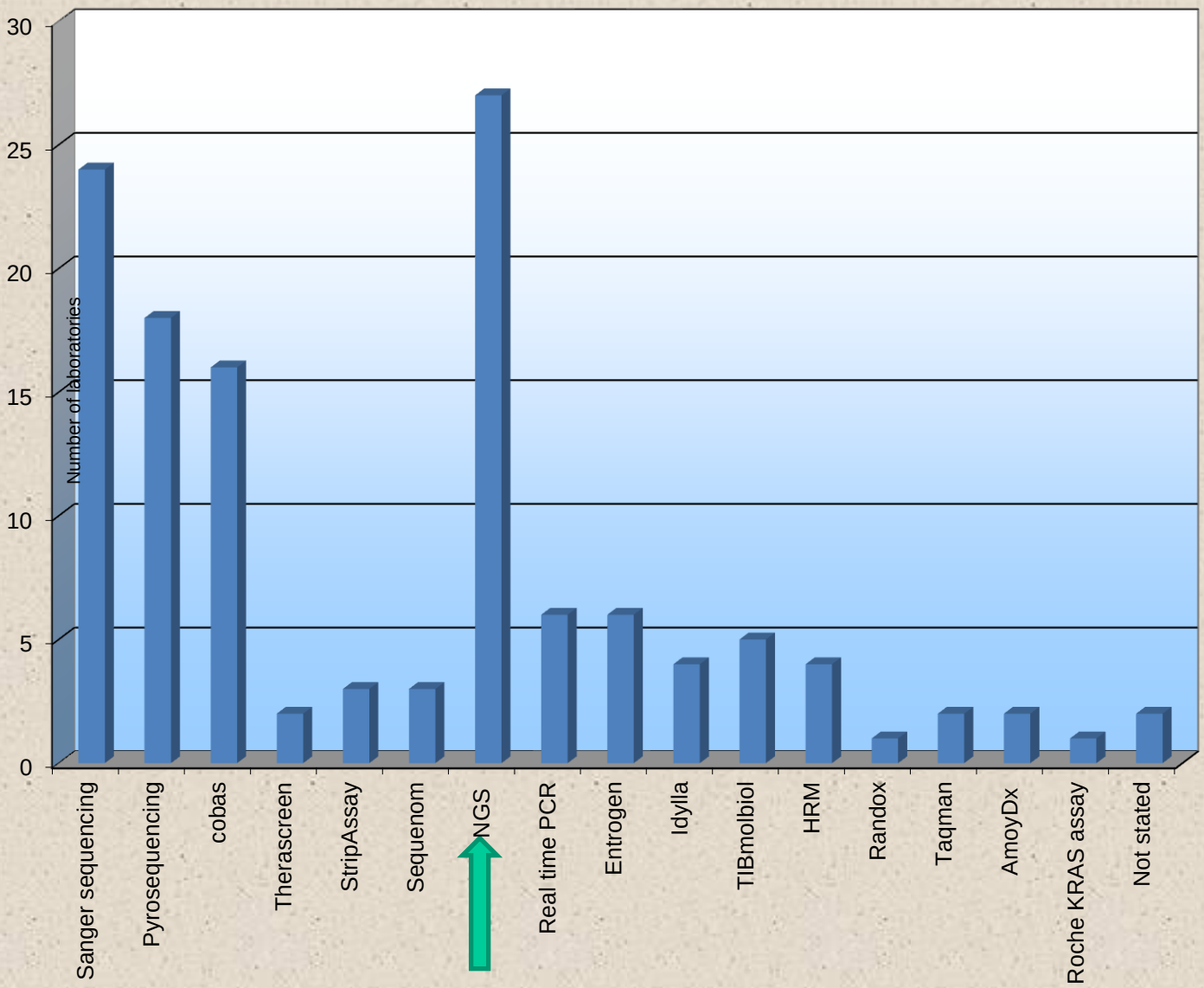
KRAS 2014-15



KRAS 2016-17 run 1

RAS testing of colorectal carcinoma—a guidance document from the Association of Clinical Pathologists Molecular Pathology and Diagnostics Group

Newton ACS Wong,[1] David Gonzalez,[2] Manuel Salto-Tellez,[3] Rachel Butler,[4] Salvador J Diaz-Cano,[5] Mohammad Ilyas,[6] William Newman,[7] Emily Shaw,[8] Philippe Taniere,[9] Shaun V Walsh[10]

ABSTRACT

Analysis of colorectal carcinoma (CRC) tissue for *KRAS* codon 12 or 13 mutations to guide use of anti-epidermal growth factor receptor (EGFR) therapy is now considered mandatory in the UK. The scope of this practice has been recently extended because of data indicating that *NRAS* mutations and additional *KRAS* mutations also predict for poor response to anti-EGFR therapy. The following document provides guidance on RAS (i.e., *KRAS* and *NRAS*) testing of CRC tissue in the setting of personalised medicine within the UK and particularly within the NHS. This guidance covers issues related to case selection, preanalytical aspects, analysis and interpretation of such RAS testing.

whether use of EGFR inhibitors is being funded by NICE or through the CDF, *KRAS* genotyping of CRC tissue has become commonly requested within the NHS to help stratify patients for anti-EGFR therapy. Groups outside the UK have already issued guidance or recommendation documents on *KRAS* testing of CRC.[8–10] However, the following document is directed specifically at practice within the UK and especially within the NHS. Further, this guidance is one of the first to incorporate recent data on *NRAS* testing of CRC in the setting of personalised medicine. The document also reviews some technical and/or investigational aspects that impact directly on RAS testing of CRC. As a document that focuses particularly on practical

J Clin Pathol 2014;**67**:751–757. doi:10.1136/jclinpath-2014-202467

Box 1 Main recommendations for RAS testing of colorectal carcinoma to guide anti-EGFR therapy

- Network arrangements should be established to ensure rapid and robust tissue pathways from referral centres to testing laboratories.
- Either primary or metastatic CRC tissue can be used for RAS testing.
- Either biopsy or resection specimen tissue can be used for RAS testing, though if both are equally available, use of resection tissue is preferable.

J Clin Pathol 2014;**67**:751–757. doi:10.1136/jclinpath-2014-202467

RAS testing of CRC – Issues

- Heterogeneity:
  - i.e. more than one clone in the same CRC (wild type vs. RAS mutant; different RAS mutants)
  - Its extent is controversial
  - If majority clone is wild type, only more sensitive assay may pick up the mutant clone.

RAS testing of CRC – Issues

- Heterogeneity:
  - Explains emerging resistance
  - cfDNA to detect resistant clone?
  - Clinical dilemma: at what level of RAS mutant clone do you deny the patient anti-EGFR rx?

RAS testing of CRC – Practical points

- Be updated with what RAS genes and exons are tested.
- Prioritise dispatch of tissue blocks to RAS testing labs (inclusion in CRC dataset).
- Read the recommendations of the JCP 2014 guidance doc!

MMR/MSI testing of CRC – Why?

- Screening for hereditary disease
- (Personalised medicine)

In Men and Women

Colon

Risk with Lynch Syndrome: **>25%** by age 50[1] **82%** by age 70[1,2]

General Population Risk[3]: 0.2% by age 50, 2% by age 70

Stomach

Risk with Lynch Syndrome: **13%** by age 70[2]

General Population Risk[3]: <1% by age 70

Though the following cancers are rare, their risk also increases with Lynch Syndrome:

Small Intestine, **7.2%**[1,2]; Urinary Tract, **4%**; Brain, **3.7%**; Biliary Tract, **2%**; all by age 70.[2]

In Women Only

Uterine (Endometrial)

Risk with Lynch Syndrome: **~20%** by age 50[1] **71%** by age 70[1,2]

General Population Risk[3]: 0.2% by age 50, 1.5% by age 70

Ovary

Risk with Lynch Syndrome: **12%** by age 70[2]

General Population Risk[3]: 2% by age 70

References: 1. Vasen HF, Wijnen J, Menko FH, et al. Cancer risk in families with hereditary nonpolyposis colorectal cancer diagnosed by mutation analysis. Gastroenterology. 1996;110(4):1020-1027. 2. Aarnio M, Sankila R, Pukkala E, et al. Cancer risk in mutation carriers of DNA-mismatch-repair genes. Int J Cancer. 1999;81(2):214-218. 3. Pause EJ, Wun LM. DEVCAN: Probability of developing or dying of cancer software, version 4.0. National Cancer Institute 1995. Available at http://www.cancer.gov/. 4. Schulmann K, Bruech HE, et al. HNPCC-associated small bowel cancer: clinical and molecular characteristics. Gastroenterology. 2005;128(3):580-588. 5. Vasen HF, Stormorken A, et al. MSH2 mutation carriers are at higher risk of cancer than MLH1 mutation carriers: a study of hereditary nonpolyposis colorectal cancer families. J Clin Oncol. 2001;19(20):4074-4080.

If one or more of the following applies to you or a family member, ask your doctor about Lynch Syndrome

- Colorectal cancer before age 50
- Endometrial cancer before age 50
- Two or more Lynch Syndrome cancers
- A previously identified mutation in the family

MMR/MSI testing of CRC – Why?

- Personalised medicine:
  - MMR deficient CRCs do not respond to 5-FU therapy.
  - ?Increased patient toxicity when 5-FU therapy is used for MMR deficient CRCs.
  - 'Borderline' CRC cases (e.g. high risk Dukes' B) for 5-FU therapy.

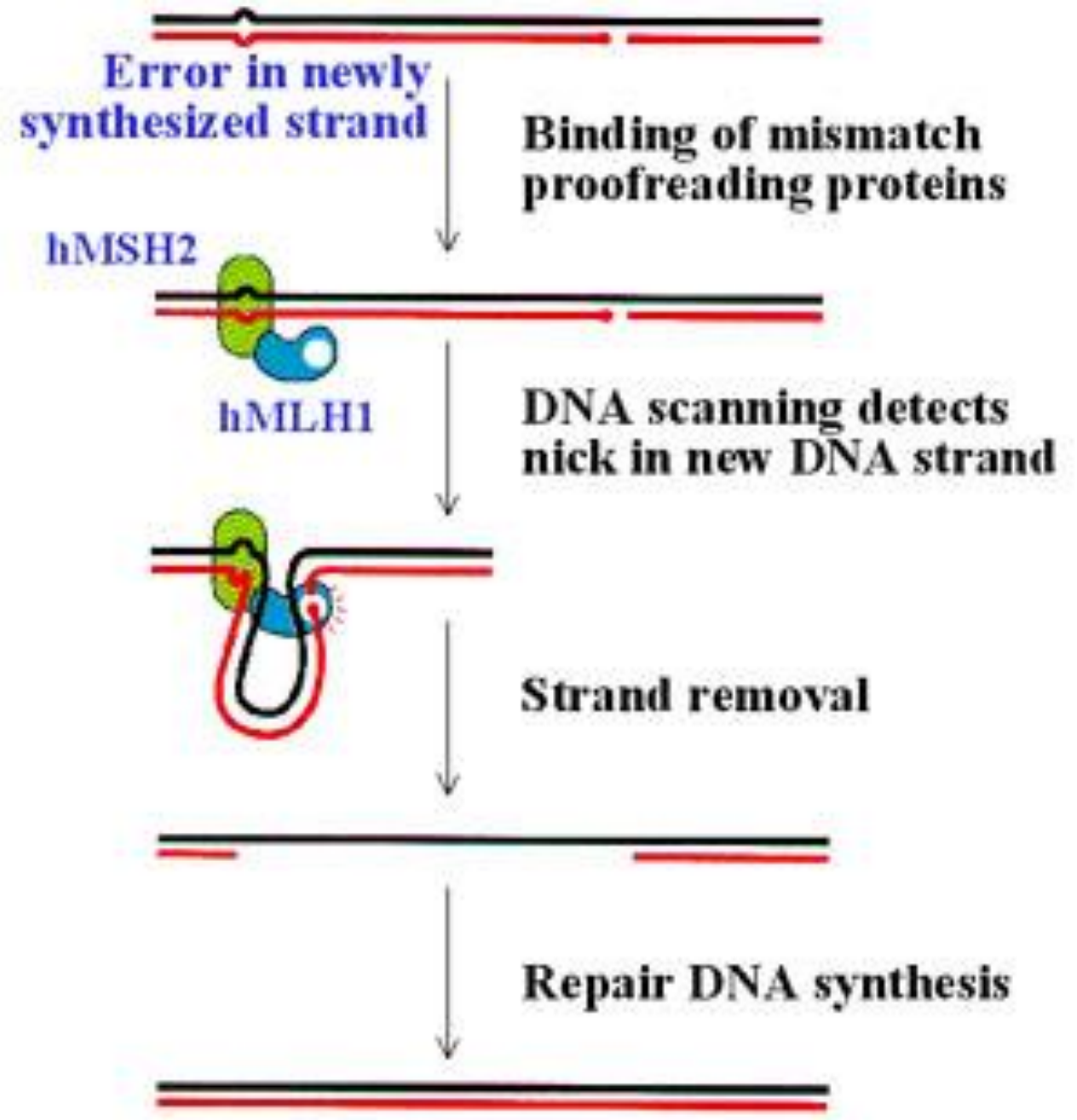
MMR/MSI testing of CRC – Why?

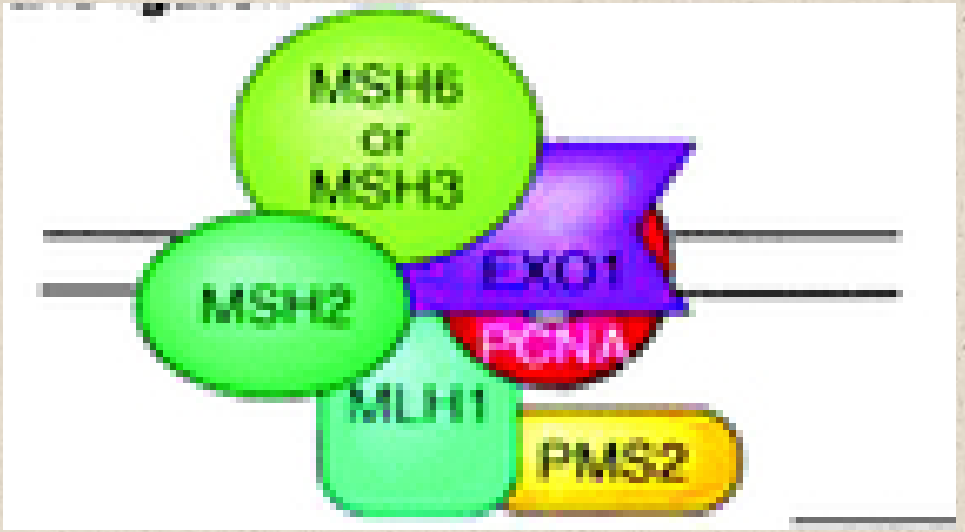
- Funding:
  - Clinical genetics for Lynch screening.
  - ?Oncologists for personalised medicine
  - ??? for Reflex testing (NICE DAP)

MMR/MSI testing of CRC – How?

- Mismatch repair → MMR proteins →Tissue sections and immunohistochemistry.

- Microsatellite instability (MSI)→ genetic change → DNA and PCR

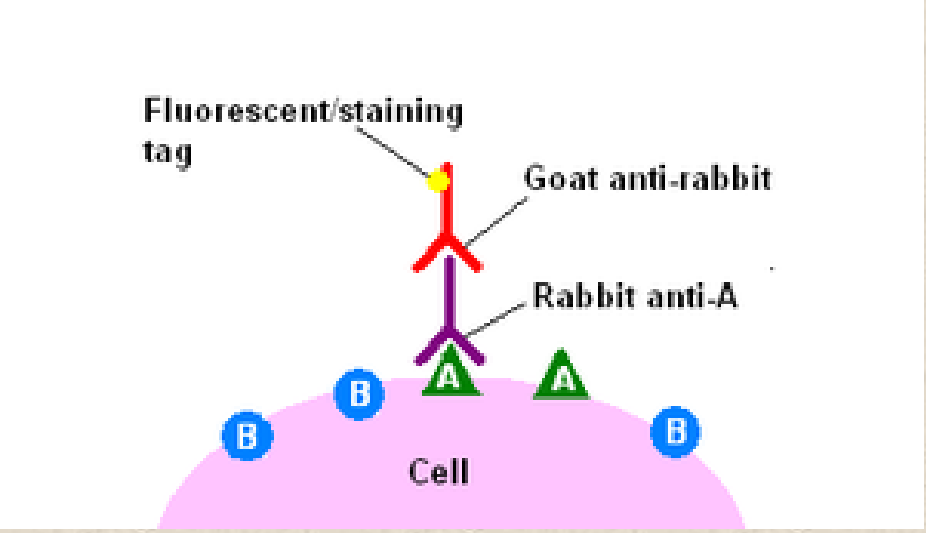

Error in newly synthesized strand
Binding of mismatch proofreading proteins
hMSH2
hMLH1
DNA scanning detects nick in new DNA strand
Strand removal
Repair DNA synthesis

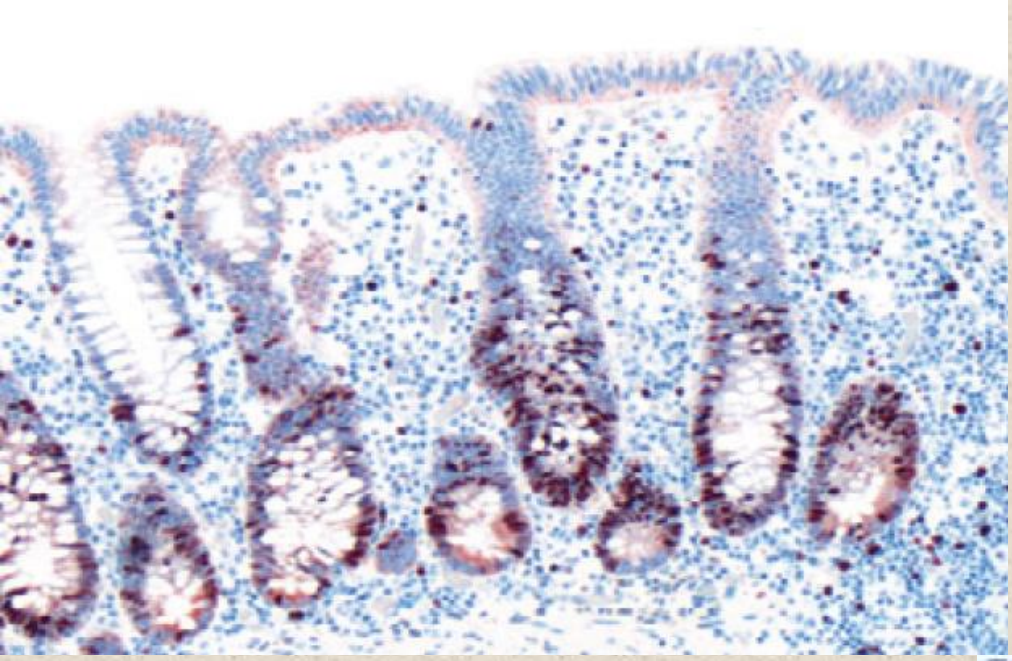

MSH6
or
MSH3
MSH2
EXO1
PCNA
MLH1
PMS2

- Protein **loss** is abnormal
- Lynch syndrome mutations:
  - MLH1 mutated → protein loss
  - MSH2 mutated → protein loss
  - MSH6 mutated → protein loss
  - PMS2 mutated → protein loss

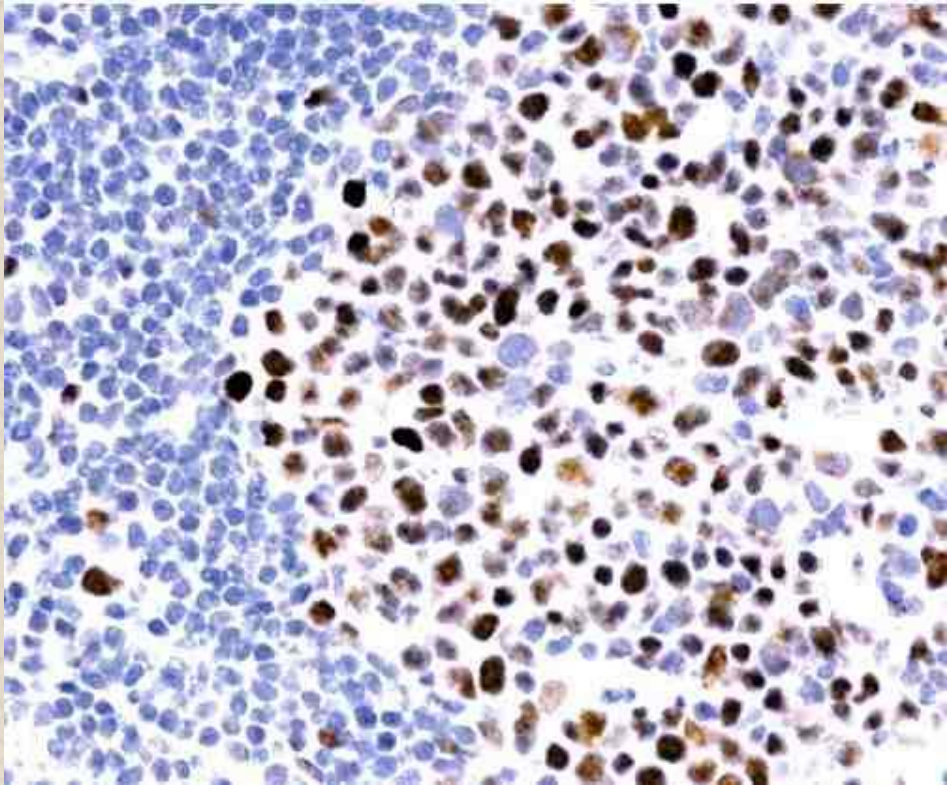
MMR immuno-histochemistry

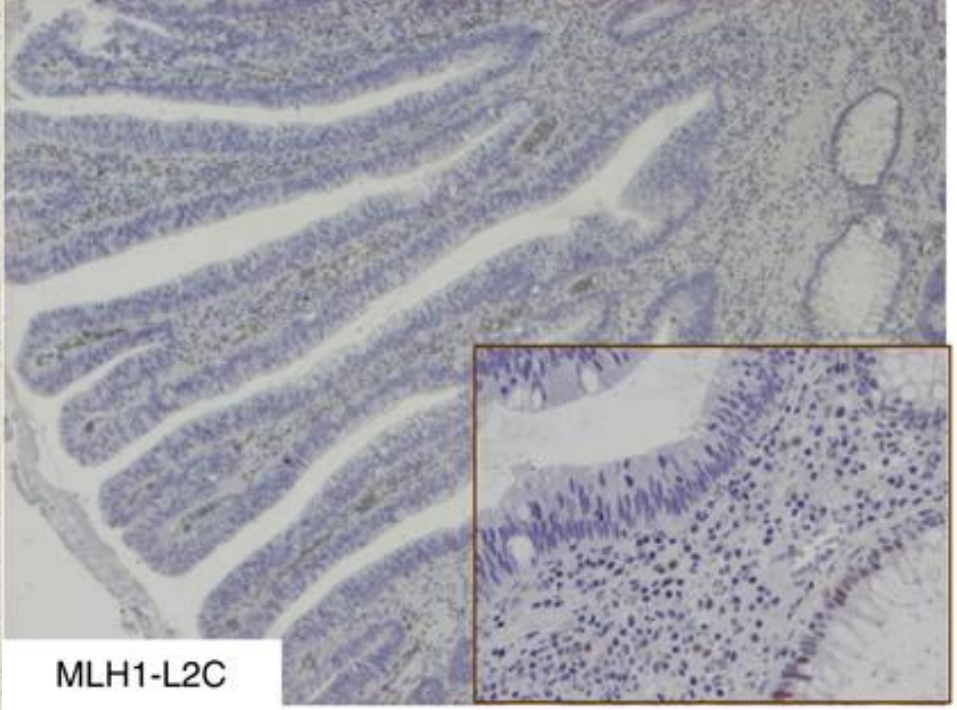
- Lynch syndrome mutations:
  - MLH1
  - MSH2
  - MSH6
  - PMS2

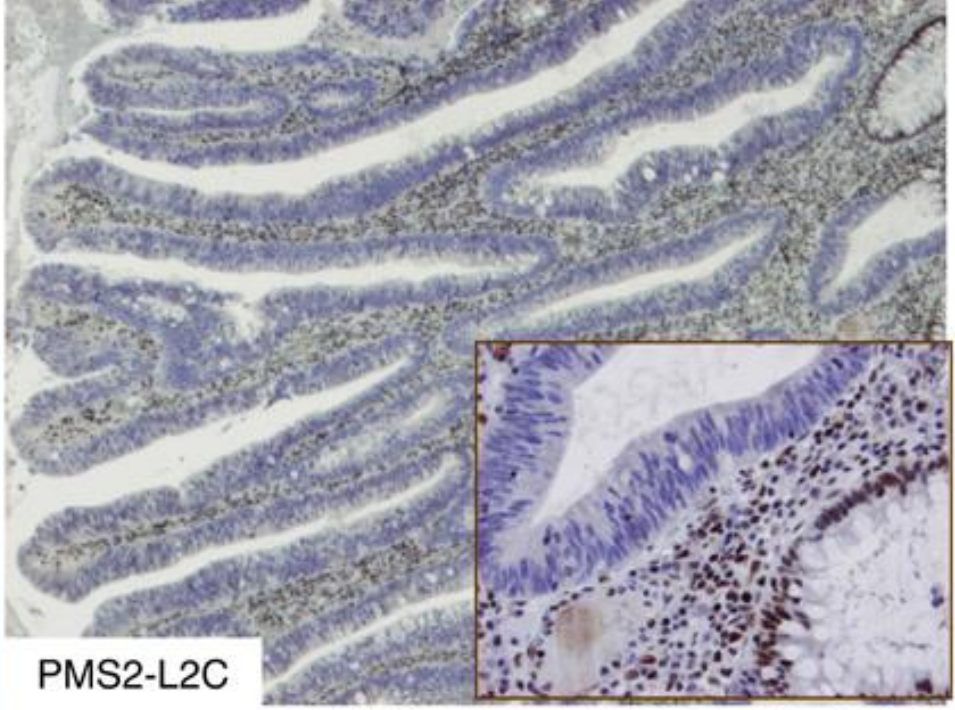


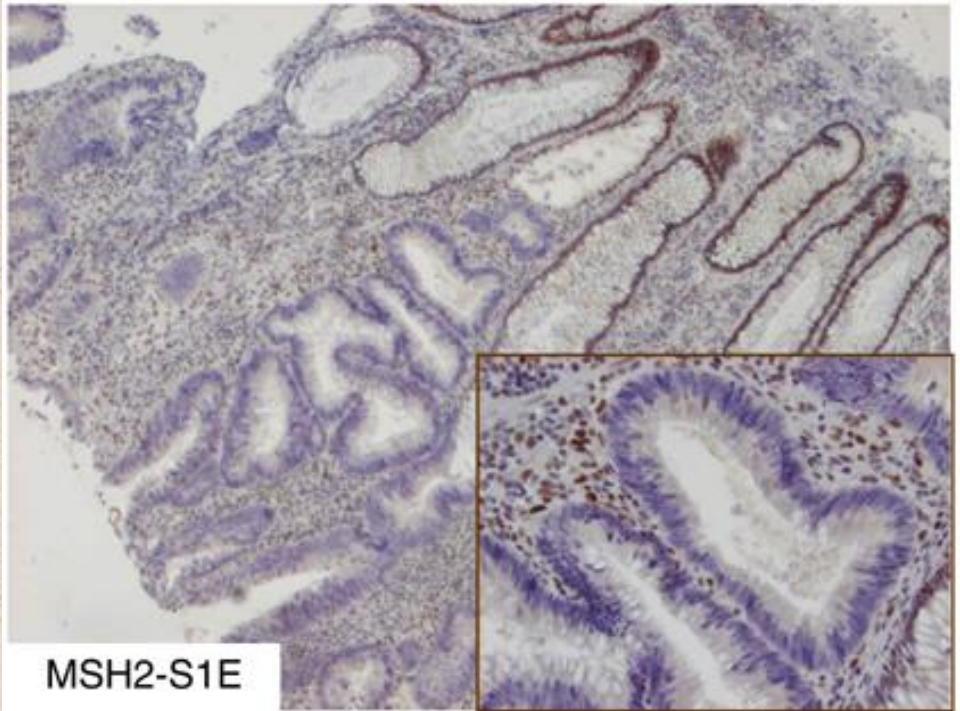


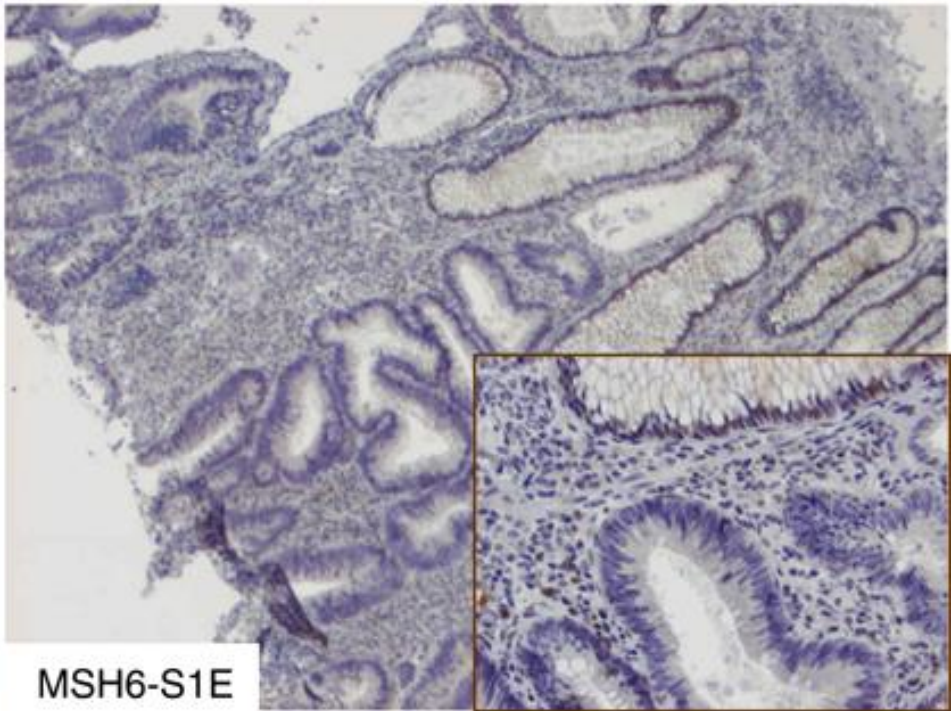
Fixation sensitive



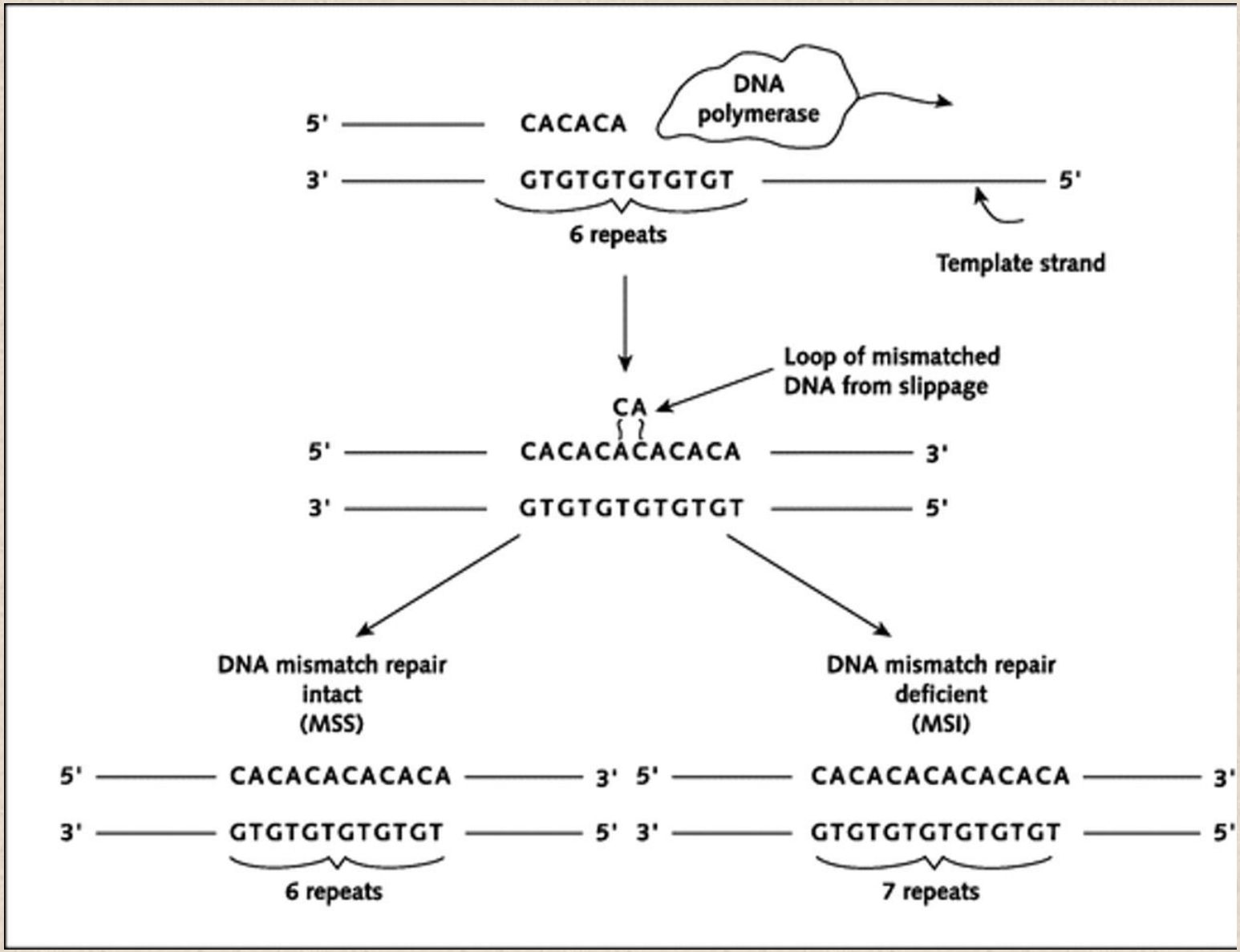

MLH1-L2C

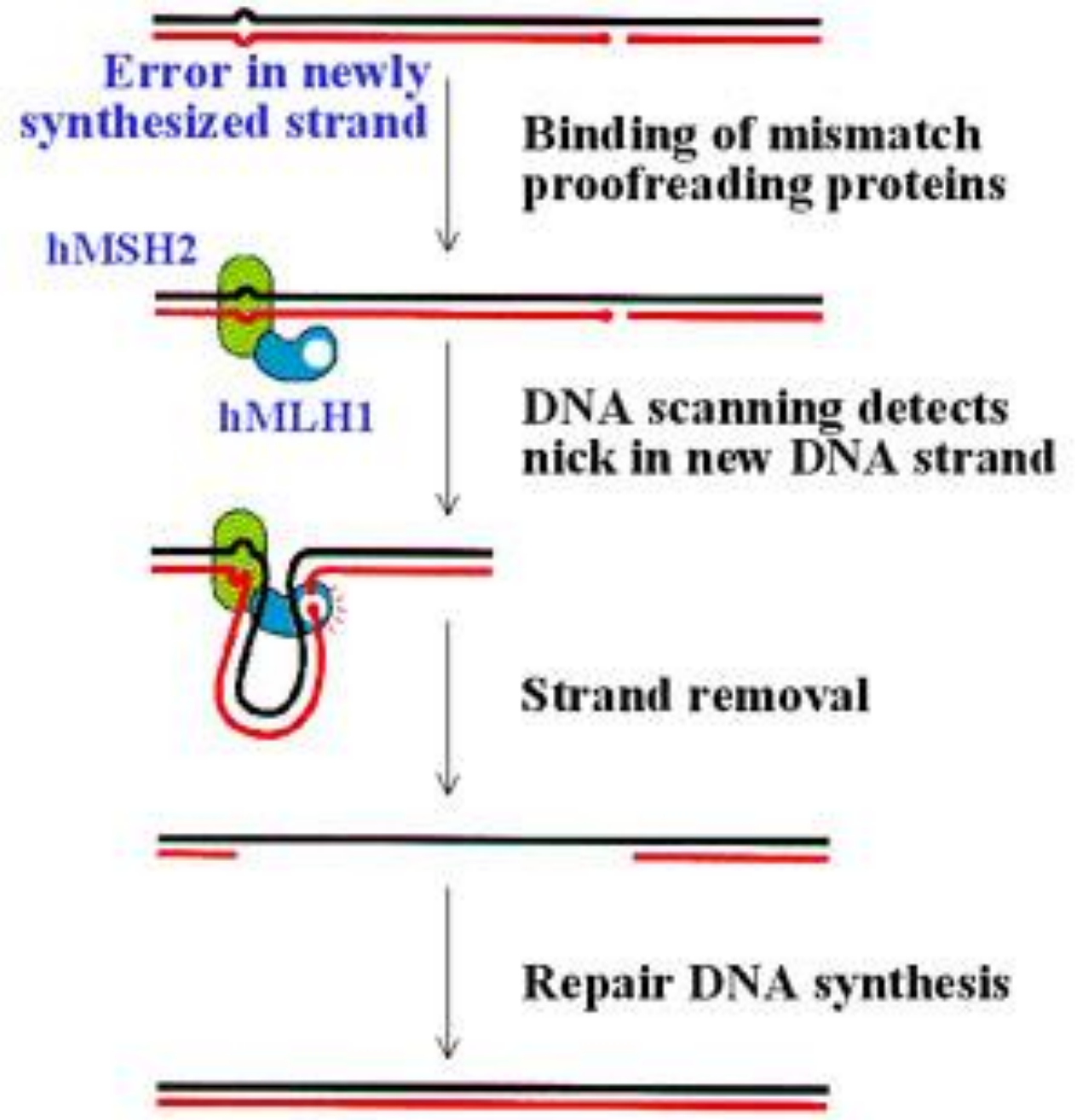

PMS2-L2C


MSH2-S1E

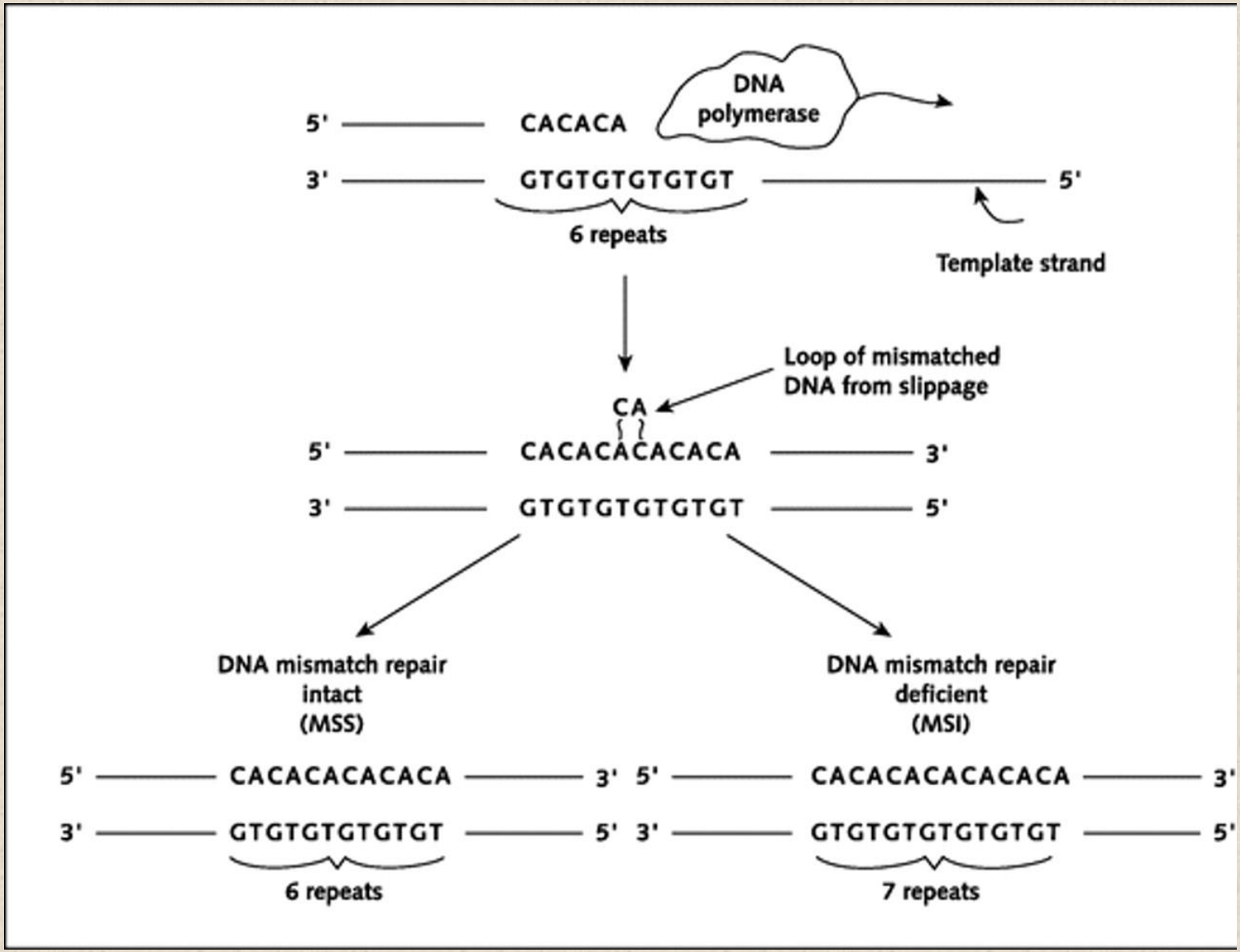

MSH6-S1E

DNA microsatellites

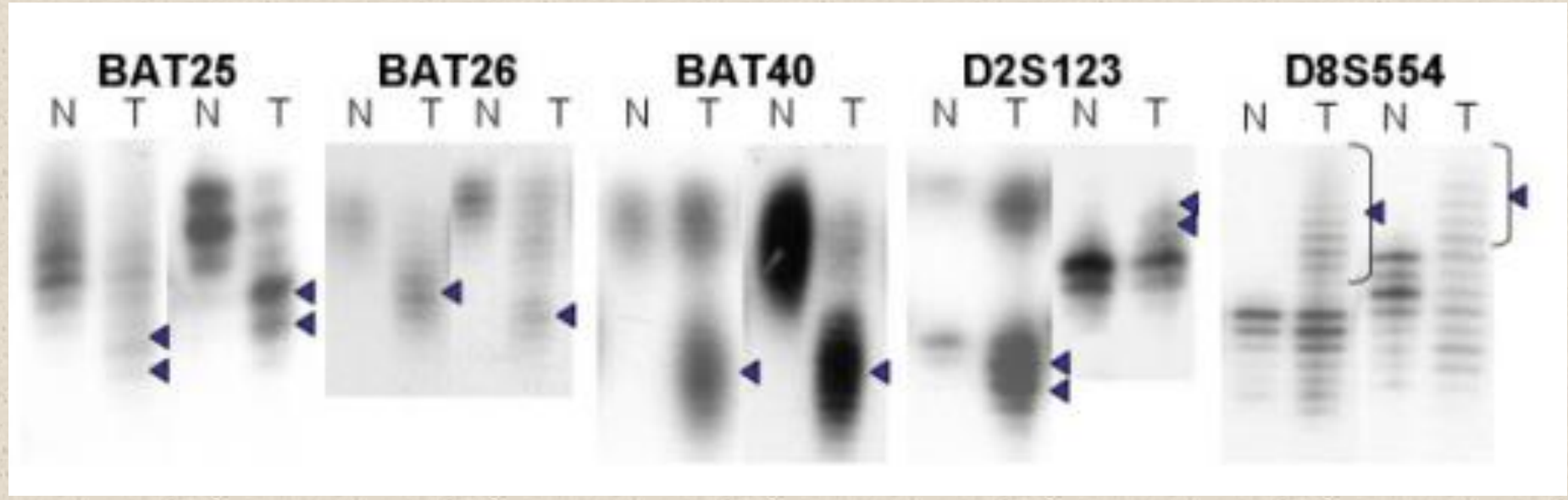



Error in newly synthesized strand
Binding of mismatch proofreading proteins
hMSH2
hMLH1
DNA scanning detects nick in new DNA strand
Strand removal
Repair DNA synthesis

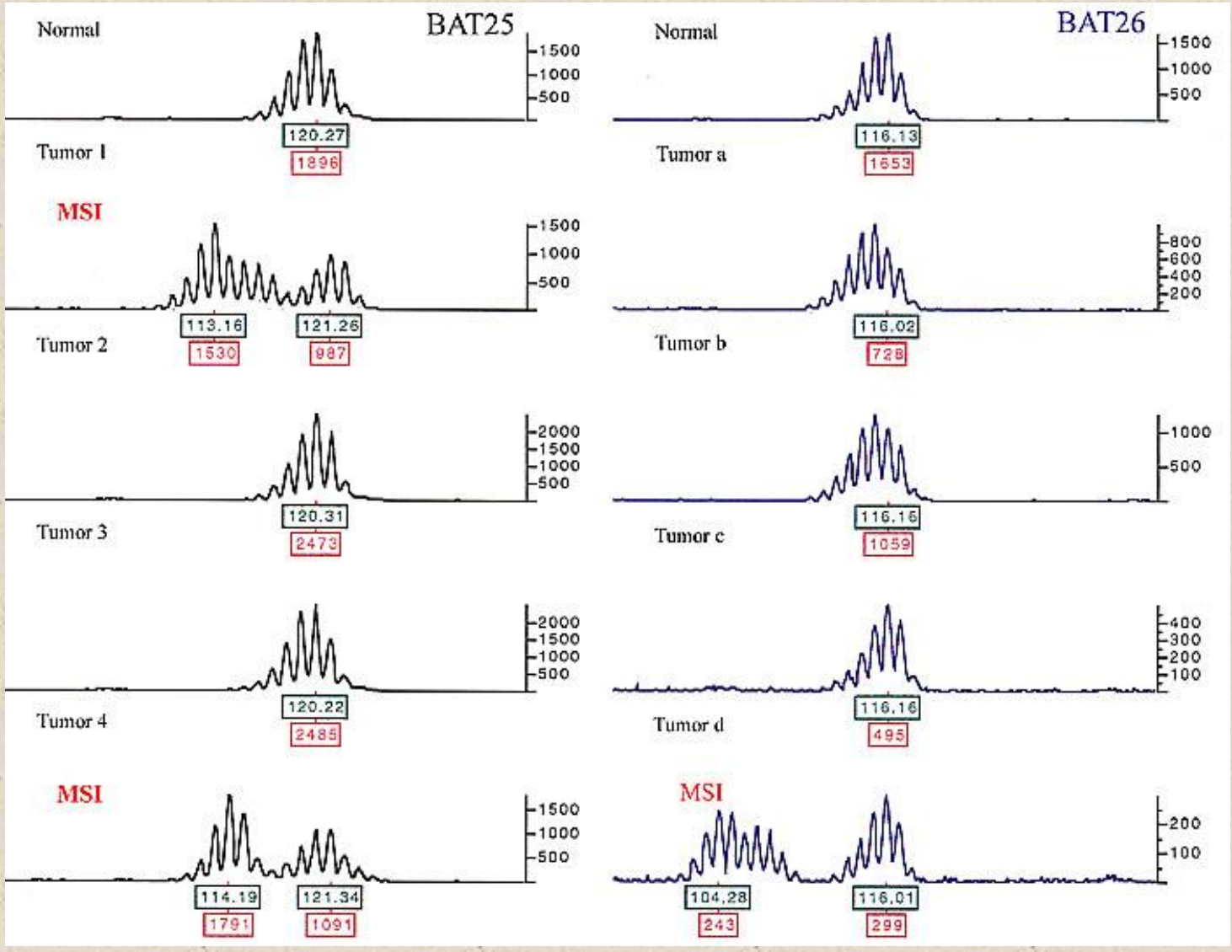
DNA microsatellites

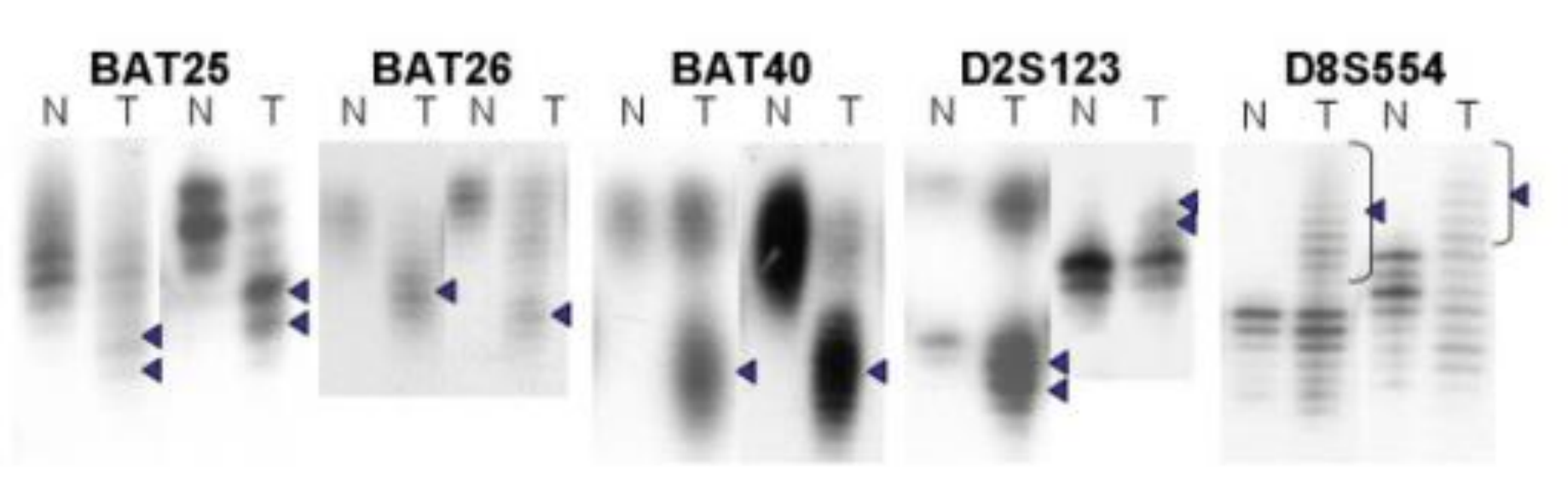


Microsatellite instability



Microsatellite instability





- MS stable (MSS) = 0/5 markers shift
- MS instability (MSI)-low = 1/5 shift
- MSI-high = 2+/5 shift

CRC & Lynch Syndrome

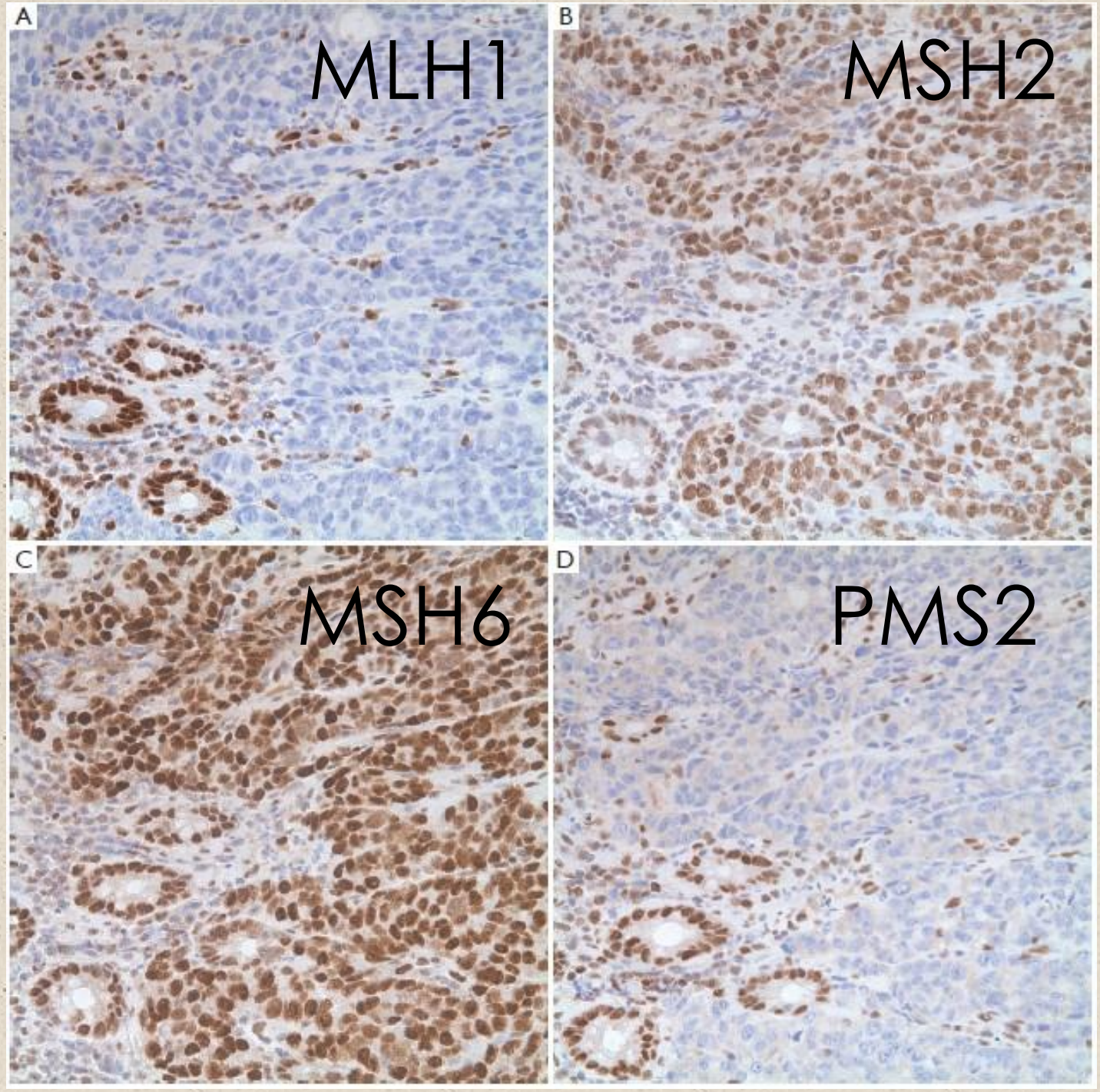
- MSI analysis – sensitivity 77-91% and specificity 90%.
- MMR IHC – sensitivity 92-94% and specificity 88-100%.
- MMR IHC is quicker, cheaper and uses less tissue.
- Only MMR IHC identifies likely mutated gene.

MLH1 wild type ← MLH1 protein present

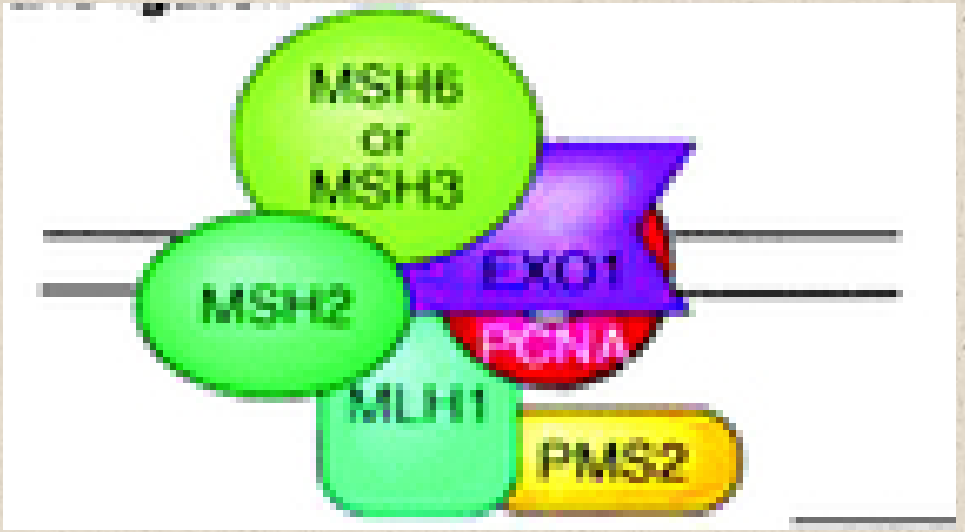
MSH2 wild type ← MSH2 protein present

MSH6 wild type ← MSH6 protein present

PMS2 mutated ← PMS2 protein loss


A
MLH1
B
MSH2
C
MSH6
D
PMS2

	CRC	Sebaceous neoplasm
MLH1-/PMS2-	66%	9%
MSH2-/MSH6-	18%	64%
MSH6-	13%	27%
PMS2-	3%	0%


MSH6
or
MSH3
MSH2
EXO1
PCNA
MLH1
PMS2

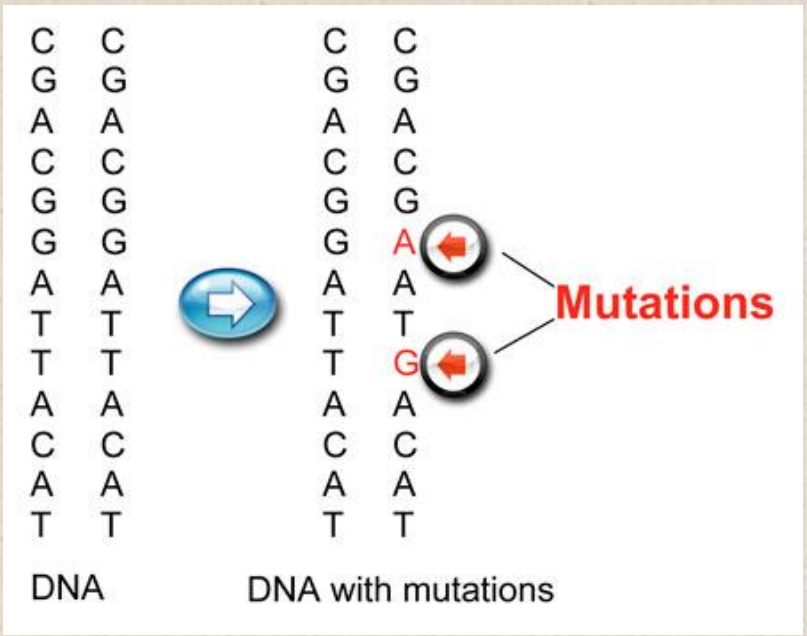
	CRC	Sebaceous neoplasm
MLH1-/PMS2-	66%	9%
MSH2-/MSH6-	18%	64%
MSH6-	13%	27%
PMS2-	3%	0%

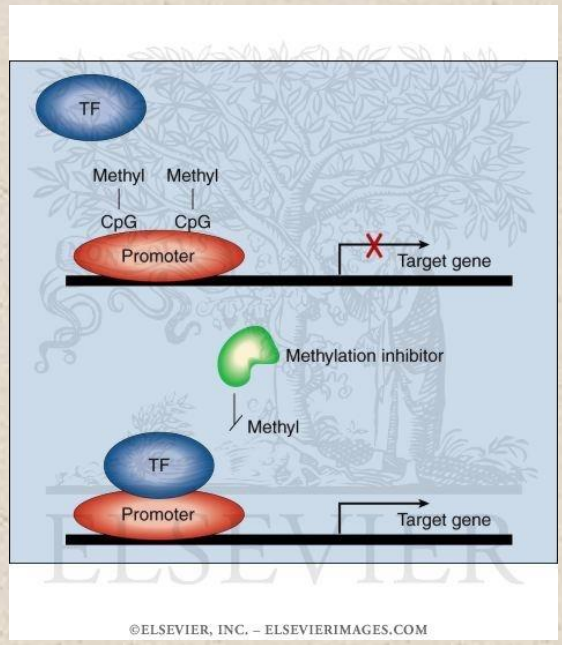
MMR/MSI testing of CRC – How?

- Reduced or loss of MSH6 expression may be due to DXT.
- *MSH2* mutation causing MSH6 loss but mutant yet immunogenic MSH2 (therefore **only MSH6 immunonegative**).

	CRC	Sebaceous neoplasm
MLH1-/PMS2-	66%	9%
MSH2-/MSH6-	18%	64%
MSH6-	13%	27%
PMS2-	3%	0%

Loss of MLH1 in CRC





20-30%

70-80%

CRC & Lynch Syndrome

- *BRAF* V600E mutation precludes Lynch syndrome
- Therefore if MLH1 loss (i.e. MLH1 and PMS2 loss):
  - *BRAF* V600E analysis (?specific IHC)
  - MLH1 hypermethylation

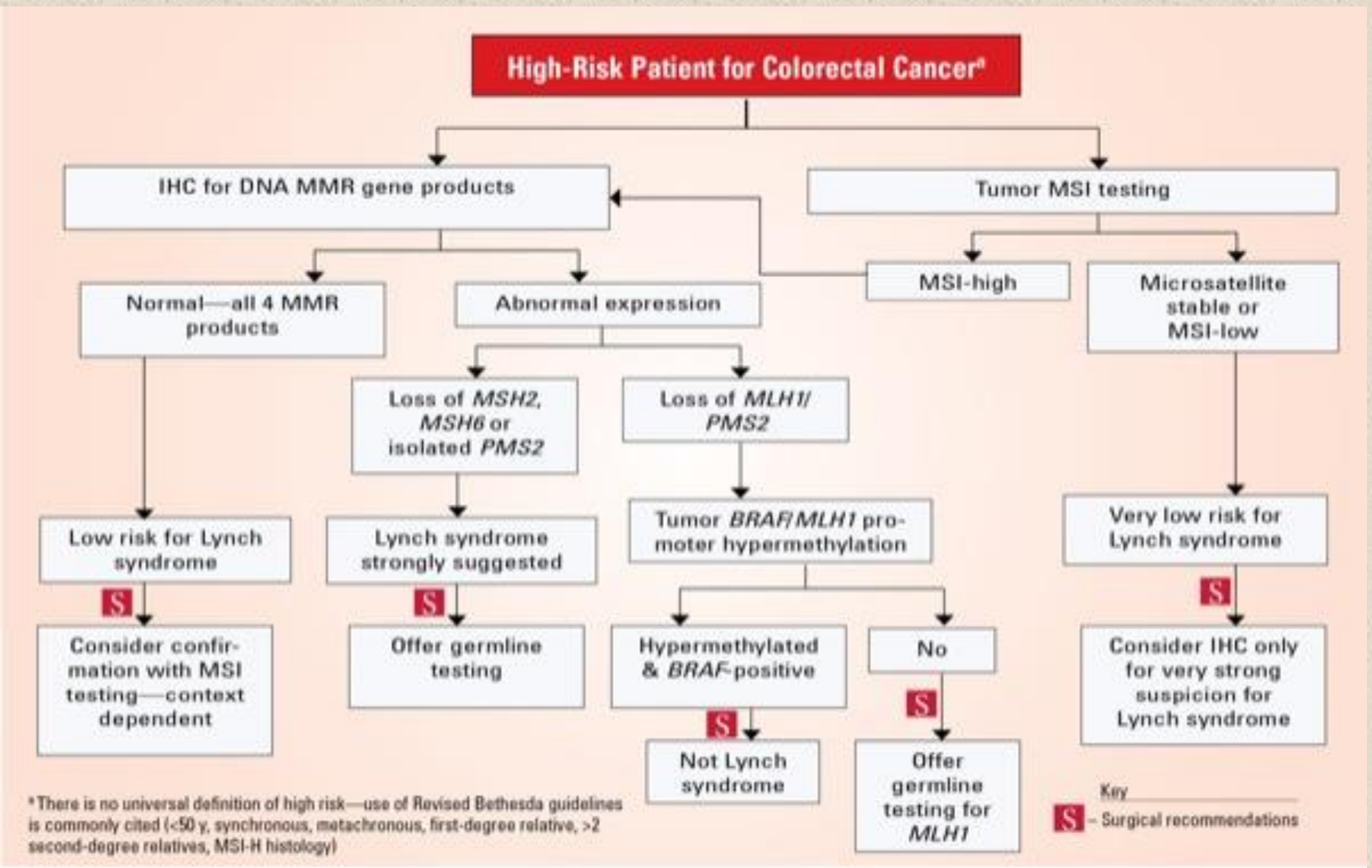


Figure. Mayo Clinic clinical pathway for patients at high risk for colorectal cancer.

IHC, immunohistochemistry; **MMR,** mismatch repair; **MIS,** microsatellite instability

Image courtesy of Mayo Clinic.

MMR/MSI testing of CRC – Issues

- Screening of resected CRC

The Royal College of Pathologists

Pathology: the science behind the cure

Standards and datasets for reporting cancers

Dataset for colorectal cancer histopathology reports

July 2014

colorectal cancers currently. As a minimum, we recommend it should be available upon request by either oncologist or geneticist on individual cases and should be performed routinely on all cases of CRC where the patient is aged less than 50 years, to detect possible Lynch syndrome (revised Bethesda guidelines[57]), and in older patients with morphological features suggesting possible MMR deficiency, for prognostication.

Standard operating protocol for reflex mismatch repair immunohistochemistry of colorectal carcinoma

This standard operating protocol addresses the guidance issued in the 2014 Royal College of Pathologists dataset for colorectal carcinoma (CRC): "In summary, MMR immunohistochemistry is currently considered a core dataset item for patients under 50 years at the time of diagnosis and for patients, in whom an assessment of prognosis is appropriate, with adenocarcinomas classified as poorly differentiated morphologically or tumours showing other morphological features of MMR deficiency".

1. Reflex mismatch repair (MMR) immunohistochemistry should be performed on CRCs resected from patients less than 50 years of age at time of diagnosis. By contrast, MMR immunohistochemistry for assessment of prognosis should be an 'on-demand' process; these requests are anticipated to come from oncologists.

2. The reflex MMR testing should be organised by the Histopathology Department that has received and reported the CRC resection specimen.

3. When the histopathology of the CRC resection specimen is presented at the local Lower GI MDT meeting, the MDT should be informed that MMR immunohistochemistry data is awaited for the patient's CRC.

4. The local Lower GI MDT should take responsibility for chasing up these data.

5. Once the completed MMR data are presented to the local Lower GI MDT and if there is evidence of MMR deficiency, the MDT should refer the patient to its local Clinical Genetics service.

6. This reflex testing does not include microsatellite instability (MSI), BRAF mutation or MLH1 hypermethylation analyses.

7. This reflex testing does not replace pre-existing local MDT protocols for identifying potential Lynch syndrome patients for referral to Clinical Genetics.

NICE National Institute for Health and Care Excellence

CONFIDENTIAL

NATIONAL INSTITUTE FOR HEALTH AND CARE EXCELLENCE

Diagnostics Assessment Programme

Molecular testing for Lynch syndrome in people with colorectal cancer

Final scope

February 2016

MMR/MSI testing of CRC – Practical points

- Check internal positive control(s).
- Specify pathways for
  - Referral
  - Testing
  - Actioning of results.

Gastric HER2 testing – Why?

- Personalised medicine

Gastric HER2 testing – Why?

Trastuzumab in combination with chemotherapy versus chemotherapy alone for treatment of HER2-positive advanced gastric or gastro-oesophageal junction cancer (ToGA): a phase 3, open-label, randomised controlled trial

Yung-Jue Bang, Eric Van Cutsem,* Andrea Feyereislova, Hyun C Chung, Lin Shen, Akira Sawaki, Florian Lordick, Atsushi Ohtsu, Yasushi Omuro, Taroh Satoh, Giuseppe Aprile, Evgeny Kulikov, Julie Hill, Michaela Lehle, Josef Rüschoff, Yoon-Koo Kang, for the ToGA Trial Investigators†*

Summary

Background Trastuzumab, a monoclonal antibody against human epidermal growth factor receptor 2 (HER2; also known as ERBB2), was investigated in combination with chemotherapy for first-line treatment of HER2-positive advanced gastric or gastro-oesophageal junction cancer.

Methods ToGA (Trastuzumab for Gastric Cancer) was an open-label, international, phase 3, randomised controlled trial undertaken in 122 centres in 24 countries. Patients with gastric or gastro-oesophageal junction cancer were eligible for inclusion if their tumours showed overexpression of HER2 protein by immunohistochemistry or gene amplification by fluorescence in-situ hybridisation. Participants were randomly assigned in a 1:1 ratio to receive a chemotherapy regimen consisting of capecitabine plus cisplatin or fluorouracil plus cisplatin given every 3 weeks for six cycles or chemotherapy in combination with intravenous trastuzumab. Allocation was by block randomisation stratified by Eastern Cooperative Oncology Group performance status, chemotherapy regimen, extent of disease, primary cancer site, and measurability of disease, implemented with a central interactive voice recognition system. The primary endpoint was overall survival in all randomised patients who received study medication at least once.

Lancet 2010; 376: 687–97

This online publication has been corrected.
The corrected version first appeared at TheLancet.com on October 15, 2010

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See Comment page 659

*These authors contributed equally

†Members listed at end of paper

Trastuzumab for the treatment of HER2-positive metastatic gastric cancer

This guidance was developed using the single technology appraisal (STA) process.

1 Guidance

1.1 Trastuzumab, in combination with cisplatin and capecitabine or 5-fluorouracil, is recommended as an option for the treatment of people with human epidermal growth factor receptor 2 (HER2)-positive metastatic adenocarcinoma of the stomach or gastro-oesophageal junction who:

- have not received prior treatment for their metastatic disease **and**
- have tumours expressing high levels of HER2 as defined by a positive immunohistochemistry score of 3 (IHC3 positive).

Gastric HER2 testing – Why?

- HER2 IHC 3+ (NICE funded)
- HER2 IHC 2+ with amplification (CDF funded)

Gastric HER2 testing – Why?

- HER2 IHC 3+ ~~(NICE funded)~~
- HER2 IHC 2+ with amplification ~~(CDF funded)~~

All funded by NHS England

Gastric HER2 testing – How

- HER2 protein expression - IHC
- HER2 gene amplification - ISH (e.g. FISH, CISH, D-DISH)

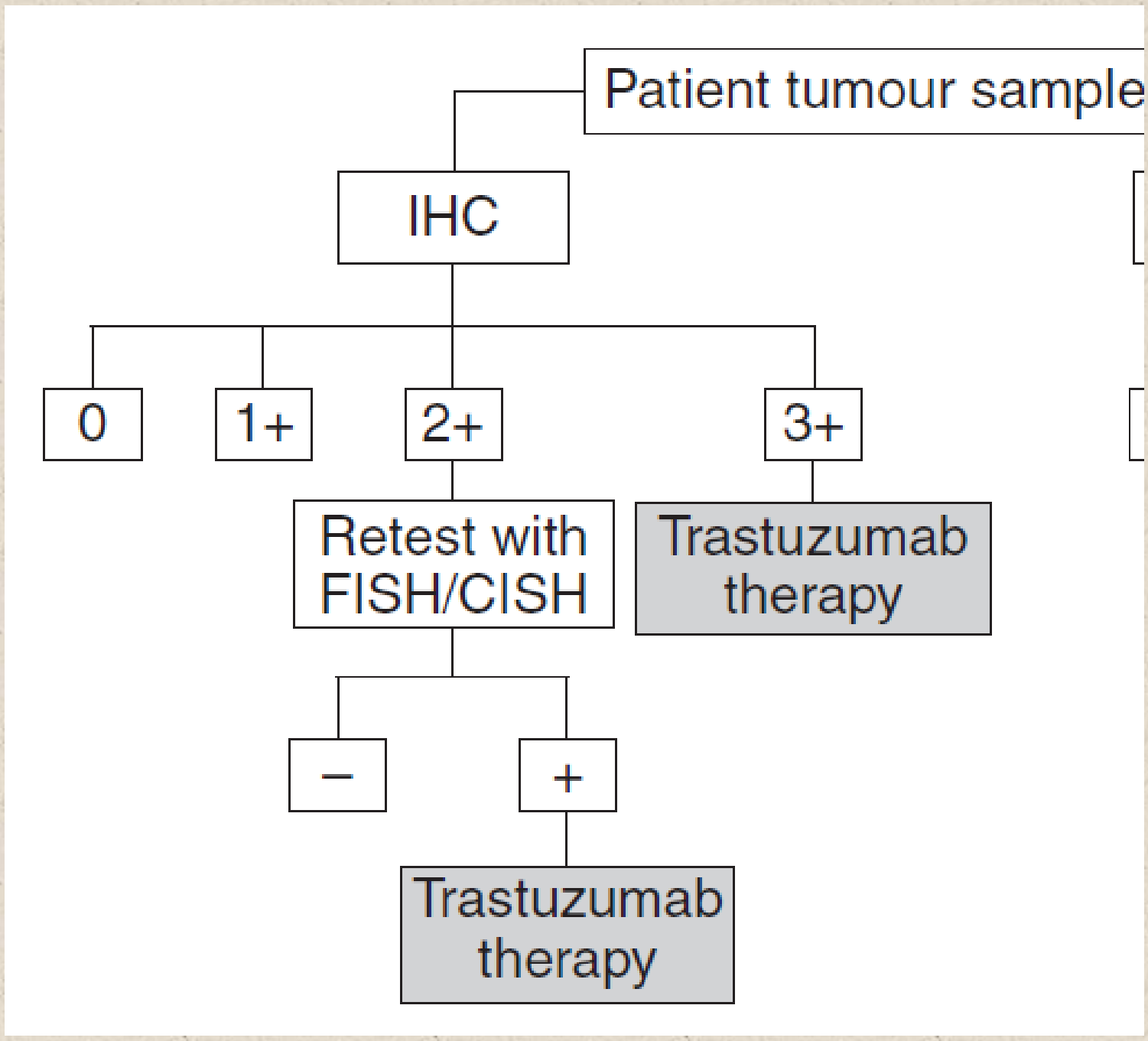
Patient tumour sample
IHC
0
1+
2+
3+
Retest with FISH/CISH
Trastuzumab therapy
–
+
Trastuzumab therapy

Table 1 Human epidermal growth factor receptor 2 (HER2) scoring criteria for gastric cancer

Score	*Surgical specimen-staining pattern*	*Biopsy specimen-staining pattern*	*HER2 overexpression assessment*
0	No reactivity or membranous reactivity in <10% of tumor cells	No reactivity or no membranous reactivity in any tumor cell	Negative
1+	Faint/barely perceptible membranous reactivity in ≥10% of tumor cells; cells are reactive only in part of their membrane	Tumor cell cluster with a faint/barely perceptible membranous reactivity irrespective of percentage of tumor cells stained	Negative
2+	Weak to moderate complete, basolateral, or lateral membranous reactivity in ≥10% of tumor cells	Tumor cell cluster with a weak to moderate complete, basolateral, or lateral membranous reactivity irrespective of percentage of tumor cells stained	Equivocal
3+	Strong complete, basolateral, or lateral membranous reactivity in ≥10% of tumor cells	Tumor cell cluster with a strong complete, basolateral, or lateral membranous reactivity irrespective of percentage of tumor cells stained	Positive

Table 2 Comparison of differences between human epidermal growth factor receptor 2 (HER2) scoring in gastric and breast cancer[29]

		Gastric cancer	*Breast cancer*
Immunohistochemical scoring	Extent	Biopsy specimens ≥5 cells	≥10% (≥30%)[a]

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HER2 testing in gastric cancer: a practical approach

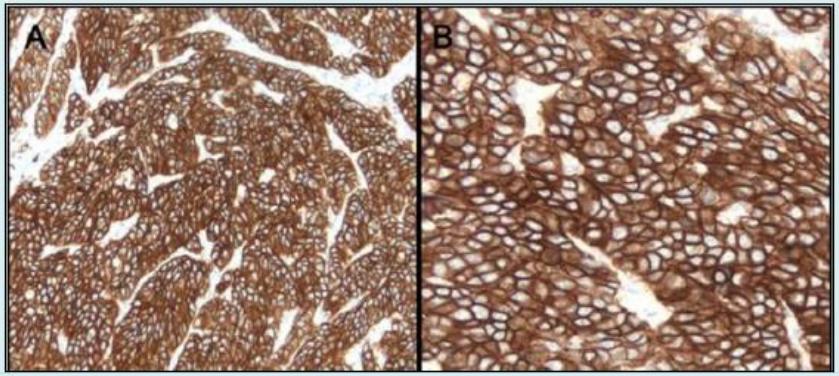
Josef Rüschoff[1,2], Wedad Hanna[3], Michael Bilous[4], Manfred Hofmann[2], Robert Y Osamura[5], Frédérique Penault-Llorca[6], Marc van de Vijver[7] and Giuseppe Viale[8]

[1]*Targos Molecular Pathology GmbH, Kassel, Germany;* [2]*Institute of Pathology Nordhessen, Kassel, Germany;*

GASTRIC HER2 IMMUNOCYTOCHEMISTRY: DIFFERENCES IN METHODOLOGY AFFECTING MEMBRANE STAINING AND INTERPRETATION: FINDINGS OF THE UK NEQAS ICC & ISH EXTERNAL ASSESSMENT SERVICE

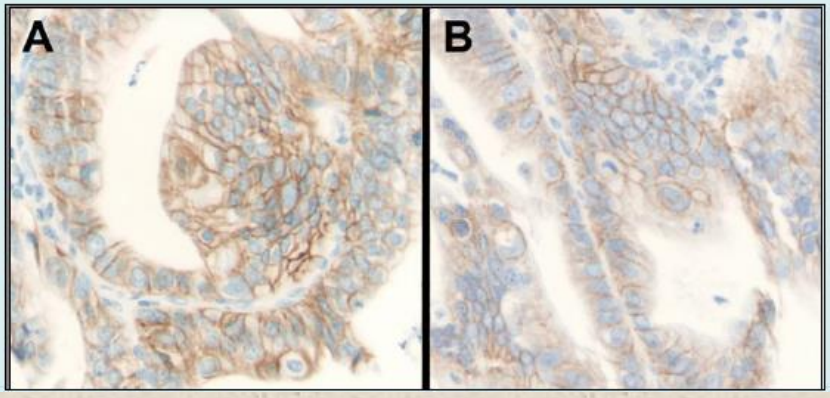
Suzanne Parry, Keith Miller, Jane Starczynski, Newton Wong, Bharat Jasani, Iris Nagelmeier, Merdol Ibrahim *UK NEQAS, University College London (UCL) UK (merdol.ibrahim@ucl.ac.uk)*

3+

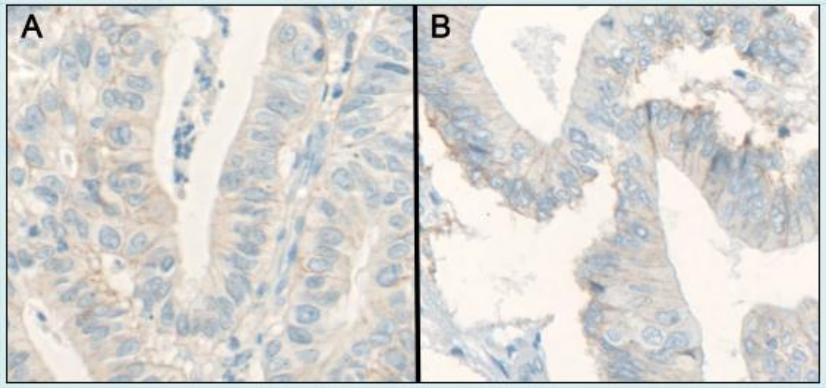


Complete membrane staining of high intensity
(A) Ventana 4B5 (B) Dako Hercept test

2+

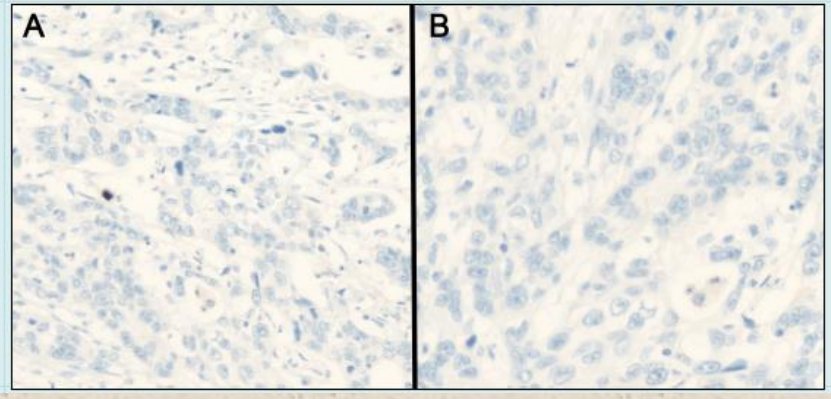


1+



Incomplete membrane staining in >10% of tumour cells. (A) Ventana 4B5 (B) CBE356 clone

0



Gastric HER2 testing – How

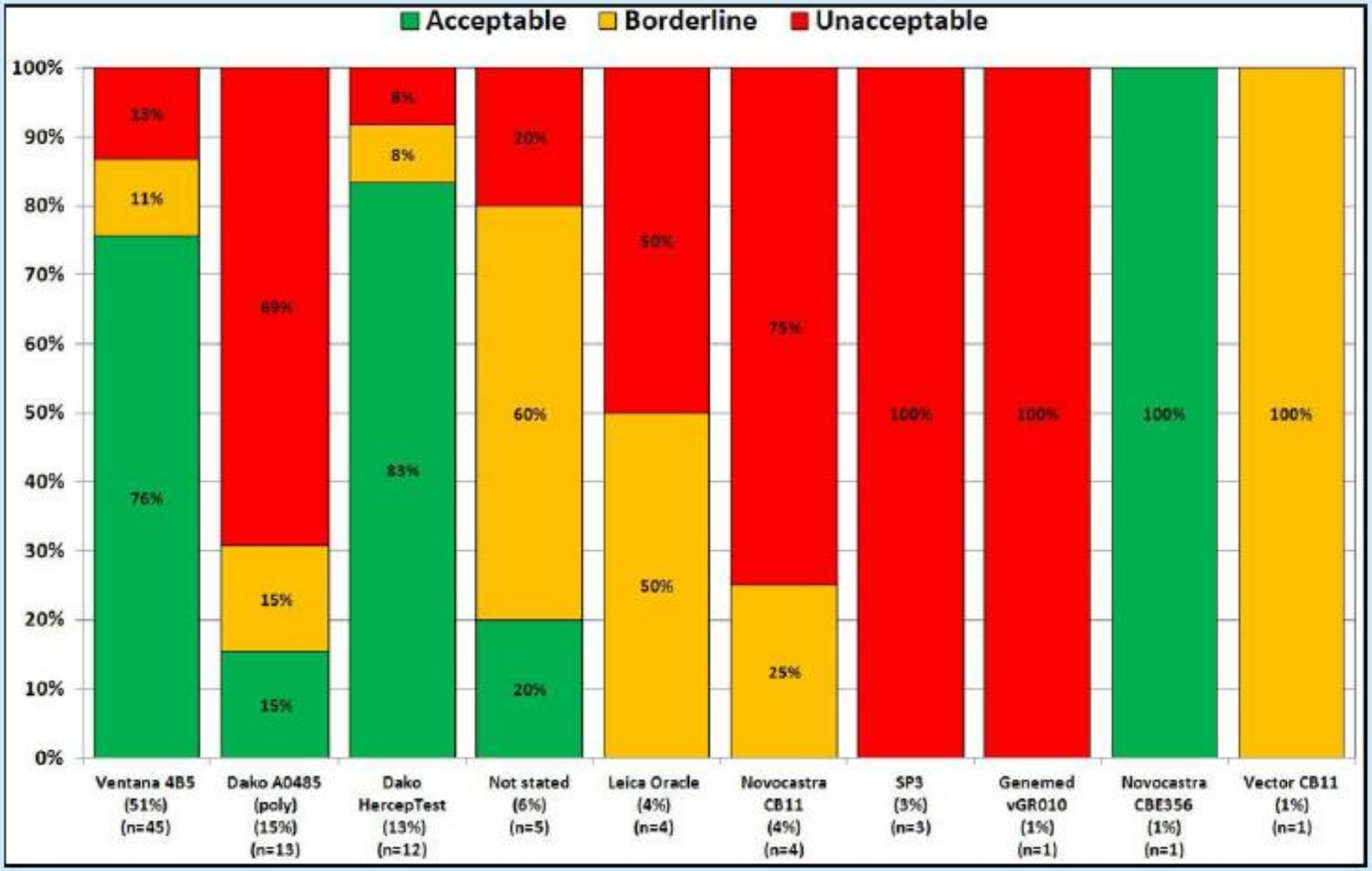
- HER2 IHC Scoring:
  - 3+ (visible at x4 obj)
  - 2+ (visible at x10-20 obj)
  - 1+ (visible at x20-40 obj)
  - 0 (not visible!!)

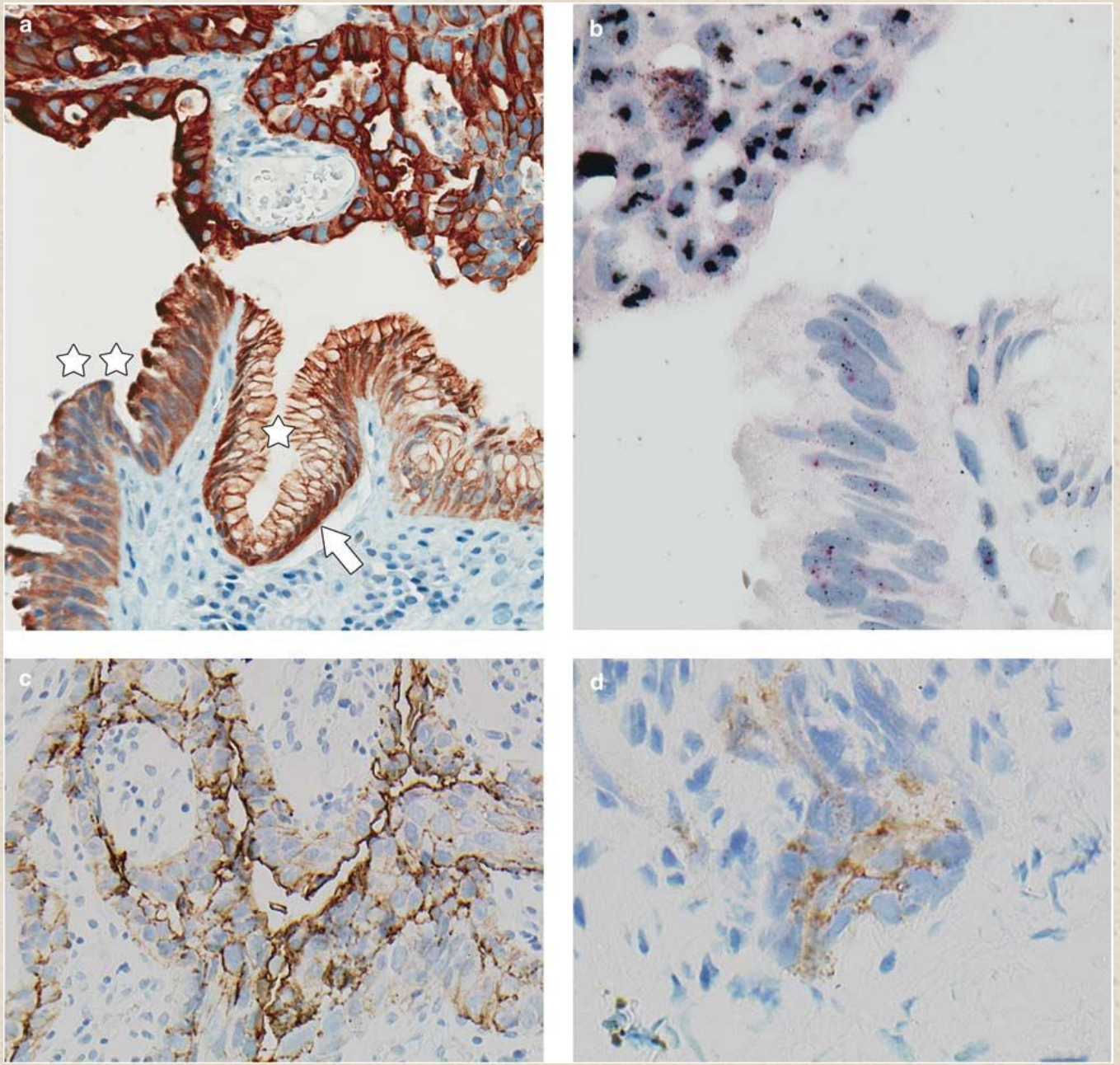
Gastric HER2 testing – How

- HER2 IHC Antibodies:
  - 4B5 (Ventana)
  - Dako HercepTest
  - Polyclonal A0845 (Dako)
  - CB11 (Novocastra)

GASTRIC HER2 IMMUNOCYTOCHEMISTRY: DIFFERENCES IN METHODOLOGY AFFECTING MEMBRANE STAINING AND INTERPRETATION: FINDINGS OF THE UK NEQAS ICC & ISH EXTERNAL ASSESSMENT SERVICE

Suzanne Parry, Keith Miller, Jane Starczynski, Newton Wong, Bharat Jasani, Iris Nagelmeier, Merdol Ibrahim *UK NEQAS, University College London (UCL) UK (merdol.ibrahim@ucl.ac.uk)*

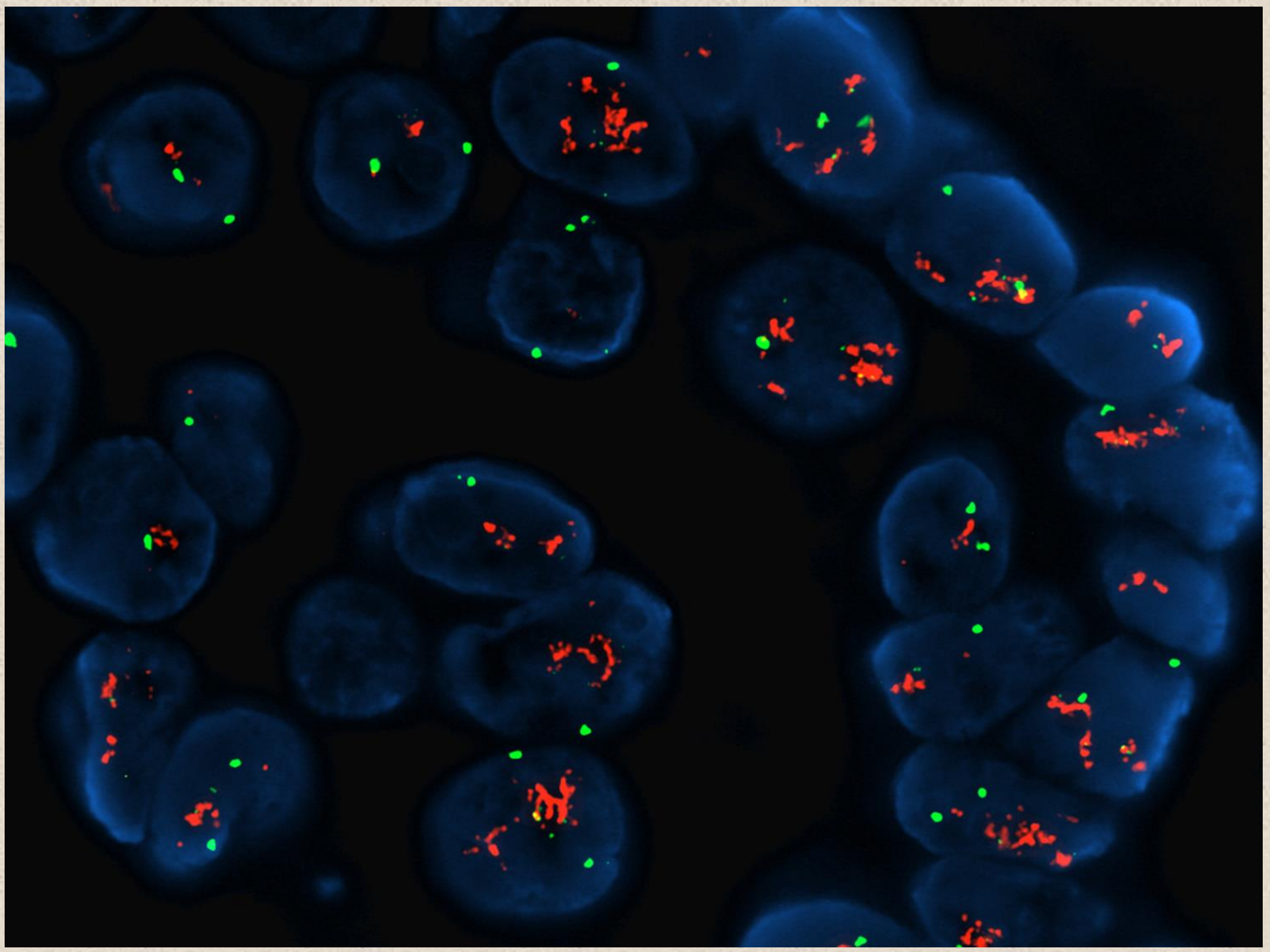




4B5 stains foveolar epithelium

Gastric HER2 testing – How

- HER2 positivity:
  - Intestinal (approx. 30%) > Mixed (approx. 15%) > Diffuse (approx, 5%)
  - OGJ/Cardiac (approx. 30%) > gastric (approx. 15%)



20 nuclei should be enumerated. If the HER2/Chr17 ratio falls between 1.8 and 2.2:
20 additional nuclei should be enumerated.

Target Area 1

☐ Heterogeneity present? (check if yes)

Cell	HER2 Count	Cell	Chr17 Count
1		1	
2		2	
3		3	
4		4	
5		5	
6		6	
7		7	
8		8	
9		9	
10		10	
11		11	
12		12	
13		13	
14		14	
15		15	
16		16	
17		17	
18		18	
19		19	
20		20	

☐Clusters Present? (Check if yes)	☐Clusters Present? (Check if yes)
Total number of HER2 signals in Target Area 1	Total number of Chr17 signals in Target Area 1
a	b

Target Area 1 HER2/Chr17 Ratio

c = a/b

☐ **Non-amplified: HER2/Chr17 < 2.0**

☐ **Amplified: HER2/Chr17 ≥ 2.0**

Target Area 2 If ratio 1.8 ≤ 2 ≥ 2.2

☐ Heterogeneity present? (Check if yes)

Cell	HER2 Count	Cell	Chr17 Count
1		1	
2		2	
3		3	
4		4	
5		5	
6		6	
7		7	
8		8	
9		9	
10		10	
11		11	
12		12	
13		13	
14		14	
15		15	
16		16	
17		17	
18		18	
19		19	
20		20	

☐Clusters Present? (Check if yes)	☐Clusters Present? (Check if yes)
Total number of HER2 signals in Target Area 2	Total number of Chr17 signals in Target Area 2
d	e

Target Areas **1 and 2** HER2/Chr17 Ratio

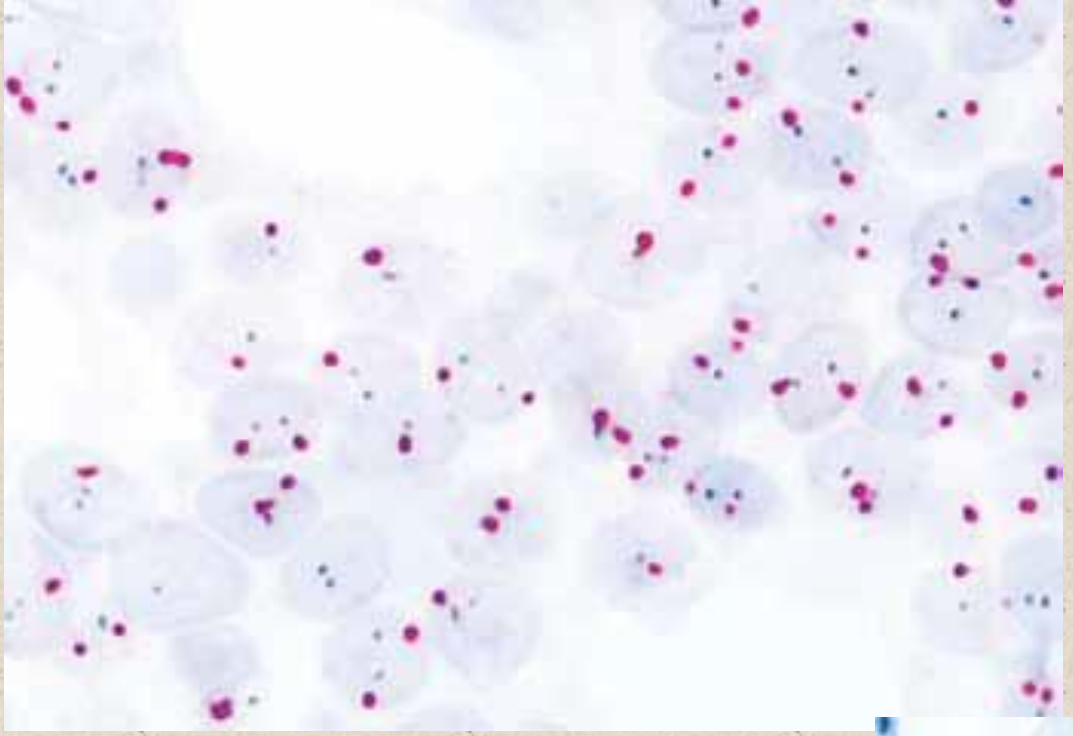
f = (a+d)/(b+e)

☐ **Non-amplified: HER2/Chr17 < 2.0**

☐ **Amplified: HER2/Chr17 ≥ 2.0**

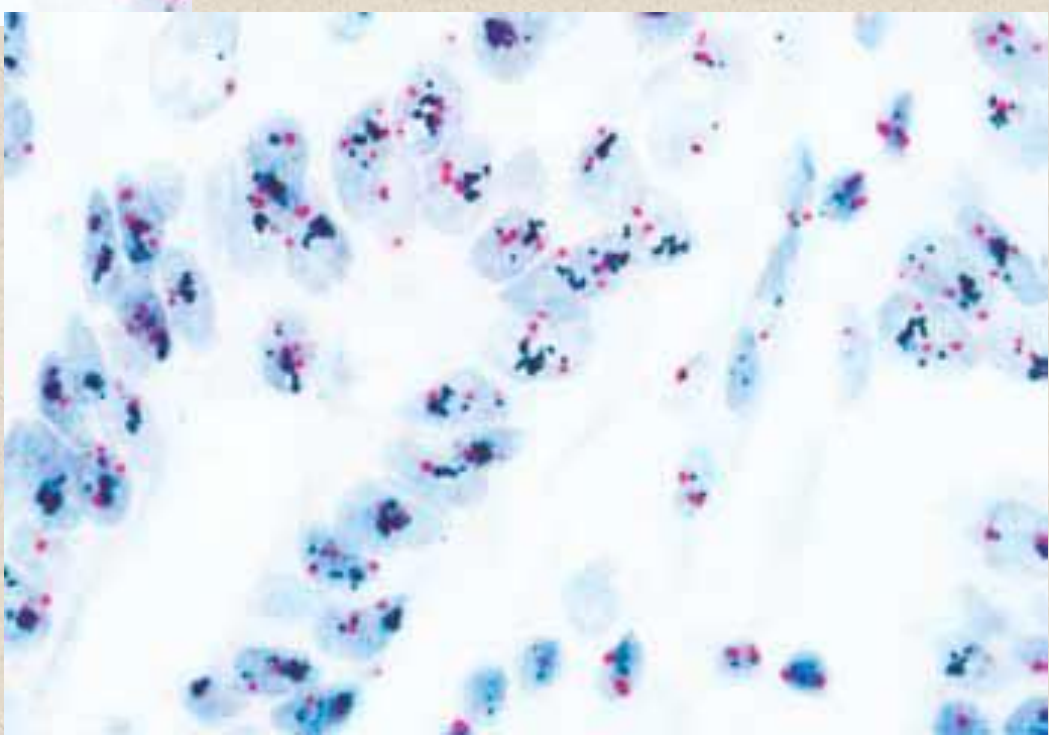
Gastric HER2 testing – How

- ISH (for 20 cells):
  - Count number of C17 signals
  - Count number of HER2 signals
  - Calculate **HER2 : C17 ratio**
  - If ratio > 2.0 = amplified
  - If ratio < 2.0 = not amplified
  - If ratio between 1.8 and 2.2 repeat with 20 other cells



Red = C17

Black = HER2



Gastric HER2 testing – Issues

- HGD vs adenocarcinoma
- Resection 10% vs. Biopsy 5 cells rule: which to use for 'intermediate' size specimens (e.g. peritoneal metastases)?

Gastric HER2 testing – How

- UK Guidance document pending

The Association of Clinical Pathologists

UK NEQAS
International Quality Expertise

Gastric HER2 testing – Practical points

- Be aware of which antibody is used.
- If possible (i.e. bxs), immunostain two sections on the same slide.
- Get some else to assess ISH!

GIST mutation testing – Why?

- Aiding histopathological diagnosis
- Screening for hereditary disease
- Personalised medicine

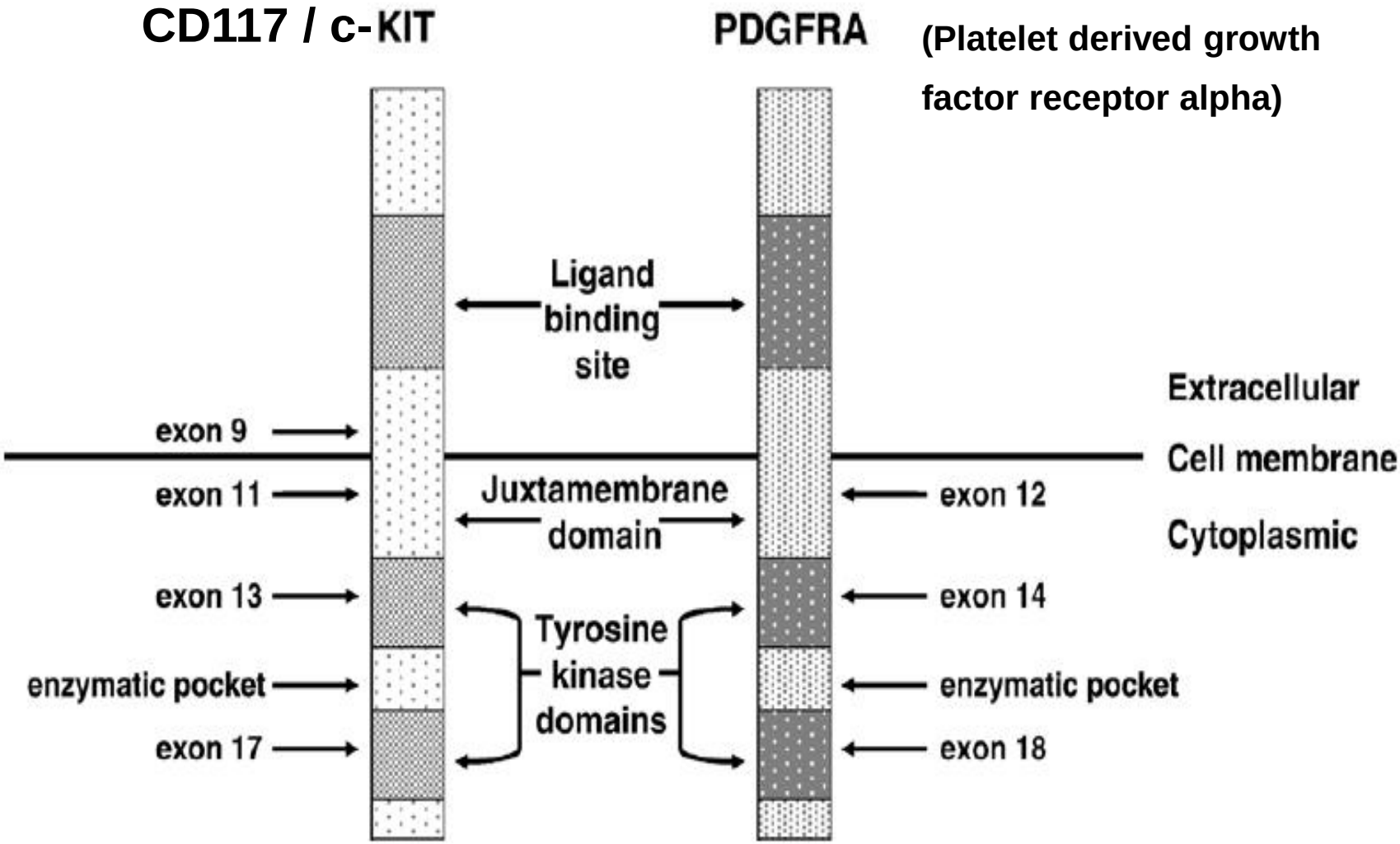
GIST mutation testing – Why?

- Funding:
  - None

GIST mutation testing – Why?

- Funding:
  - ~~None~~
  - Impending NHS England funding for *KIT* (*PDGFRA* not mentioned)

Mutations of receptor tyrosine kinase (RTKs)



Hornick *et al*. *Hum Pathol* 2007; **38:** 679.

KIT and *PDGFRA*

- In chemo-naive GISTs, only one primary mutation per neoplasm.

KIT and *PDGFRA*

- Amongst all GISTs:
  - 85% *KIT* mutation
  - 5% *PDGFRA* mutation
  - 10% 'wild-type' (i.e. no activating mutations in *KIT* exons 9,11,13,17 or *PDGFRA* exons 12,14,18)

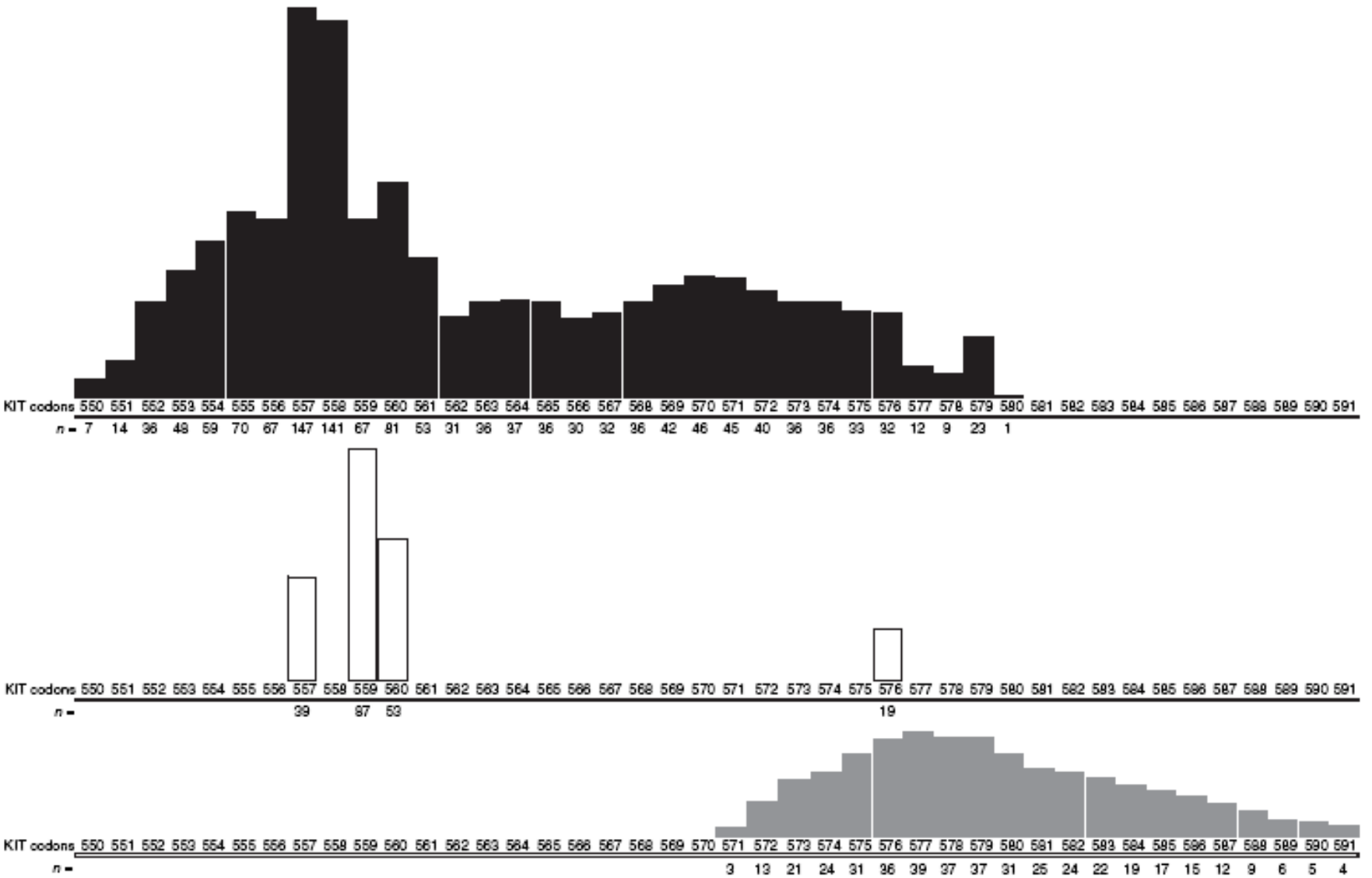
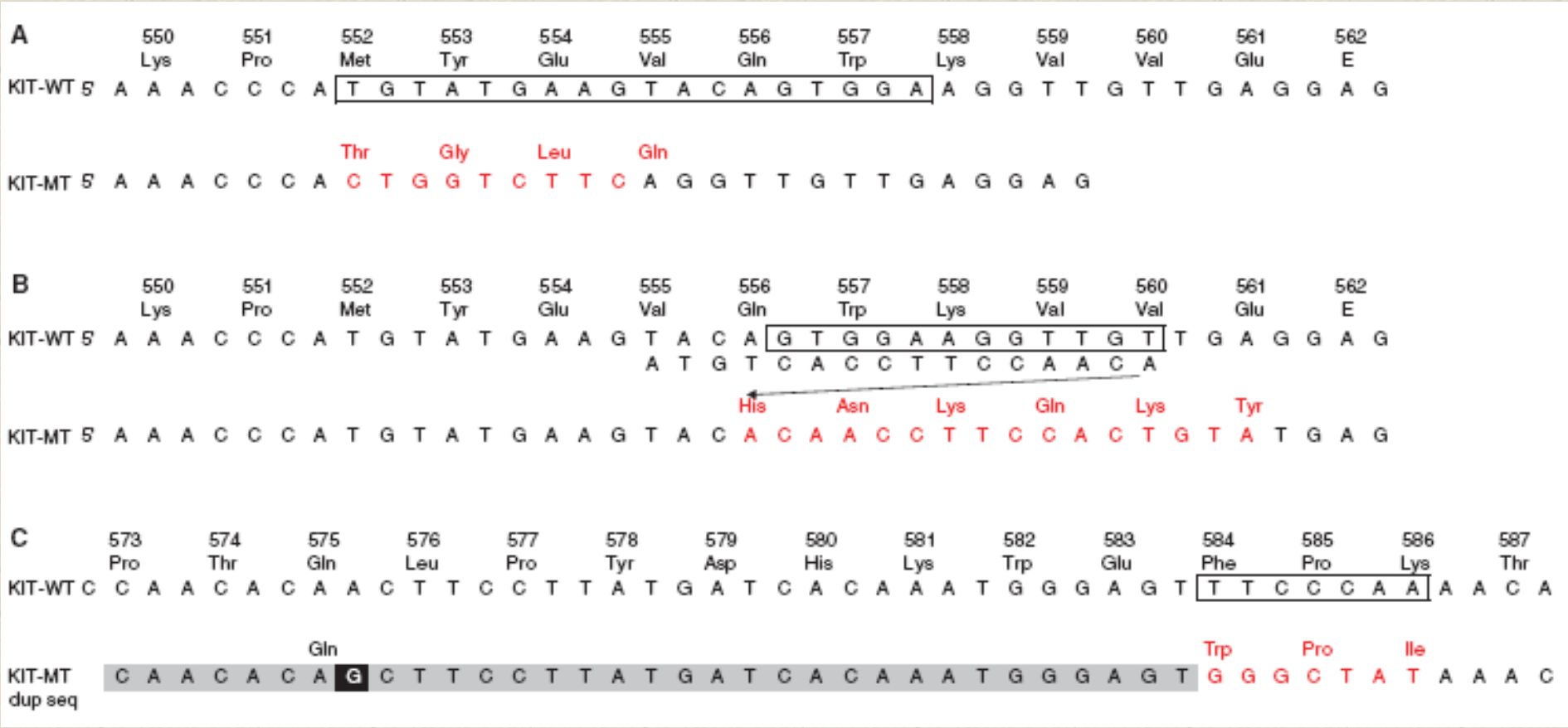


Figure 2. The involvement of KIT exon 11 codons by different mutation types. Deletions, substitutions and duplications are indicated by black, white and grey colours, respectively. Figure is based on evaluation of 546 KIT exon 11 mutants from Armed Forces Institute of Pathology collection. *n*, how many times the codon was deleted.

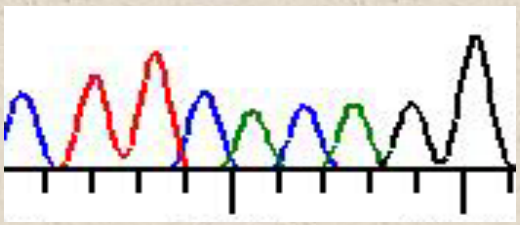
Lasota & Miettinen. *Histopathol* 2008; **53:** 245.



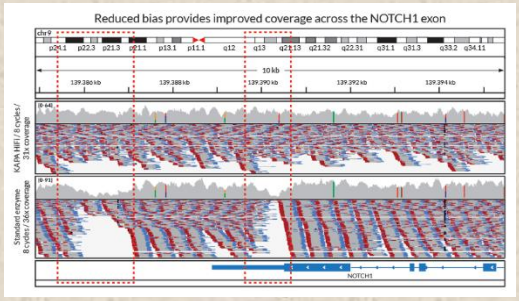
Lasota & Miettinen. *Histopathol* 2008; **53:** 245.

GIST mutation testing – How?

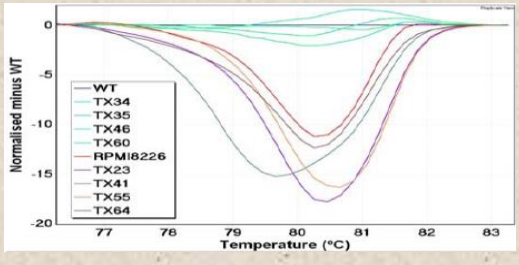
Sanger sequencing:



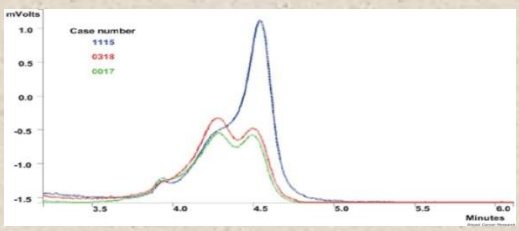
Next generation seq:



High Resolution Melting:



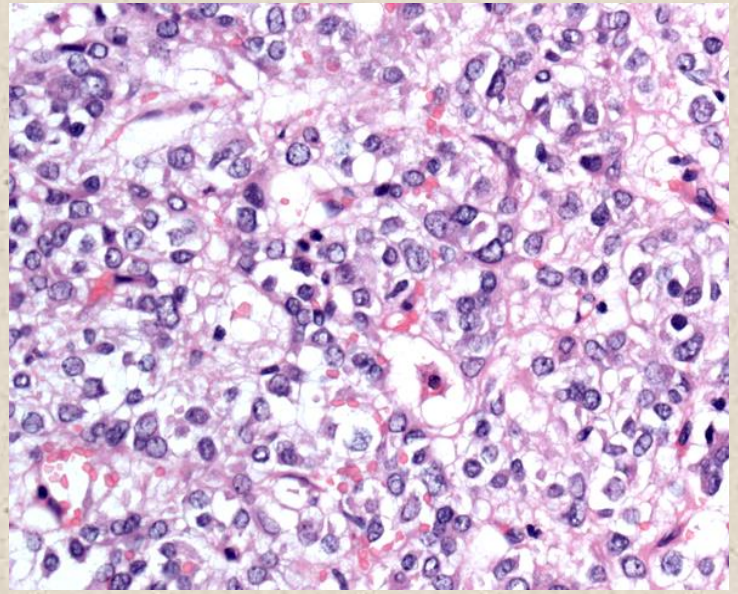
dHPLC:



GIST mutation testing – Why?

- Aiding histopathological diagnosis
- Screening for hereditary disease
- Personalised medicine

I) Diagnosis of GIST



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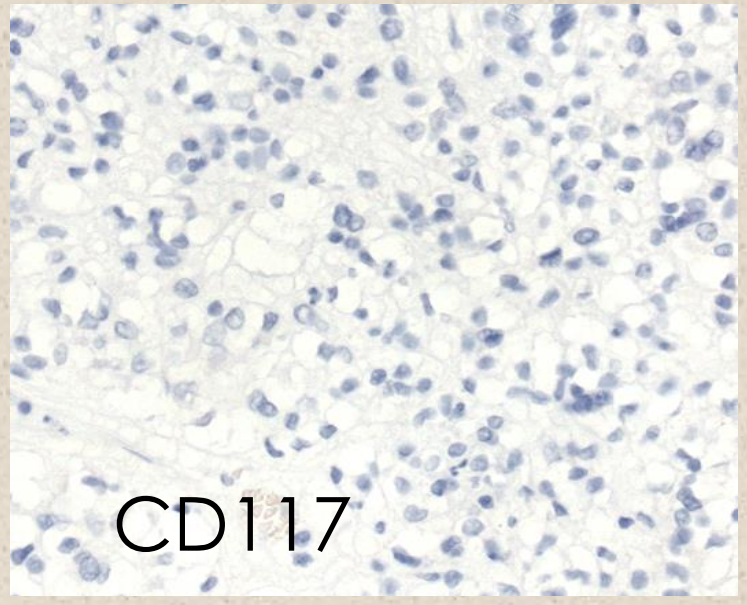
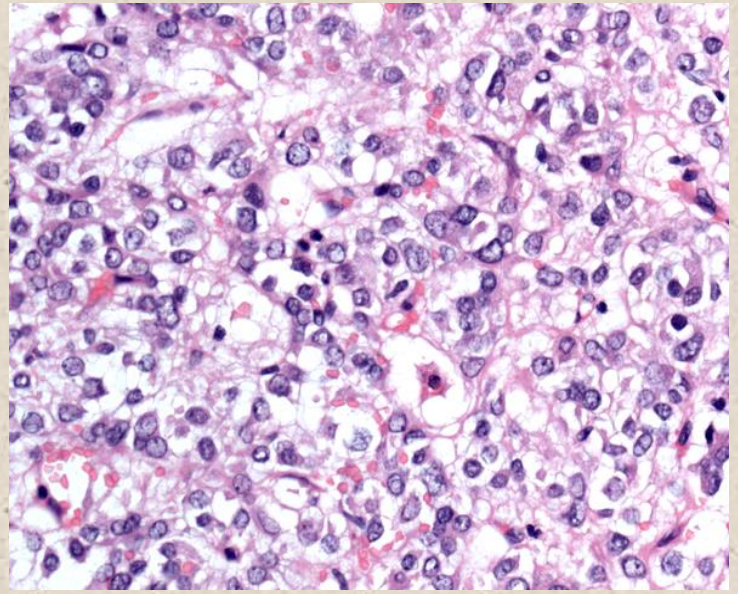


= ???

I) Diagnosis of GIST



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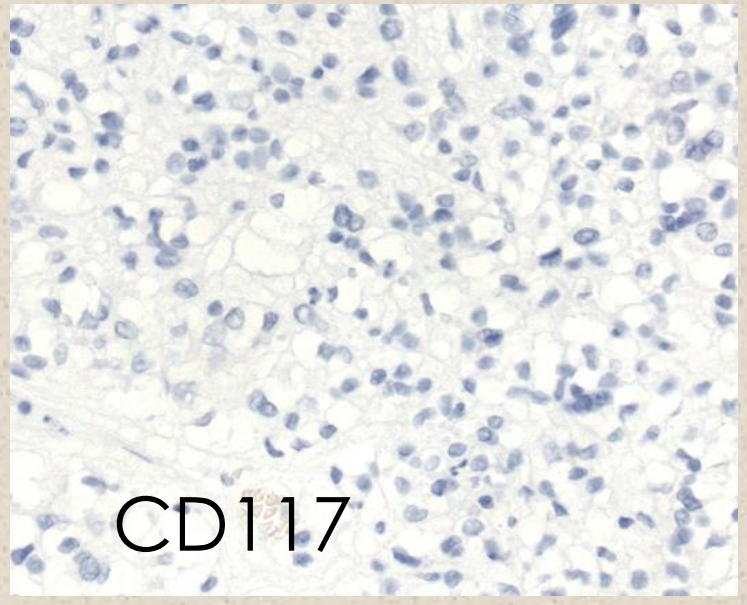
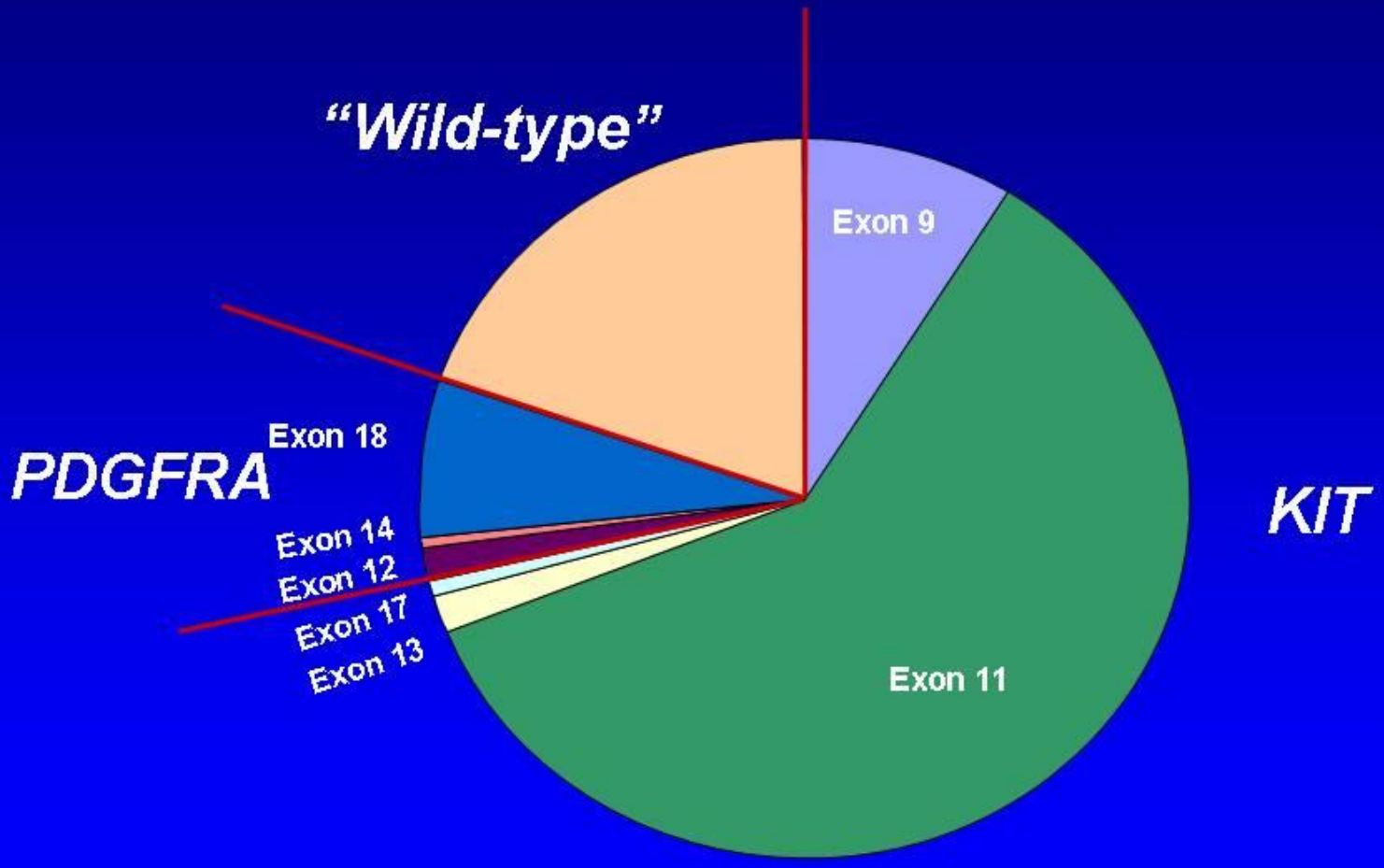


+ *PDGFRA* p.Asp842Val = GIST

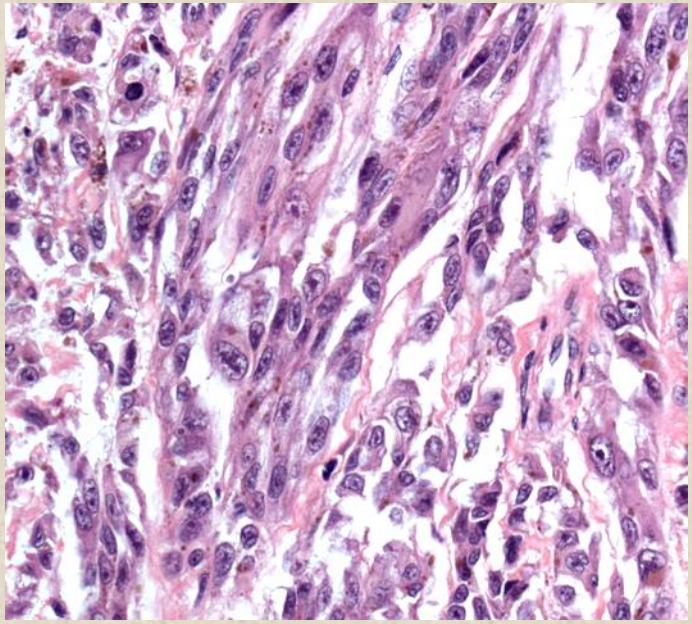
Kinase Mutations in GISTs



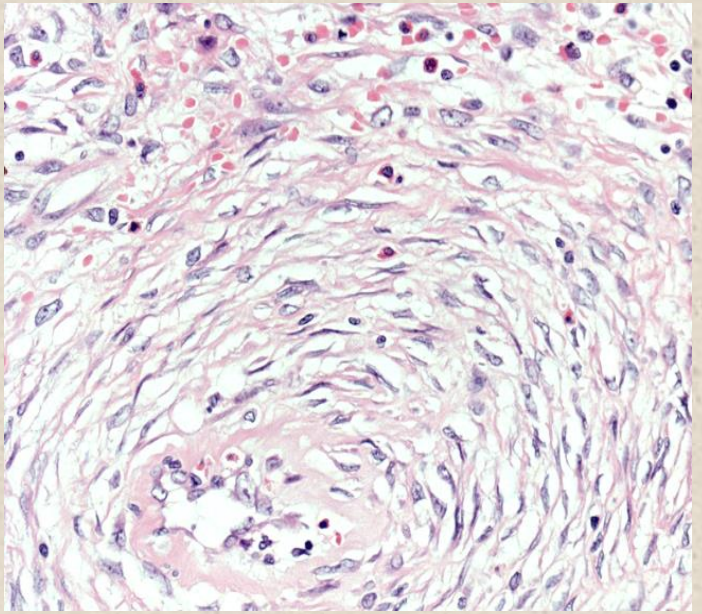
Heinrich et al. *J Clin Oncol 21:4342-4349, 2003*
Agaram et al. Genes, Chromosomes & Cancer 47:853–859, 2008
Agaimy et al. J Clin Pathol 2009;62:613–616, 2009

I) Diagnosis of GIST

- A few GIST mimics may show identical mutations:

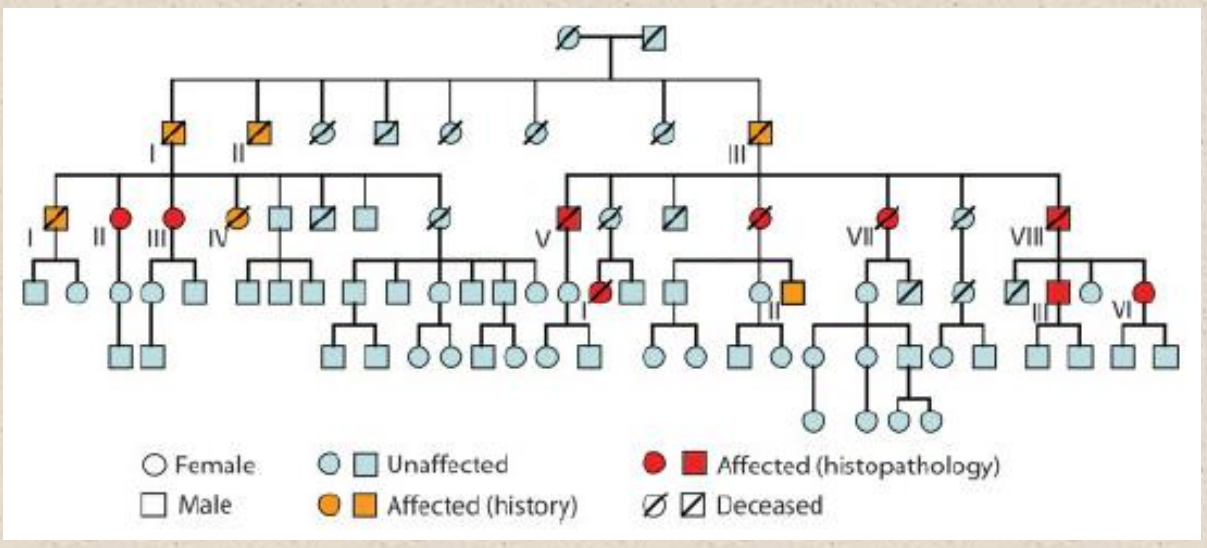


Melanoma
(*KIT* mutations)

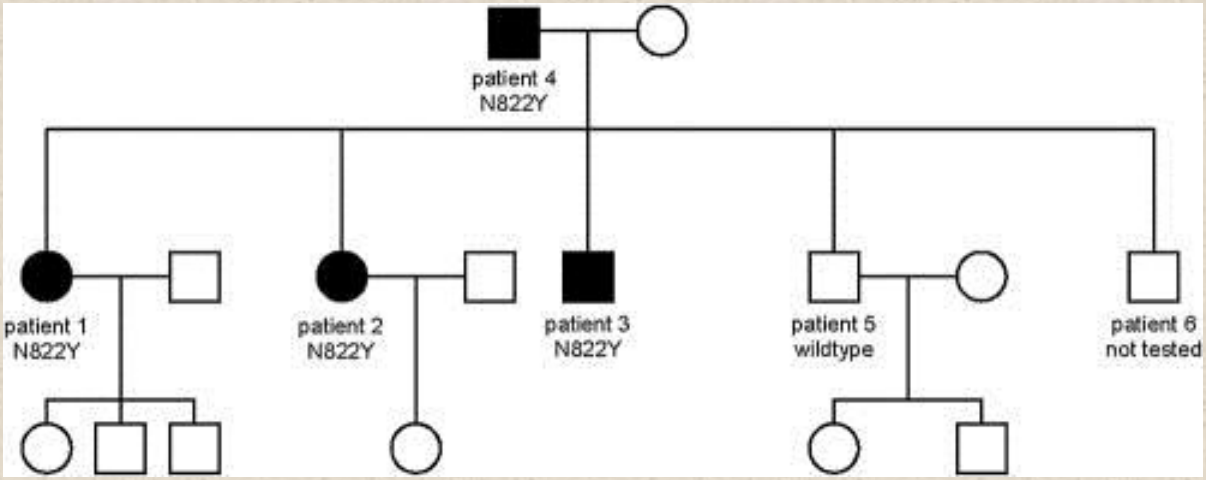


Inflammatory fibroid polyp
(*PDGFRA* mutations)

II) Investigating GIST families



Kleinbaum *et al. Int J Cancer* 2008; **122:** 711.



Thalheimer *et al. Am J Surg Pathol* 2008; **32:** 1560.

II) Investigating GIST families

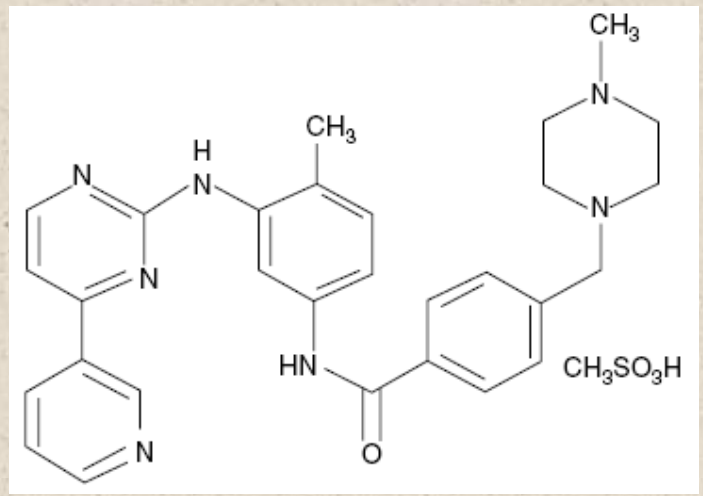
- Approx. 25 reported families with *KIT* germline mutation.
- One reported family with *PDGFRA* germline mutation.

III) Predict response to RTK inhibitors

- Imatinib for advanced GIST.
- 10% patients show progression within 6 months: primary resistance.
- 40-50% patients show progression within 24 months after response/stable disease: secondary resistance.

Chemotherapy for GIST

- RTK inhibitors
  - Imatinib



  - Sunitinib
  - Nilotinib, Dasatinib

PRIMARY RESISTANCE / SENSITIVITY

IMATINIB:

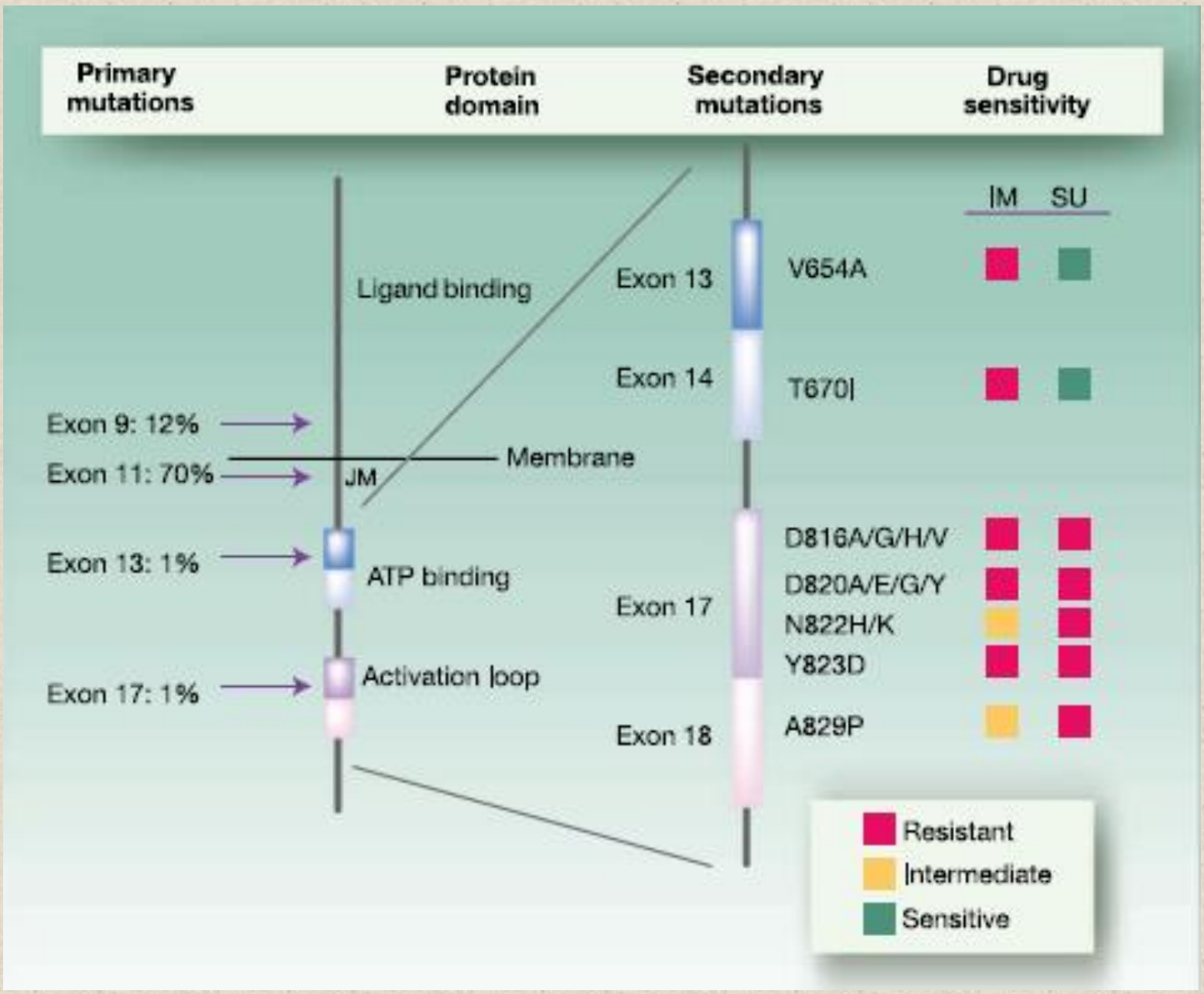
Sensitive primary mutations	Resistant primary mutations
KIT exon 11	*KIT* exon 9 (but dose escalation)
Upstream small mutations > downstream large deletions	*KIT* exon 17
	PDGFRA exon 18 (e.g. D842V)
	Wild type

SUNITINIB:

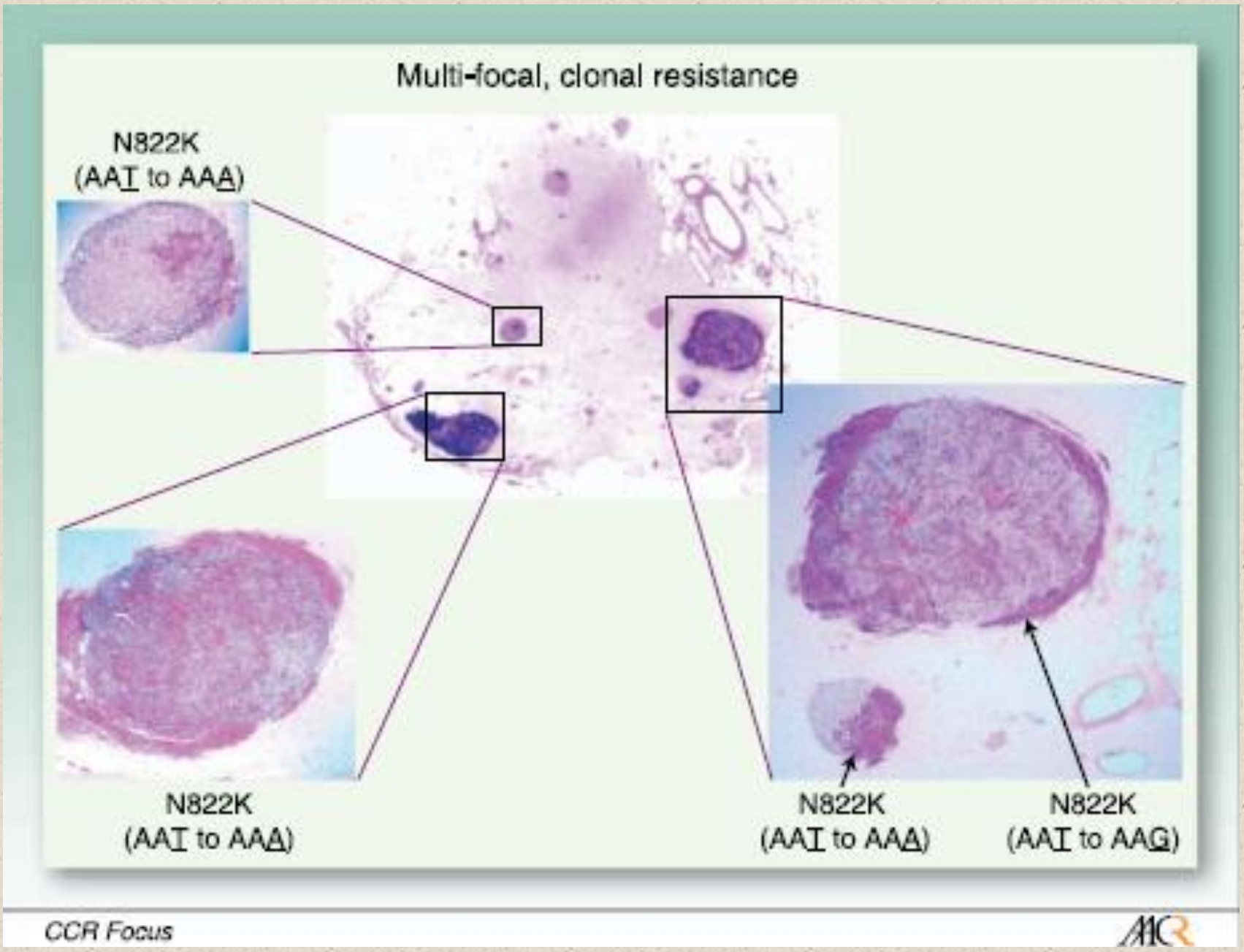
Sensitive primary mutations	Resistant primary mutations
KIT exon 9	*KIT* exon 11
Wild type	*PDGFRA* exon 18 (D842V)

III) Predict response to RTK inhibitors

- Imatinib for advanced GIST.
- 10% patients show progression within 6 months: primary resistance.
- 40-50% patients show progression within 24 months after response/stable disease: secondary resistance.



Gramza *et al*. *Clin Cancer Res* 2009; **15:** 1750.



Gramza *et al*. *Clin Cancer Res* 2009; **15:** 1750.

GIST mutation testing – Issues

- Drug licensing:
  - Imatinib is the only licensed first line therapy (advanced disease therapy or as adjuvant therapy) for GIST in the UK.

GIST mutation testing – Issues

- Drug licensing:
  - Imatinib is the only licensed first line therapy (advanced disease therapy or as adjuvant therapy) for GIST in the UK.
  - However, if imatinib resistant mutation, may switch to second line therapy sooner.

GIST mutation testing – Issues

- Rare mutations: *KIT* exon 8

MODERN PATHOLOGY (2013) 26, 1004–1012
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OPEN

A subset of gastrointestinal stromal tumors previously regarded as wild-type tumors carries somatic activating mutations in *KIT* exon 8 (p.D419del)

Sebastian Huss[1], Helen Künstlinger[1], Eva Wardelmann[1], Michaela A Kleine[1], Elke Binot[1], Sabine Merkelbach-Bruse[1], Thomas Rüdiger[2], Jens Mittler[3], Wolfgang Hartmann[1], Reinhard Büttner[1] and Hans-Ulrich Schildhaus[1]

Int J Clin Exp Pathol 2014;7(11):8024-8031
www.ijcep.com /ISSN:1936-2625/IJCEP0002704

Original Article

Gastrointestinal stromal tumors with exon 8 c-*kit* gene mutation might occur at extragastric sites and have metastasis-prone nature

Takashi Ito[1,2], Masahiro Yamamura[3], Toshihiro Hirai[4], Takashi Ishikawa[5], Tatsuo Kanda[6], Takuya Nakai[7], Mizuka Ohkouchi[1], Yuka Hashikura[1], Koji Isozaki[1], Seiichi Hirota[1]

GIST mutation testing – Issues

- Rare mutations: *KIT* exon 8
  - 0.3% of all GISTs
  - 1 to 2% of wild type GISTs
  - Imatinib sensitive

GIST mutation testing – Practical points

- Remember limitations of GIST mutation testing.
- Impending RCPath GIST dataset.
- Know your local GIST mutation testing centre.

Conclusion

- RAS testing of CRC.
- MMR/MSI testing of CRC.
- HER2 testing of oesophago-gastric carcinoma.
- GIST mutation testing.
- [Melanoma testing]