



External quality control of
Immunohistochemistry: from
yesterday to tomorrow
(setting the scene)

Keith Miller



Some history of the development of IHC
over the last 30 years



Professor Clive Taylor,
co-founder of
Immunohistochemistry on
Formalin fixed Paraffin wax
sections

Taylor CR & Burns J. The demonstration of plasma cells and other immunoglobulin-containing cells in formalin fixed, paraffin embedded tissues using peroxidase labelled antibody *J.Clin.Pathol.* 1974; 27: 14 - 20

Trypsinisation

- **Huang S, Minissain H, More JD.** Application of immunofluorescent staining in paraffin sections improved by trypsin digestion. *Lab Invest* 1976; 35; 383 - 391



1982

- Professor Peter Isaacson became Head of Department

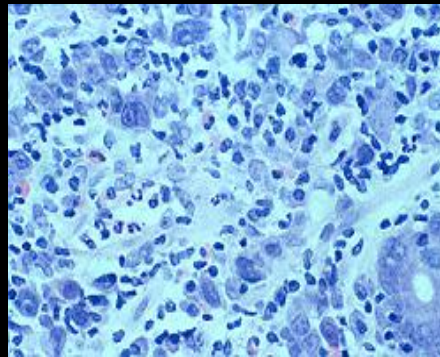
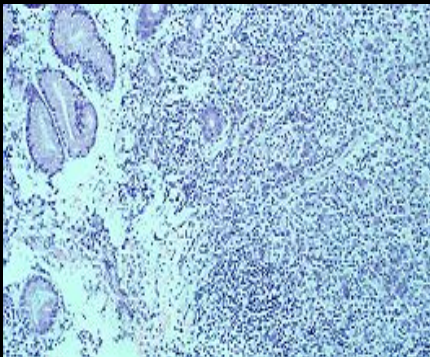


- CAM5.2, LCA, **Light Chain Immunoglobulins**
- Alpha -1-Antitrypsin
- Lysozyme

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H&E

?diagnosis



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Makin CA, Bobrow LG, Bodmer WF.
Monoclonal antibody to cytokeratin for use
in routine histopathology.

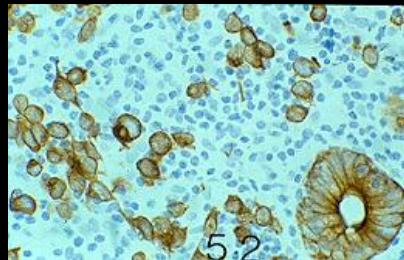
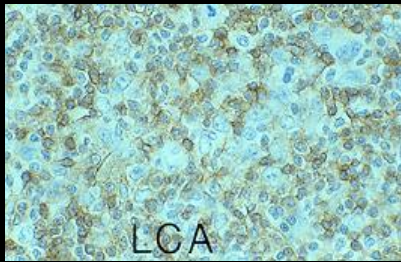
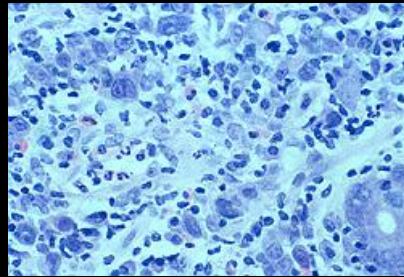
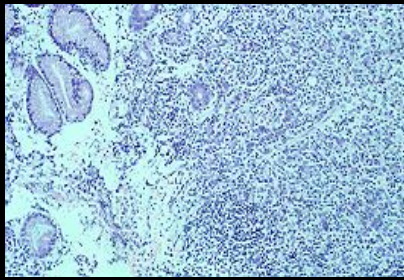
J Clin Pathol. 1984 Sep;37(9):975-83

CAM5.2

Warnke RA, Gatter KC, Falini B, Hildreth P, et al.
Diagnosis of human lymphoma with monoclonal
antileukocyte antibodies. N Engl J Med. 1983 Nov
24;309(21):1275-81.

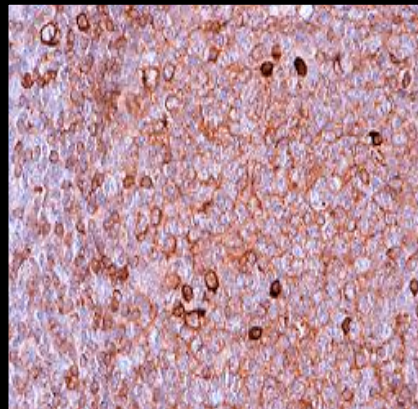
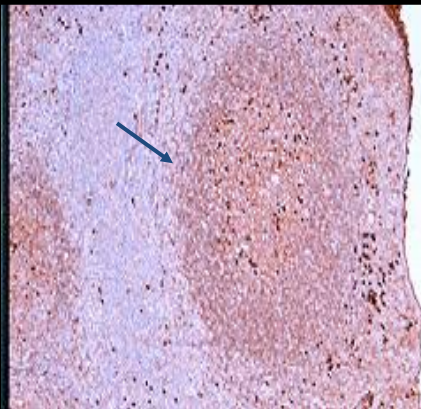
Clones PD7/26 & 2B11

1983 case



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Kappa Light Chain Ig

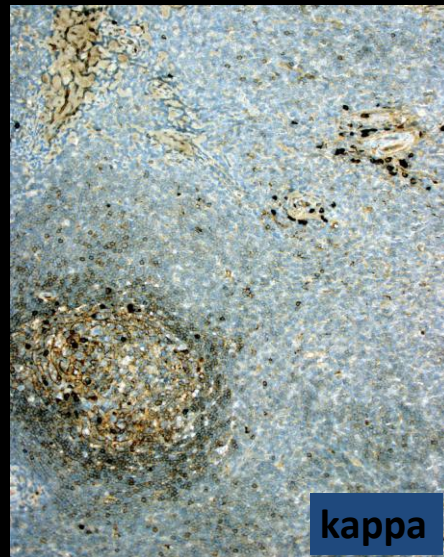
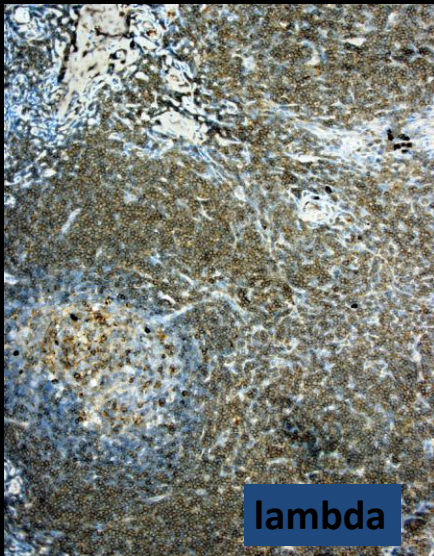


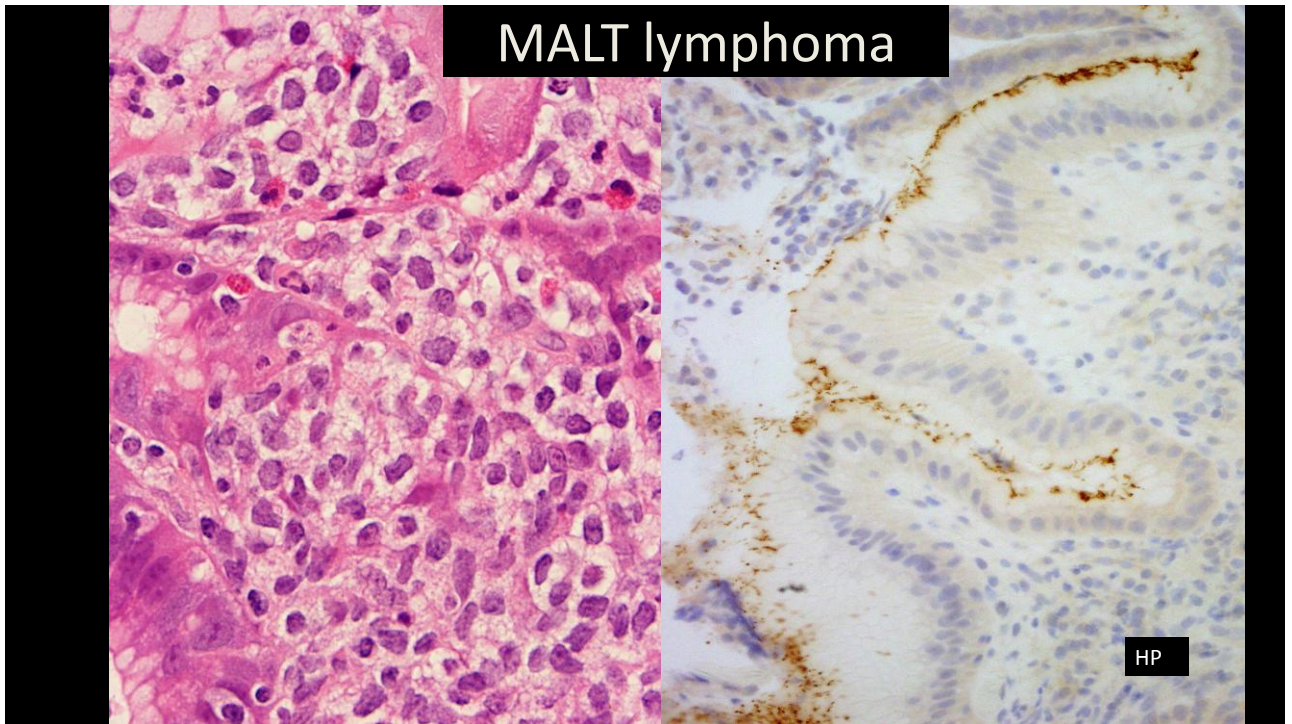
The most powerful tool in immunocytochemistry!
light chain Ig staining



- Kappa light chain on tonsil.
- Tonsil was sliced and fixed overnight in 10% formol saline.

Gastric MALT lymphoma





**Wotherspoon AC, Doglioni C, Diss TC, Pan L,
Moschini A, de Boni M, Isaacson PG.**

**Regression of primary low-grade B-cell
gastric lymphoma of mucosa-associated
lymphoid tissue type after eradication of
Helicobacter pylori.**

Lancet 1993 Sep 4;342(8871):575-7

Dr Robin Warren & Dr John Chan

Adelaide, May 2008

Joint Nobel
prize winner
with Dr Barry
Marshall for
discovering
**Helicobacter
Pylori**



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Professor Peter Isaacson



- Fellow of the Royal Society of Medicine 2009
- Others who are Fellows of the Royal Society in London include **Charles Darwin**

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Antigen Retrieval in the 1990s



1995 - Helix



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2004 - Bonds: Sean, Pierce & Roger!



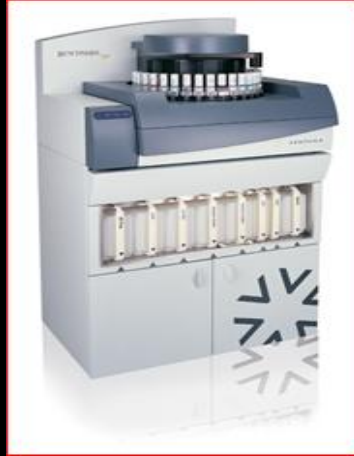
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Since 2010 – Bond 3



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Ventana – Roche Benchmark XT



The UK National External Quality
Assessment Scheme for
Immunocytochemistry and ISH

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History of the EQA Programme

- Began in 1984 at a meeting held at University College London
- Meeting Organiser – Mr Gerry Reynolds from Mount Vernon Hospital
- Approximately 50 laboratories from across the south east of England were represented
- Agreement was reached for EQA to begin in 1985

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UK NEQAS

- Code of Practice
- “Not for Profit”
- Charitable Status
- Independent of all Manufacturers/Suppliers
- Accreditation given under ISO 17043 in March by UK Accreditation Services

www.uclad.com & UKNEQAS.org.uk

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Outline of Assessment Procedure



Headquarters



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Scoring System

- 4 & 5 Points
Expected quality of immunostaining for the antigen in question
- 3 Points
Sub-optimal immunostaining, but still useful diagnostically
- 1 & 2 Points
Poor quality immunostaining with much improvement required

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Good quality needs courses too!

SCHOOL OF LIFE SCIENCES

MODERN TISSUE PATHOLOGY AT THE UNIVERSITY OF WESTMINSTER

The University of Westminster and UK NEQAS invite you to attend a short course in Modern Tissue Pathology on 5-8 September 2011, which will cover basic histology, diagnostic histopathology, diagnostic immunohistochemistry, protein and genetic biomarking, and digital technology.

The aim of this course is to mix practical and theoretical sessions to bring you up to date with the latest developments in the pathology laboratory. Vendors will also give presentations and provide practical demonstrations of their products and equipment. The course will be chaired by Dr Tony Madgwick from University of Westminster.

Monday 5 September – Beginners histology and immunocytochemistry

- For those who are less experienced in the practice of histology and immunohistochemistry (IHC)
- Peter Jackson and David Blythe from Leeds Teaching Hospitals NHS Trust will present basic histology and immunocytochemistry in formal lectures and a practical wet workshop

Tuesday 6 September – Breast cancer

Biomarkers in breast cancer	Michael Gandy (UCL-Advanced Diagnostics)
Overview of breast cancer	Dr Clive Wells (UCLH NHS Trust)
Interactive session: Examining sample slides using Leica SlidePath Technology	Dr Corrado D'Arrigo (Dorset County Hospital)
Presentations by vendors	

- HER2 staining facilities are expected to be available for a maximum of 80 slides (IHC in the morning and *in situ* hybridisation (ISH) overnight)

Wednesday 7 September – The gastrointestinal tract

Gastrointestinal (GI) tract pathology including gastric HER-2 and hereditary non-polyposis colorectal cancer (HNPCC)	Dr Manuel Rodriguez-Justo (UCLH NHS Trust)
Interactive session: Examining slides of gastric HER-2 cases prepared using Roche digital slide technology	Dr Manuel Rodriguez-Justo (UCLH NHS Trust) and Michael Gandy (UCL-Advanced Diagnostics)
UK NEQAS HER-2 data for IHC and ISH	Dr Merdol Ibrahim (UCL)
Presentations by vendors	

C.U.K./LIFESCIENCES

5th to 8th
September 2011
In London

Proliferation of primary Antibodies & the start of companion diagnostics/biomarking

• Primary antibodies for diagnoses
now number in hundreds

• Biomarkers such as ER & PR only
became available from the early
1990's

Today & Tomorrow

Modernising the IHC service for the benefit of patients

- Improving Education
- **Example of new antibodies**
- Improving turnaround time of the biomarking service for patients with advanced disease
- **Important markers related to new targeted therapy**



A unique combination in a single building:

- A dedicated **independent** slide-based Cancer Testing Laboratory
- An Education Centre for the pathology community

Based at:

The Poundbury Cancer Institute,
Newborough House,
Queen Mother Square,
Poundbury, Dorset, UK

Where is CADQAS?

- In Poundbury Cancer Institute, Dorset



Poundbury is an experimental new town on land owned by the Duchy of Cornwall (The Prince of Wales)



Cancer Diagnostic Quality Assurance
Services Community Interest Company

Who is involved?

Keith Miller

Director of UK NEQAS
ICC&IHC & CADQAS CIC



Cancer Diagnostic Quality Assurance
Services Community Interest Company

Sarah Wedden

Director of CADQAS
CIC



Corrado D'Arrigo

Founder of Poundbury Cancer
Institute



BAP-1

BRCA Associated Protein-1

- Part of the ubiquitin C-terminal hydrolase subfamily
- Binds specifically to: BRCA-1 and other associated proteins
- Has tumour suppressor activity
- mesothelioma, uveal and cutaneous melanoma, RCC & squamous carcinoma of the lung.
- BAP-1 mutations:(melanoma) there may be association with BRAF V600E mutation

nature
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Germline *BAP1* mutations predispose to malignant mesothelioma

Joseph R Testa, Mitchell Cheung, Jianming Pei, Jennifer E Below, Yinfei Tan, Eleonora Sementino, Nancy J Cox, A Umran Dogan, Harvey I Pass, Sandra Trusa, Mary Hesdorffer, Masaki Nasu, Amy Powers, Zeyana Rivera, Sabahattin Comertpay, Mika Tanji, Giovanni Gaudino, Haining Yang & Michele Carbone

[Affiliations](#) | [Contributions](#) | [Corresponding authors](#)

Mesothelioma: Assists with diagnosis

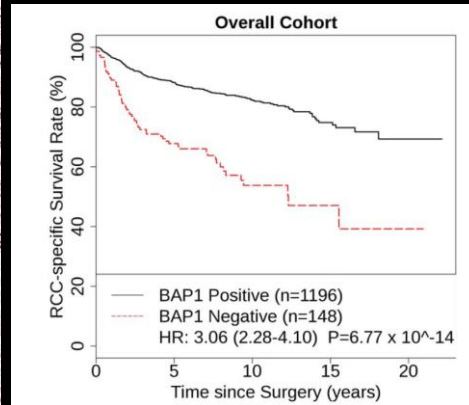
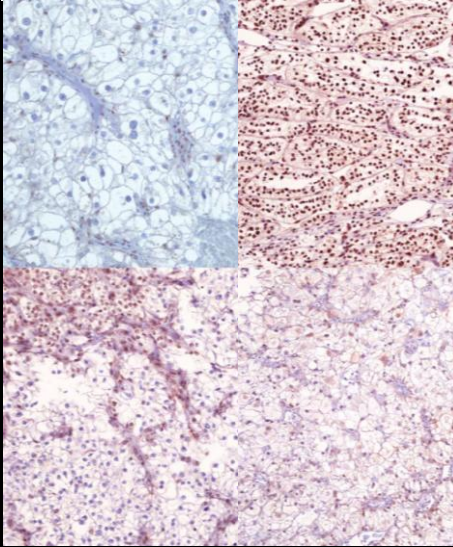
benign mesothelial proliferation:
 BAP-1 preserved in 100%
 p16 preserved in 100%

mesothelioma:
 BAP-1 lost in 26%
 p16 lost in 52%



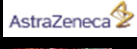





Original Article

Loss of BAP1 Protein Expression Is an Independent Marker of Poor Prognosis in Patients With Low-Risk Clear Cell Renal Cell Carcinoma

Richard W. Joseph, MD¹; Payal Kapur, MD²; Daniel J. Serin, BS³; Jeanette E. Eckel-Passow, Ph.D⁴; Mansi Parasramka, Ph.D⁵; Thai Ho, MD, Ph.D⁶; John C. Chevillet, MD⁷; Eugene Frenkel, MD⁷; Dinesh Rakheja, MD⁸; James Brugarolas, MD, Ph.D⁹; and Alexander Parker, PhD³



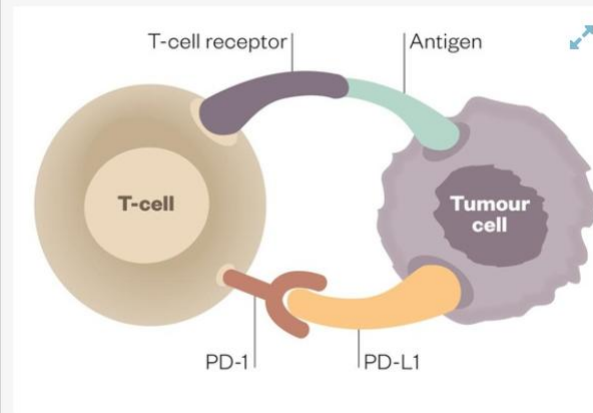
PD-L1 CDx Landscape

	Ventana (SP142)	Ventana (SP263)	Dako (28-8)	Dako (22C3)
Type	Rabbit Monoclonal	Rabbit Monoclonal	Rabbit Monoclonal	Rabbit Monoclonal
Commercial Availability	RUO	CE-IVD Analytical RTU	PD-L1 IHC 28-8 pharmDx	PD-L1 IHC 22C3 pharmDx
Pharma Partner	Roche / Genentech	AstraZeneca / MedImmune	BMS	Merck
Pharma	 	 	 	 
Drug name	Atezolizumab	Durvalumab	Nivolumab	Pembrolizumab
CDx Configuration	<ul style="list-style-type: none"> BenchMark Series RTU CDx Antibody OptiView Detection OptiView Amp 			

PD-1/PD-L1: the target of many drug companies

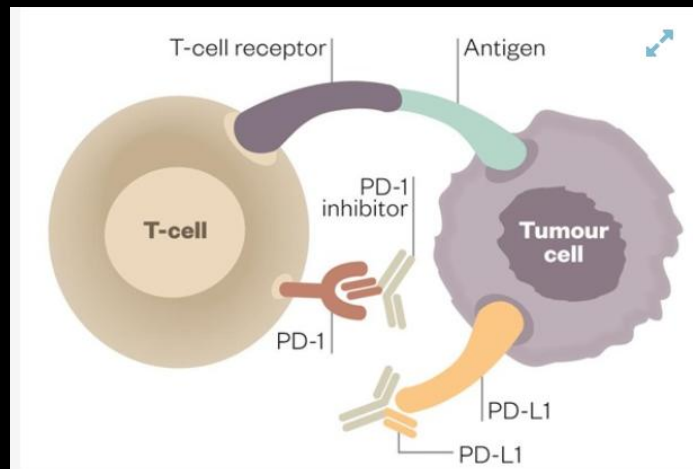
Taking off the brakes

Tumour cells can evade the body's immune system by turning it off just as it begins to mount a response against them. However, scientists have discovered how to block this "immune checkpoint" and let the body continue its attack.



Deactivated T cell

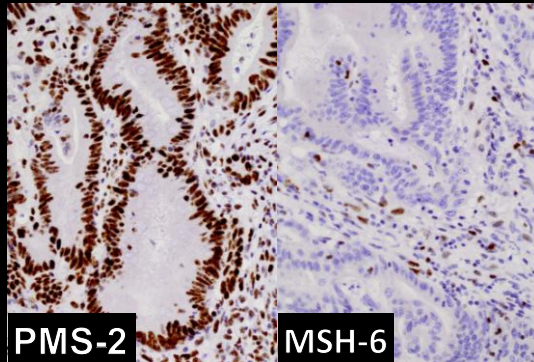
When programmed-death receptor (PD-1) on the T cell binds to programmed death-ligand 1 (PD-L1) on the tumour cell, the T cell becomes deactivated, allowing the cancer cell to evade immune attack



Activated T cell

Inhibitors of programmed-death receptor (PD-1) and programmed death-ligand 1 (PD-L1) can prevent the tumour cell from binding to PD-1, enabling the T cell to remain active and co-ordinate an attack

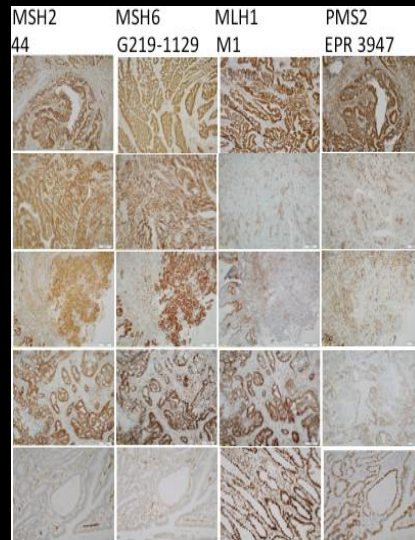
MMR staining – relatively easy to interpret in well fixed colorectal cancer specimens



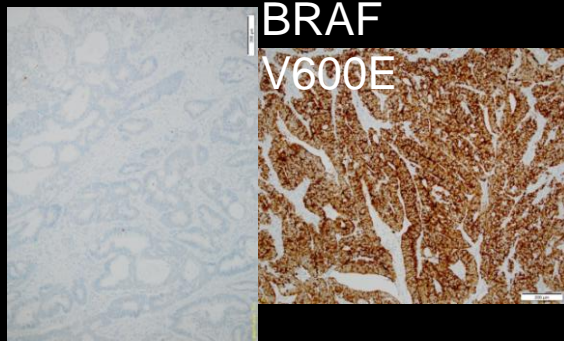
How to identify dMMR

IHC for presence or absence of MMR proteins

- all 4 expressed (but 2% of MSI-H tumours have normal IHC)
- **Loss of MLH1 and PMS2, normal MSH2 and MSH6**
2/3 MLH1 promoter hypermethylation
1/3 LS with germline mutation in MLH1
- **Loss of MSH2 and MSH6**
most have germline MSH2 mutation
- **Loss of MSH6**
- **Loss of PMS2**
- **Rare: missense mutation (usually in MLH1)**
gives antigenically intact but non functional protein

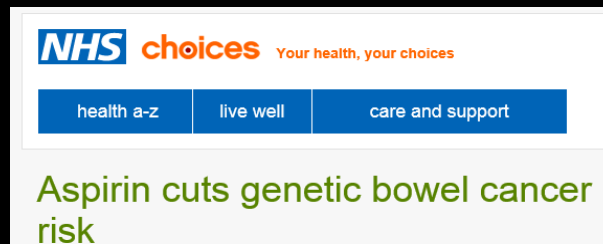


Mutation-specific antibodies



colorectal cancer

Why do MMR?



NHS choices Your health, your choices

health a-z | live well | care and support

Aspirin cuts genetic bowel cancer risk

Checkpoint inhibitors provide very effective treatment for MMR deficient colorectal cancers e.g Pembrolizumab

