Developments in Mismatch Repair IHC, Lynch / HNPCC and related syndromes

Mark Arends
Professor of Pathology, Edinburgh University
Hon. Consultant Pathologist

M.Arends@ed.ac.uk

Ian M Frayling
Consultant in Genetic Pathology, University Hospital of Wales
Honorary Senior Clinical Research Fellow, Institute of Cancer & Genetics, Cardiff University

Fraylingim@cf.ac.uk
Sporadic or Lynch or Familial?
Outline

- Predisposition to colorectal cancer
- Lynch syndrome
  - What is it?
  - What does it do?
  - What can be done about that?
  - How to diagnose it?
  - MSI, IHC, BRAF
  - Interpreting inherited mutations as pathogenic
  - Systematic case finding – guidelines

- New developments
  - Just how does LS predispose to CRC?
  - Advances in
    - Treatment
    - Prevention
Colorectal cancer: Familial risk

**High Risk:** >1:2 (FAP, LS, PJS, JPS)

**High-Moderate Risk:** ~1:6

**Low-Moderate Risk:** ~1:12

**Average Risk:** ~1:20

**Below Average Risk:** <1:20
Colorectal Cancer: Genetic Risk

- Familial Adenomatous Polyposis (FAP)
- Peutz-Jeghers Syndrome (PJS)
- Juvenile Polyposis Syndrome (JPS)
- “Cancer Family Syndrome” ~ “Hereditary Non-Polyposis Colorectal Cancer” (HNPCC)
Colorectal Cancer: Genetic Risk

- Familial Adenomatous Polyposis (FAP)
- Attenuated FAP (AFAP)
- MUTYH-Associated Polyposis (MAP)
- Serrated Polyposis Syndrome (SPS)
- Peutz-Jeghers Syndrome (PJS)
- Juvenile Polyposis Syndrome (JPS)
- Hereditary Mixed Polyposis Syndrome (HMPS)
- Familial Colorectal Cancer / Syndrome X (FCCX)
- Lynch-like Syndrome (LLS)

Lynch Syndrome (LS)
Colorectal Cancer: Genetic Risk

- Familial Adenomatous Polyposis (FAP)
- Attenuated FAP (AFAP)
- MUTYH-Associated Polyposis (MAP)
- NTHL1-Associated Polyposis (NAP)
- Polymerase-Associated Polyposis (PPAP)
- Serrated Polyposis Syndrome (SPS)
- Peutz-Jeghers Syndrome (PJS)
- Juvenile Polyposis Syndrome (JPS)
- Hereditary Mixed Polyposis Syndrome (HMPS)
- Familial Colorectal Cancer / Syndrome X (FCCX)
- Lynch-like Syndrome (LLS)

- ~1.5%

- ~3.3% Lynch Syndrome (LS)

  MSH2, MLH1, MSH6, PMS2, EPCAM
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~3.3% Lynch Syndrome (LS)

*MSH2, MLH1, MSH6, PMS2, EPCAM*
Outline

• Lynch syndrome
  – What is it?
• Lynch syndrome
  – What is it?

An avoidable form of often young onset cancer that untreated costs time, lives, money and resources.
Lynch Syndrome: 1895

- Warthin: Family ‘G’

Warthin, A. S. (1913). Heredity with reference to carcinoma: As shown by the study of the cases examined in the pathological laboratory of the University of Michigan, 1895-1913. Archives of Internal Medicine, 12(5), 546-555.
Lynch Syndrome: 1966

• “Cancer family syndrome”
• Hereditary Non-Polyposis Colorectal Cancer Syndrome

Molecular genetics and clinical-pathology features of HNPCC (Lynch Syndrome)

Historical Journey from Pedigree Anecdote to Molecular Genetic Confirmation.

Lynch HT, Smyrk T, Lynch JF.
Lynch Syndrome: 2016

• Genetics:
  – Autosomal Dominant
  – Partially penetrant, variably expressed, sex limited and phenocopied
  – CRC risks start 20s – 40s; average age of CRC ~42y
  – Due to constitutional ("germline") pathogenic mutations in a DNA mismatch repair (MMR) gene
    • *MSH2, MLH1, MSH6, PMS2*
    • *EPCAM, LRRFIP2*
  – Risks vary with each gene …
  – Common, as rare diseases go - ~1:1000 x 4 = 1:250

  – Biallelic / recessive (inheritance of two mutations in the same gene) = *Constitutional MisMatch Repair Disorder (CMMR-D)*
MSH2 MSH6

ADP


MSH2

ADP

MSH6

MLH1

PMS2

Lynch syndrome: mutations

- Constitutional (‘germline’) mutations come in all sorts
  - Methylation: *MLH1* *(LRRFIP2)*, *MSH2* *(EPCAM)*
  - Point
    - Truncating
    - Splice
    - Missense
  - Indels
  - Large del/dup
  - Chromosomal
    - 46,XX,inv(2)(p21.1p22.2).arr(1-22,X)x2
    - Undetectable on seq, MLPA, aCGH …
Outline

• Lynch syndrome
  – What does it do?
Lynch Syndrome Risks

• **Cancers**
  – **principal:** colon & rectum
  – **major:** endometrium (lower segment)
  – **minor:**
    • Ovary (non-serous)
    • Stomach
    • Small intestine
    • Pancreas
    • Hepato-biliary tract
    • Urinary pelvis/ureter; bladder (TCC)
    • Skin (sebaceous adenoma/carcinoma & keratoacanthoma - *Muir-Torre*)
    • CNS glioblastoma
    • Prostate
    • Breast
    • ...

  – Gene dependent
Lynch Syndrome Risks

- **Cancers**
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    - Prostate
    - Breast
    - ...

- **Gene dependent**
Cancer incidence and survival in Lynch syndrome patients receiving colonoscopic and gynaecological surveillance: first report from the prospective Lynch syndrome database

Pål Møller, Toni Seppälä, Inge Bernstein, Elke Holinski-Feder, Paola Sala, D Gareth Evans, Annika Lindblom, Finlay Macrae, Ignacio Blanco, Rolf Sijmons, Jacqueline Jeffries, Hans Vasen, John Burn, Sigve Nakken, Eivind Hovig, Einar Andreas Rødland, Kukatharmini Tharmaratnam, Wouter H de Vos tot Nederveen Cappel, James Hill, Juul Wijnen, Kate Green, Fiona Laloo, Lone Sunde, Miriam Mints, Lucio Bertario, Marta Pineda, Matilde Navarro, Monika Morak, Laura Renkonen-Sinisalo, Ian M Frayling, John-Paul Plazzer, Kirsti Pylvanainen, Julian R Sampson, Gabriel Capella, Jukka-Pekka Mecklin, Gabriela Möslin

in collaboration with The Mallorca Group (http://mallorca-group.eu)

http://www.lscarisk.org

LS Risks

Stomach, small bowel, biliary tract and pancreas, both sexes

Kidney, ureter and bladder, both sexes

Lynch Syndrome

• How can it be managed?
Management of LS

- Colonoscopic surveillance, 3 yrly
  - Reduces CRC mortality by ~50%

**Figure 25** Cumulative risks of CRC for people with and without MMR mutations.

**Figure 26** Impact of colonoscopy (age 25–75 years) in reducing cumulative risk of CRC for people with LS.

Management of LS

**TABLE 78** Stage distribution of CRCs for individuals undergoing colonoscopic surveillance, from Mecklin and colleagues (2007)

<table>
<thead>
<tr>
<th>Dukes' stage</th>
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<td>C</td>
<td>5 (0.122)</td>
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<tr>
<td>D</td>
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*Source: Mecklin and colleagues.*

**FIGURE 33** Stage distribution of CRCs for people with and without LS surveillance.

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Other Management of LS

• Colon cancer
  – ? More extensive surgery if known LS at time of CRC diagnosis

• Endometrial / ovarian cancer
  – No proven efficacious surveillance, but mort. is low
  – Surgical prophylaxis, but disbenefits

• Gastric, urothelial, brain, small bowel etc
  – No proven efficacious surveillance

• ...

LS: Diagnosis

- Multidisciplinary team effort
- Family history
  - Not bad, but poor sensitivity and specificity
- Constitutional mutation testing
  - Defines LS
- Tests on tumours:
- Microsatellite instability (MSI)
  - MSI in 12% of ‘sporadic’ colon cancers – not due to LS
  - MSI in 4% of colon cancers – due to LS (or somatic MMR mutations etc.)
  - MSI in 1-2% of rectal cancers – due to LS
    - Optimised for colon cancers
    - Technique fairly well standardized (& EQA)
    - Adenomas and Ca at other sites: reduced sensitivity
    - MSH6 and PMS2 tumours: reduced sensitivity
LS: Diagnosis

• Immunohistochemistry (IHC) for abnormal MMR protein expression
  – May exhibit ‘non-standard’ patterns
  – Techniques/Interpretation not standardised (NEQAS)

• *BRAF* V600E
  – Present in most sporadic CRC with MSI; absent in LS

• Methylation of the *MLH1* gene in sporadic MSI+ colon cancers
  – Some cases are constitutional

• (Tumour whole genome sequencing)
“Doctor, is it better to use MSI or IHC, and what about BRAF?”

“Well, MSI and IHC testing are complementary and not exclusive.

I will prescribe you both, and some BRAF as well to be applied as required, but ultimately sequencing will be needed.

But are we talking about a rectal or colonic cancer?”
Microsatellite Instability (MSI)

Normal (black)

BAT25

BAT40

Microsatellite Instability (MSI)

Normal (black)

Tumour (red & blue)

Recent Advances in LS

- Updated UK guidance for genetics laboratories

ACGS best practice guidelines for genetic testing and diagnosis of Lynch syndrome

Prepared and edited by Ian Frayling¹, Ian Berry², Andrew Wallace³, Stewart Payne⁴ and Gail Norbury⁵

¹ All Wales Medical Genetics Service, ² Leeds Genetics Laboratory, ³ Manchester Centre for Genomic Medicine, ⁴ North West Thames Regional Genetics Service ⁵ Guy’s & St Thomas’ Genetics Service.

Guidelines reviewed & updated March 2016 following a CMGS workshop held 30th November 2009

http://www.acgs.uk.com/media/998715/ls_bpg_approved.pdf
Recent Advances in LS

• Updated UK guidance for genetics laboratories
  – Significance of MSI by age
  – Mutation interpretation
MSI and LS and age

Recent Advances in LS

- Significance of MSI by age

\[ P(\text{MSI colon cancer is due to LS}) \]

*Age vs. Probability of MSI colorectal cancer being due to LS
Immunohistochemistry
MMR Immunohistochemistry

Mucinous colonic cancer

MSH2

MLH1
MMR Immunohistochemistry

MSH2

adenoma-carcinoma

MLH1
MMR Immunohistochemistry

Colonic cancer

Gastric cancer

MSH2

MLH1
Skin Sebaceous Adenoma: MMR Immunohistochemistry

Muir-Torre Syndrome: sebaceous tumour & internal malignancy
Bladder Neoplasms: MMR Immunohistochemistry

- TCPapilloma case 563
- TCCarcinoma case 564

Patchy/weak MLH1
MMR Immunohistochemistry

endom. cancer

caecal cancer

Patchy/weak MLH1

MSH2
Caecal Carcinoma: MMR Immunohistochemistry

Patchy / weak MLH1

MLH1 & PMS2 heterodimeric binding partners
Endometrioid Carcinoma: MMR Immunohistochemistry

MSH2 & MSH6 heterodimeric binding partners
Colonic Adenoma: MMR Immunohistochemistry

Compare adjacent tumour & normal/stroma – beware poor fixation
Lynch Syndrome

- **IHC - issues**
  - Antibodies are fixation-sensitive – must interpret a well fixed area
  - Many CRC patchily fixed or poorly fixed – may try several blocks or pre-operative biopsy
  - Must use all 4 Ab: MSH2, MLH1, MSH6, PMS2 – secondary loss of binding partners (MSH2-MSH6 & MLH1-PMS2) useful
  - Mostly loss of expression
  - ~5% patchy / weak expression
  - ~5% CRC due to Lynch Syndrome show normal IHC expression
  - Must take part in NEQAS (Alimentary Tract - HNPCC/MMR module – started 2007) for quality control

- **Other tests useful – MSI & BRAF**
  - Must take part in NEQAS (MSI Mol Gen module) for quality control
2007: Pilot module for DNA MMR proteins

Technical, not interpretative

NEQAS ICC send tumour alongside normal appendix (a)
- Laboratories stain for the MMR protein using their protocol
- Returning the slides and their protocol to NEQAS ICC
- Technical and pathologist feedback on staining is also returned

Laboratories select and stain a control tissue and/or a tumour of their choice: “In-house” (b/c)
- Returning two unstained sections of the same tissue, which are stained by NEQAS ICC using a reference method (d/e)

A panel of four examiners assesses and scores each slide
Scoring criteria

0  No slide submitted; no staining

1 - 2  Very weak demonstration of target antigen in normal epithelium, &/or false positive staining of tumour

3  Weak demonstration of requested target antigen. Although clinically readable, improvements can still be made in the staining

4 - 5  Good / Excellent demonstration of the target antigen
• Marks are deducted when the assessors feel that correct clinical interpretation of staining may be hindered due to other factors such as:
  – False positive / false negative / non-specific or inappropriate staining
  – Excessive cytoplasmic staining
  – Diffuse nuclear staining
  – Excessively strong or weak haematoxylin counter-stain
  – Poor quality of in-house control tissue, including:
    • Poor / inadequate choice of control tissue
    • Poor / inadequate fixation
    • Damaged cell morphology (over retrieval) etc.
• Examiners also make free-text comments
### Scoring database analyses examiner variance

#### Immunocytochemistry

**Assessor Scoresheet Details for the iNPCC module**

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</table>

**Below for Office Use Only**

**ANTIBODY NOT STOCKED**

**COMMENTS**

- Weak demonstration of antigen
- Very weak demonstration antigen
- Antigen is not demonstrated or only very weakly
- Diffuse staining of antigen
- Inappropriate / non-specific staining of some cells
- Slight background staining
- Excessive background staining
- Insufficient pre-treatment (enzymatic or heat mediated)
- Excessive pre-treatment (enzymatic or heat mediated)
- Pre-treatment not recommended
- Counter stain is pale
- Counter stain is excessive
- Uneven staining
- Poor choice of control tissue
- Poor tissue quality
- Cytological preparation is recommended
- Endogenous peroxidase not blocked sufficiently
- Polymer Detection Recommended

**Assessor:** Dr Meriol Ibrahim

Version 1.02
Appendix control: MLH1

Good
Appendix control: MLH1

Borderline poor
Appendix control: MLH1

Poor, non-specific
Appendix control: MLH1

Poor

Poor, cytoplasmic only
Tonsil control: MLH1

Too strong
NEQAS Tumour: MLH1

Good (LP)
NEQAS Tumour: MLH1

Good (HP)
NEQAS Tumour: MLH1

Poor, non-specific
NEQAS Tumour: MLH1

Poor, non-specific
NEQAS Tumour: MLH1
Poor, EDTA
Recommendations

• Automated staining, *not* manual
• Appropriate control tissue: appendix or colon
• Test for MSH2, MLH1, MSH6 and PMS2
• Beware poor fixation or variable fixation – compare adjacent stromal cells & tumour cells
• Do not use quantitative scoring systems, e.g. ++++, ++, +, - instead indicate: “Normal” or “Negative expression”
• Indicate “Weak/patchy expression” with great caution (fixation)
• Full interpretation can only be made in the wider context of e.g. family history data and mutation detection, preferably by means of a multidisciplinary team including geneticists. Only then can a diagnosis of Lynch syndrome be made.
• Remember: 15% sporadic CRC – MLH1 silenced by prom meth
• **ALL UK LABORATORIES SHOULD PARTICIPATE**
LS: Diagnostic tests

- Family history has very limited sensitivity and very poor specificity

- MSI and IHC are much more sensitive and specific
  - MSI is slightly more sensitive (100 – 88% vs. 100 – 73%) but less specific than IHC (84 – 68% vs. 92 – 77%) in CRC
  - Both cost about the same

- BRAF/meMLH1 are sensitive and specific for non-LS colon cancers

- Sequencing is highly sensitive (although there’s always some undetectable mutations) and uniquely specific
  - Used to be much more expensive, but with NGS it is now possible to test many genes at the same time, so increasing sensitivity
  - Often needs tumour results etc to interpret findings, as part of an MDT, so is limited if done in isolation
Lynch Syndrome

• Mutation interpretation
Clinical consequences of mutations identified by genetic tests

- Not pathogenic
- Uncertain significance
- Pathogenic
Clinical consequences of mutations identified by genetic tests

Not pathogenic

Uncertain significance

Pathogenic
Recent Advances in LS

• Updated UK guidance: Mutation interpretation

The International Society for Gastrointestinal Hereditary Tumours (InSiGHT) maintains a helpful resource on variants and the InSiGHT LOVD, as reflected on ClinVar, Ensembl and Decipher, is now recognised by the HVP/GA4GH as the sole global repository for MMR gene mutations and their interpretation. [http://chromium.lovd.nl/LOVD2/colon_cancer/home.php]. For reasons of quality and to minimise disparity in interpretation between centres this should be regarded as the primary source of interpretations of MMR gene mutations.

The current criteria used for the interpretation of MMR mutations\(^\text{30}\) are given on the InSiGHT website [http://insight-group.org/criteria/\(^\text{}\)]. The evidence supporting each classification is given on the LOVD listings and can include segregation and tumour analyses (incorporated in a multifactorial Bayesian model), as well as mRNA and other analyses. The prior probability of pathogenicity of all possible MMR gene missense mutations has been calculated using a customised version of PolyPhen v2.1 in conjunction with MAFF (a LS-specific in vitro model)\(^\text{31}\). Using conditional probabilities of pathogenicity from e.g. tumour tests, posterior probabilities of pathogenicity can be established.

http://www.acgs.uk.com/media/998715/ls_bpg_approved.pdf
A Systematic Approach to Clinical Classification of DNA Sequence Variants in Mismatch Repair Genes:

The InSiGHT Variant Interpretation Committee

Established Yokohama, 2007

San Antonio, Mar 2011
IARC 5-tiered Classification System:
www.insight-group.org/criteria/

Class 5: Pathogenic
Class 4: Likely pathogenic
Class 3: Uncertain
Class 2: Likely not pathogenic
Class 1: Not pathogenic
Bayes’ Rule

Prior probability

Updated by

Likelihood ratios -or- odds ratios of causality

Posterior probability

Thompson et al., Human Mutation 34: 200-209, 2012.
Classification of MMR gene UVs:

Prior probability: $LR = \frac{\Pr(\text{Data}_i | \text{HR})}{\Pr(\text{Data}_i | \text{N})}$

Empirical:
- in silico missense analysis
- in silico splicing analysis

Current LRs (Data):
- LR1: Co-segregation
- LR2: Microsatellite instability
- LR3: BRAF V600E

Tba:
- Summary family history
- MLH1 promoter methylation
- In vitro MMR assays

Classification: IARC 5-grade model with posterior probability cutoffs

Outcomes and Significance

• Submission of data for variant classification
  – **Tumour test results are critical**
  – “not on the InSiGHT database” is no longer a valid statement on a genetics report!

• Put data in and you will get an output:  
  
• **APC, MUTYH, POLD1, POLE …**
Application of a 5-tiered scheme for standardized classification of 2,360 unique mismatch repair gene variants in the InSiGHT locus-specific database.
From the highest rated international genetics journal to the UK newspaper with the lowest required reading age in <48 h.

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CANCER’S DNA HOPE

SCIENTISTS have found a more accurate test for bowel and womb cancer which they say will save lives.

Their research identified the genes that cause Lynch Syndrome – the most common form of hereditary bowel cancer. Sufferers also have an increased risk of developing other cancers, including in the womb.

Patients could now have more idea if the cancers run in their family – meaning they can be screened earlier.

Bowel cancer kills 16,000 people each year and womb cancer 2,000. Dr Ian Frayling, from Cardiff University, said: “This study will help to save lives.”
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http://www.insight-database.org/genes
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Outline

• Lynch syndrome
  – Systematic case finding – guidelines
Case finding
Learn from others … keep it simple and smart -
Case finding

Learn from others … keep it simple and smart -
Case finding
Learn from others … keep it simple and smart -
Case finding

Learn from others ... keep it simple and smart -

Use technology to find hereditary cancer which doesn’t depend on people remembering anything, and when it is found, tell them what to do.

http://www.journalslibrary.nihr.ac.uk/hta/volume-18/issue-58

Evaluated according to the NICE reference case.
# Testing Strategies

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<td>Universal Genetic Testing</td>
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* Family history = AC

Within Genetic Testing: *MLH1+MSH2+MSH6* is considered separately from *PMS2*. 

Within Genetic Testing: *MLH1+MSH2+MSH6* is considered separately from *PMS2*.
## Figure 73. Incremental net health benefit compared to Strategy 1(1) [Age 50y]

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<td>6</td>
<td>MSI BRAF IHC</td>
</tr>
<tr>
<td>7</td>
<td>IHC MSI BRAF</td>
</tr>
<tr>
<td>8</td>
<td>Universal GT</td>
</tr>
</tbody>
</table>

The figure illustrates the incremental net health benefit compared to Strategy 1(1) for various test combinations at age 50 years. The vertical bars represent the confidence intervals for each strategy.
Screening for Lynch Syndrome

• NIHR HTA Report – Net Health Benefit of Lynch Screening
  – Compared no testing vs testing – IHC or MSI or BRAF or comb.

• Royal College of Pathologists:
  – 2014 Colorectal Cancer dataset: new guidelines
  – Test all CRCs <50y, plus those considered by the pathologist to be worthwhile, for loss of MMR – by IHC (or equivalent)
    • e.g. those with 2 LS-associated tumours at any age, characteristic tumours/histology, Stage II/IIIs considering 5FU chemotherapy etc.
  – If IHC then **MSH2, MLH1, MSH6 & PMS2 should all be tested**
  – Testing up to 70y for LS is warranted but not yet obligatory
  – Endometrial cancer screening – under discussion (BRAF inappropriate)

• Recent Bowel Cancer UK / RCPath FOI survey

• Implementation in Scotland
  – Co-ordinated approach in major centres – Edinburgh, Dundee, Glasgow, Aberdeen
  – Agreed document on molecular pathology of CRC – current practice in Edinburgh (starting point)
  – Currently: IHC – MSI – BRAF
Scotland: Lynch Screening

**Request testing**

Clinical Criteria for LS: Amsterdam II/Revised Bethesda

**Reflex testing**

CRC: <60 yr or clinical or pathological features suggestive of MMR defect (multiple tumours, premenopausal endometrial cancers)

**Analysis for:**

MMR protein expression (MLH1, MSH2, MSH6 and PMS2), MSI and BRAF mutation

- **No MSI and normal MMR protein expression**
  - Sporadic cause of tumour

- **MSI-H and/or loss of MMR protein expression**
  - No BRAF mutation
  - Tumours likely to be Lynch Syndrome
  - Genetics counselling and germline testing

- **Loss of MMR protein expression and/or MSI-H + BRAF mutation**
  - Sporadic cause of tumour
Outline

• New developments
  – When IHC abnormality doesn’t tally with inherited MMR gene mutations
  – Just how does LS predispose to CRC?
  – Advances in
    • Treatment
    • Prevention
Underlying causes of microsatellite instability in colorectal and endometrial cancers in genetics clinic patients, by associated pattern of MMR IHC abnormality.

<table>
<thead>
<tr>
<th></th>
<th>MLH1 (alone, or in combination with PMS2)</th>
<th>MSH2 (alone, or in combination with MSH6)</th>
<th>MSH6 (alone)</th>
<th>PMS2 (alone)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constitutional MLH1 mutation</td>
<td>12%</td>
<td></td>
<td></td>
<td>2%</td>
</tr>
<tr>
<td>Constitutional MLH1 methylation</td>
<td>0.4%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Constitutional MSH2 mutation</td>
<td></td>
<td>14%</td>
<td>0.4%</td>
<td></td>
</tr>
<tr>
<td>Constitutional EPCAM mutation</td>
<td></td>
<td></td>
<td>2%</td>
<td></td>
</tr>
<tr>
<td>Constitutional MSH6 mutation</td>
<td></td>
<td></td>
<td>1%</td>
<td>10%</td>
</tr>
<tr>
<td>Constitutional PMS2 mutation</td>
<td></td>
<td></td>
<td></td>
<td>6%</td>
</tr>
<tr>
<td>Acquired MLH1 methylation</td>
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<td></td>
<td>24%</td>
<td></td>
</tr>
<tr>
<td>Acquired MLH1 mutation</td>
<td></td>
<td></td>
<td>7%</td>
<td></td>
</tr>
<tr>
<td>Acquired MSH2 mutation</td>
<td></td>
<td></td>
<td>2%</td>
<td></td>
</tr>
<tr>
<td>Unexplained</td>
<td>10%</td>
<td>6%</td>
<td>2%</td>
<td>2%</td>
</tr>
<tr>
<td>Total</td>
<td>53%</td>
<td>25%</td>
<td>12%</td>
<td>9%</td>
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</table>


Underlying causes of microsatellite instability in colorectal and endometrial cancers in genetics clinic patients, by associated pattern of MMR IHC abnormality.

<table>
<thead>
<tr>
<th>IHC abnormality</th>
<th>MLH1 (alone, or in combination with PMS2)</th>
<th>MSH2 (alone, or in combination with MSH6)</th>
<th>MSH6 (alone)</th>
<th>PMS2 (alone)</th>
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</thead>
<tbody>
<tr>
<td>Constitutional MLH1 mutation</td>
<td>12%</td>
<td>2%</td>
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<td></td>
</tr>
<tr>
<td>Constitutional MLH1 methylation</td>
<td>0.4%</td>
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</tr>
<tr>
<td>Constitutional MSH2 mutation</td>
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<td>0.4%</td>
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<tr>
<td>Constitutional EPCAM mutation</td>
<td>2%</td>
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<tr>
<td>Constitutional MSH6 mutation</td>
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<td>10%</td>
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<tr>
<td>Constitutional PMS2 mutation</td>
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<td>Acquired MLH1 methylation</td>
<td>24%</td>
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<tr>
<td>Acquired MSH2 mutation</td>
<td>2%</td>
<td>2%</td>
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<tr>
<td>Unexplained</td>
<td>10%</td>
<td>6%</td>
<td>2%</td>
<td>2%</td>
</tr>
<tr>
<td>Total</td>
<td>53%</td>
<td>25%</td>
<td>12%</td>
<td>9%</td>
</tr>
</tbody>
</table>

IHC is a pointer to, but not an absolute indicator of the underlying genetic defect / inherited mutation.

All four MMR proteins need to be tested.


Colon Cancer: types

- **Hypermutable**
  - MSI + BRAF wt
  - MSI + BRAF V600E

- **~1%** POL gene mutations
  - Lynch syndrome
  - Lynch-like syndrome, inc. MAP
  - Hypermutant

- **~3.3%** MSI: MMR gene mutations
  - Lynch syndrome
  - Lynch-like syndrome, inc. MAP

- **~10%** Sporadic (often serrated pathway)

- **CIN**
### Colon Cancer: types

<table>
<thead>
<tr>
<th>Type</th>
<th>MSI</th>
<th>POL gene mutations</th>
<th>PPAP</th>
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</thead>
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<tr>
<td>Hypermutilant</td>
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<tr>
<td>MSI + BRAF wt</td>
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</tr>
<tr>
<td>MSI + BRAF V600E</td>
<td>~3.3%</td>
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<tr>
<td>Lynch syndrome</td>
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<td></td>
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<tr>
<td>Lynch-like syndrome, inc. MAP</td>
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<td></td>
</tr>
<tr>
<td>Sporadic (often serrated pathway)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**NB** MSI is rare in rectal cancer, but when it occurs usually indicates LS


Recent Advances in LS

- Lynch-like syndrome
Recent Advances in LS

- Lynch-like syndrome
  - Strong family history of LS-type tumours
  - <10 adenomas
  - MSI in the tumours
  - Abnormal MMR protein immunohistochemistry
  - But no inherited MMR gene mutation on testing
Recent Advances in LS

• Lynch-like syndrome
  – Strong family history of LS-type tumours
  – <10 adenomas
  – MSI in the tumours
  – Abnormal MMR protein immunohistochemistry
  – But no inherited MMR gene mutation on testing

• Familial Colorectal Cancer (FCC-X syndrome)
  – Strong family history
  – Maybe >10 adenomas
  – No MSI
  – Tend not to get MMR gene testing
Recent Advances in LS

• Lynch-like syndrome
Clinico-pathological features of biallelic MUTYH carriers

N = 225 Lynch-like syndrome patients

15 MUTYH mutation carriers

- 8 monoallelic
- 7 biallelic

So MUTYH is a *bona fide* Lynch-like syndrome gene

[and as these cases had <10 adenomas, so they didn’t have MAP …]
### Constitutional variants in CRC-associated genes

**18 MSH2 deficient LLS**

6 carriers of predicted pathogenic variants in other genes

<table>
<thead>
<tr>
<th>Patient ID</th>
<th>Gene</th>
<th>Transcript/cDNA change</th>
<th>Predicted protein change</th>
<th>Variant calling</th>
<th>Splicing</th>
<th>Protein function</th>
<th>Protein stability</th>
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</thead>
<tbody>
<tr>
<td>105</td>
<td>MUTYH</td>
<td>NM_001128425.1:c.1227_1228dup</td>
<td>p.Glu410GlyfsX43</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>105</td>
<td>SETD2</td>
<td>NM_014159.6:c.1204C&gt;T</td>
<td>p.Arg402Trp</td>
<td>Gain of acceptor splicing site</td>
<td>Damaging</td>
<td>Destabilizing</td>
<td>N.D.</td>
</tr>
<tr>
<td>115</td>
<td>MLH3</td>
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<td>Inconclusive</td>
<td>Inconclusive</td>
<td>Inconclusive</td>
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<tr>
<td>115</td>
<td>BUB1</td>
<td>NM_004336.4:c.3005C&gt;G</td>
<td>p.Thr1002Ser</td>
<td>Loss of acceptor splicing site</td>
<td>Benign</td>
<td>Destabilizing</td>
<td>N.D.</td>
</tr>
<tr>
<td>111</td>
<td>BUB3</td>
<td>NM_004725.3:c.*1124G&gt;A</td>
<td>p.?</td>
<td>Loss of donor splicing site</td>
<td>Damaging</td>
<td>N.D.</td>
<td></td>
</tr>
<tr>
<td>111</td>
<td>MLH3</td>
<td>NM_001040108.1:c.*2058G&gt;T</td>
<td>p.?</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>107</td>
<td>SETD2</td>
<td>NM_014159.6:c.2798G&gt;T</td>
<td>p.Gly933Val</td>
<td>Loss of donor splicing site</td>
<td>Benign</td>
<td>N.D.</td>
<td></td>
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<tr>
<td>107</td>
<td>ENG</td>
<td>NM_000118.3:c.1712G&gt;A</td>
<td>p.Arg571His</td>
<td>Inconclusive</td>
<td>Benign</td>
<td>N.D.</td>
<td></td>
</tr>
<tr>
<td>107</td>
<td>EPCAM</td>
<td>NM_002354.2:c.-280G&gt;C</td>
<td>p.?</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>107</td>
<td>MLH3</td>
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<td>p.?</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>110</td>
<td>SETD2</td>
<td>NM_014159.6:c.2508T&gt;G</td>
<td>p.Cys836Trp</td>
<td>No change</td>
<td>Damaging</td>
<td>Destabilizing</td>
<td>N.D.</td>
</tr>
<tr>
<td>109</td>
<td>MSH2</td>
<td>NM_000251.2:c.211G&gt;C</td>
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<td>Loss of donor splicing site</td>
<td>Inconclusive</td>
<td>N.D.</td>
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<tr>
<td>109</td>
<td>PMS1</td>
<td>NM_000534.4:c.2186A&gt;G</td>
<td>p.Asn690Ser</td>
<td>Inconclusive</td>
<td>Benign</td>
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<tr>
<td>109</td>
<td>TP53</td>
<td>NM_000546.5:c.*1175A&gt;C</td>
<td>p.?</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>109</td>
<td>APC</td>
<td>NM_000038.4:c.*1684A&gt;G</td>
<td>p.?</td>
<td>-</td>
<td>-</td>
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<tr>
<td>109</td>
<td>ENG</td>
<td>NM_000118.3:c.*704delAGTT</td>
<td>p.?</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

**Possible oligogenic effect**

**Custom NGS panels help in the identification of other possibly associated genes**

Courtesy of Dr Gardenia Vargas
### Double somatic hits in DNA repair genes in MSH2/6- MAP

<table>
<thead>
<tr>
<th>Patient ID</th>
<th>Tumor tested</th>
<th>Variant calling</th>
<th>Gene Transcript/cDNA change</th>
<th>Predicted protein change</th>
<th>In silico predictions</th>
<th>Protein function</th>
<th>LOH in MSH2 locusº</th>
</tr>
</thead>
<tbody>
<tr>
<td>108_C2</td>
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<td></td>
<td><strong>BUB1B</strong> NM_001211.5:c.1738G&gt;T</td>
<td>p.Glu580*</td>
<td>Inconclusive</td>
<td>Benign</td>
<td>Possible</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><strong>MLH1</strong> NM_001167618.1:c.1253G&gt;A</td>
<td>p.Arg418Gln</td>
<td>Inconclusive</td>
<td>Benign</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><strong>MSH6</strong> NM_000179.2:c.2625G&gt;T</td>
<td>p.Met875Ile</td>
<td>No change</td>
<td>Damage</td>
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<tr>
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<td></td>
<td><strong>BMPR1A</strong> NM_0043429.2:c.878C&gt;T</td>
<td>p.Ala293Val</td>
<td>No change</td>
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<td><strong>POLE</strong> NM_006231.2:c.2284C&gt;T</td>
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<td>Damage</td>
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<td>108_C1</td>
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<td><strong>SETD1B</strong> NM_015048.1:c.22del</td>
<td>p.H8fs*27</td>
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<td>Possible</td>
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<td><strong>MSH3</strong> NM_002439.4:c.1141delAA</td>
<td>p.Lys383Argfs*32</td>
<td>Inconclusive</td>
<td>Benign</td>
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<td><strong>PMS2</strong> NM_000535.5:c.1501G&gt;A</td>
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<td>Damage</td>
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<td></td>
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<tr>
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<td></td>
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<td><strong>MSH2</strong> NM_000251.2:c.1741delA</td>
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<td><strong>MLH3</strong> NM_001040108.1:c.1755del</td>
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<td>Benign</td>
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<td><strong>BMPR1A</strong> NM_0043429.2:c.419del</td>
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<tr>
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<td><strong>MLH1</strong> NM_001167618.1:c.443G&gt;A</td>
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<td>No change</td>
<td>Benign</td>
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<tr>
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<td><strong>POLE</strong> NM_006231.3:c.2375A&gt;G</td>
<td>p.Lys792Arg</td>
<td>Gain of donor</td>
<td>Damage</td>
<td></td>
</tr>
</tbody>
</table>
Final remarks

Germline
- 6/18 MSH2/6- Lynch syndrome
- 2 constitutional MLH1 methylation
- 3 reclassified MSH2 VUS
- 1 previously unidentified MSH2 mutation

Somatic
- 7/18 MSH2/6- Predicted germline pathogenic variants
- 3 in FAN1
- 3 in SETD2
- 1 in BUB1
- 2 double somatic MMR hits
- 2 double heterozygous DNA repair

3.5% MAP syndrome

260 LS-suspected

Courtesy of Dr Gardenia Vargas
Implications for how we test in the future …

<table>
<thead>
<tr>
<th>Gene</th>
<th>Polyps</th>
<th>No polyps</th>
<th>MSS</th>
<th>MSI</th>
</tr>
</thead>
<tbody>
<tr>
<td>APC (FAP/AFAP)</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓ (?)</td>
</tr>
<tr>
<td>AXIN2</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓ (?)</td>
</tr>
<tr>
<td>BUB1</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>MUTYH (MAP)</td>
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<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
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<td>✓</td>
<td>✓</td>
</tr>
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<td>✓</td>
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</tr>
<tr>
<td>POLE (PPAP)</td>
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<td>✓</td>
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</tr>
<tr>
<td>MSH2</td>
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<td>✓</td>
<td>✓ (✓)</td>
<td>✓</td>
</tr>
<tr>
<td>MLH1</td>
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<td>✓</td>
<td>✓ (✓)</td>
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<td>MSH6</td>
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<td>✓</td>
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<tr>
<td>FAN1</td>
<td>✓</td>
<td>✓</td>
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<td>✓</td>
</tr>
<tr>
<td>SETD2</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
</tbody>
</table>
Panels: what does it all mean?

- Implications for how we test in the future …
- We [used to] use polyps and MSI/IHC testing to guide which gene/s to test, but better now to test all cases with a panel and use tumour tests to interpret genetic findings - not to limit which genes to test.

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<td>✓</td>
</tr>
<tr>
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<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
</tbody>
</table>
Panels: what does it all mean?

- Implications for how we test in the future …
- And, logically, if we are **screening** for hereditary causes of CRC, better to test with a gene panel, and not exclude those without LS by testing first with MSI and IHC.

<table>
<thead>
<tr>
<th>Strategy</th>
<th>Polyps</th>
<th>No polyps</th>
<th>MSS</th>
<th>MSI</th>
</tr>
</thead>
</table>
| 1(1) No testing | ✔ | ✔ | ✔ | ✔ (?)
| 1(2) Family history* | ✔ | ✔ | ✔ | ✔ (?)
| 2 | IHC | ✔ | ✔ | ✔ | ✔ | ✔ |
| 3 | BRAF | ✔ | ✔ | ✔ | ✔ |
| 4 | MSI | ✔ | ✔ | ✔ | ✔ |
| 5 | BRAF | ✔ | ✔ | ✔ | ✔ |
| 6 | MSI | ✔ | ✔ | ✔ | ✔ |
| 7 | IHC | ✔ | ✔ | ✔ | ✔ |
| 8 | Universal GT | ✔ | ✔ | ✔ | ✔ |

### Incremental net health benefit

- **APC (FAP/AFAP)**: ✔ ✔ ✔ ✔ (?)
- **AXIN2**: ✔ ✔ ✔ ✔ (?)
- **BUB1**: ✔ ✔ ✔ ✔
- **MUTYH (MAP)**: ✔ ✔ ✔ ✔
- **NTHL1 (NAP)**: ✔ ✔ ✔ ✔
- **POLD1 (PPAP)**: ✔ ✔ ✔ ✔
- **POLE (PPAP)**: ✔ ✔ ✔ ✔
- **MSH2**: ✔ ✔ (✔) ✔ ✔
- **MLH1**: ✔ ✔ (✔) ✔ ✔
- **MSH6**: ✔ ✔ ✔ ✔
- **PMS2**: ✔ ✔ ✔ ✔
- **FAN1**: ✔ ✔ ✔ ✔
- **SETD2**: ✔ ✔ ✔ ✔
Recent Advances in LS

Take homes:

• Loss of MMR / abnormality of IHC may not be caused by LS, but may well be caused by some other form of predisposition, e.g. LLS, MUTYH, POLD/E etc …

• We use tumour tests to help direct (and limit) which gene/s to test, but maybe in the future we will test the genes first and then we will really need tumour tests to help us interpret the genetic findings
Recent Advances in LS

From a Professor of Surgery, 3 days ago …

“Hi Ian, I am happy to say that we are marching ahead with panel testing, probably with 64 genes.

The good thing is that finally (!) we will do this as a fast track service, meaning that I will have the results prior to the surgery for the cancer patients.

This allows for patients informed consent as to which surgical option they are interested in, including perhaps hysterectomy for females after family completion.

We will probably go for all CRC < 70 and endometrial cancer < 70 without any tumor testing, but trying to get the family histories.

There will be a big interdisciplinary meeting here in September. The topic is relevant to the hospital chain and [as] we deal with about 3000 CRC per year, so we could quickly get results [and show] how well this works in a country like Germany ;-)

Recent Advances in LS

• So, how does LS predispose to CRC?
The Adenoma - Carcinoma Sequence

Intestinal epithelial crypts → Aberrant crypt focus → Adenoma → Carcinoma

- APC
- KRAS
- Other oncogenes?
- SMAD2/SMAD4 Chromosome 18q LOH
- TP53 Chromosome 17p LOH

Nuclear β-catenin levels and chromosomal instability

http://www.pathologyoutlines.com/topic/colontumoradenomacarcinoma.html

The Adenoma - Carcinoma Sequence

Chromosomal Instability (CIN)
How sporadic dMMR colon cancers develop

Microsatellite Instability (MSI)
How sporadic dMMR colon cancers develop

1. APC

2. MLH1 methylation

3. BRAF

4. TGFβIIR

5. BAX

Microsatellite Instability (MSI)
1. **CIMP-high, MSI-H**, methylation of MLH1, BRAF mutation, chromosomally stable, MSI-H, origin in serrated polyps, known generally as sporadic MSI-H (12%).

2. **CIMP-high**, partial methylation of MLH1, BRAF mutation, chromosomally stable, MSS or MSI-L, origin in serrated polyps (8%).

3. **CIMP-low**, KRAS mutation, MGMT methylation, chromosomal instability, MSS or MSI-L, origin in adenomas or serrated polyps (20%).

4. **Chromosomal instability, MSS**, CIMP-negative, origin in adenomas (57%).

5. **Lynch syndrome**, MSI-H, CIMP-negative, BRAF mutation negative, chromosomally stable, origin in adenomas (3%).

1. **CIMP-high, MSI-H**, methylation of MLH1, BRAF mutation, chromosomally stable, MSI-H, origin in serrated polyps, known generally as sporadic MSI-H (12%).

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4. **Chromosomal instability, MSS**, CIMP-negative, origin in adenomas (57%).

5. **Lynch syndrome**, MSI-H, CIMP-negative, BRAF mutation negative, chromosomally stable, origin in adenomas (3%).

Lesion (UK)
How do LS bowel cancers develop?
In the colorectal mucosa of LS mutation carriers, ~1 crypt / cm$^2$ is deficient in MMR, i.e. ~10,000 in total.

Colorectal cancers with immediate invasive growth in Lynch syndrome – evidence from histology

β-catenin staining

Possible pathways of CRC development in Lynch syndrome

- Normal epithelial cell
  - APC mutation
  - MMR gene inactivation
  - MMR-DCC
- Adenoma
  - Polypous growth
  - MMR gene inactivation
  - MMR-deficient cancer
  - Alternative molecular alterations (CTNNB1?)
- Non-polypous growth

How some LS cancers may develop …

1. β-catenin

2. K-ras

3. TGFβIIIR

4. Bax, TCF4, ACVRII, Caspase 5 …

5. Microsatellite Instability (MSI)

MSH2*, MLH1*, MSH6*
Recent Advances in LS

• How does LS predispose to CRC?

• Well, if half of the CRCs in LS arise from non-polypoid sub-mucosal immediately invasive lesions that arise at a very slow rate from the 1,000s of crypts which have lost MMR, then this may explain why colonoscopy removes many polyps, but makes little difference to the numbers of cancers arising, and yet prevents half of LS CRCs

• Hence, we’ve been fooled into thinking LS patients must get CRC from “very rapidly growing adenomas”, because an understandable assumption was made at the outset that all CRCs derive from adenomas …
Recent Advances in LS

• Treatment & Prevention
• The immune system and LS: Potential future therapies
Management of MSI cancers

- **5-FU response**

![Graph showing recurrence rates for stage II/III patients with dMMR (MSI) and pMMR (MSS).](image)

- **Recurrence**
  - dMMR (MSI)
  - pMMR (MSS)

- **Alive and recurrence-free**
  - dMMR (MSI)
  - pMMR (MSS)
Future Management of MSI cancers

- PD-1 Blockade
Management of MSI cancers

• PD-1 Blockade
  – Phase 2 Study of MK-3475 in Patients With Microsatellite Unstable (MSI) Tumor
  – Pembrolizumab Combined With INCB039110 and/or INCB050465 in Advanced Solid Tumors [including MSI CRCs]
  – Pilot Study of Using Epigenetic Modulators to Enhance Response to MK-3475 in Microsatellite Stable Advanced Colorectal Cancer
Management of LS

- Colonoscopic surveillance, 3 yrly
  - Reduces CRC mortality by ~50%

**Figure 25** Cumulative risks of CRC for people with and without MMR mutations.

**Figure 26** Impact of colonoscopy (age 25–75 years) in reducing cumulative risk of CRC for people with LS.
Future Prevention in LS

• Aspirin
  – CAPP2: “600 mg aspirin/day for 2 yrs reduced all Lynch syndrome cancers at 5 yrs by over 60%”
  – CaPP3 dose-determination trial in progress http://www.capp3.org/

• Vaccines
  – Micoryx http://clinicaltrials.gov/show/NCT01461148
  – Monocyte-derived dendritic cells https://clinicaltrials.gov/ct2/show/NCT0188572
Summing up

• Lynch syndrome accounts for 1:30 of all CRCs
• The role of the histopathologist in diagnosing LS is paramount
• The quality and interpretation of IHC is critical

• LS demands particular management, including identification through systematic testing of incident cases
• Treatment of LS may soon include prophylaxis by aspirin and vaccination …
• Tumours with MSI, whether due to LS or not, warrant different treatment
• New treatments like PD-1 blockade may be especially effective
• It teaches us there are more ways than one of causing CRC


http://insight-group.org/
Acknowledgements

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David Adams (Sanger, Hinxton)  
Mike Mueller, Ying Zhou (Edinburgh)  
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Matthias Kloor (Heidelberg)  
Gabriela Moeslein (Wuppertal)  
Tristan Snowsill (PenTAG, Exeter)  

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Rick Fishel (Ohio, USA)  
Pat Lynch (MD Anderson, Houston)  
The Mallorca Group  
Lynch Syndrome UK
Detection and interpretation of PD-L1 expression in the management of non-small cell lung cancer; what does it all mean?

Professor JR Gosney

Royal Liverpool University Hospital; University of Liverpool
Disclosure

JRG is a paid advisor to and speaker for AstraZeneca, Boehringer-Ingelheim, Bristol-Myers Squibb, Dako, Lilly & Co, Merck Sharp and Dohme, Novartis, Pfizer and Roche
The biology
The cells of a malignant neoplasm are an insurgent army

- Carpet-bombing
  - Chemotherapy
- Selective destruction by drones
  - Targeted therapy against specific genetic aberrations
- Disablement of command, control and weapons
  - Immune modulation
Inflamed tumour
Patterns of immune cell infiltration

immune desert

immune excluded

immune infiltrated
Immune cell populations and prognosis

T-cell populations

1. CD4 T Cells
   - High expression, n = 251
   - Low expression, n = 82
   - Disease-Specific Survival
   - Time (months)
   - P < 0.001

2. CD8 T Cells
   - High expression, n = 61
   - Low expression, n = 268
   - Disease-Specific Survival
   - Time (months)
   - P = 0.002

3. CD56+ NK cells
   - High expression, n = 37
   - Low expression, n = 295
   - Disease-Specific Survival
   - Time (months)
   - P = 0.014

4. Regulatory T cells
   - Foxp3+ cells ≥3, n = 51
   - Foxp3+ cells <3, n = 49
   - Recurrence-Free Survival
   - Time (months)
   - P = 0.004

References:
T-cell responses are regulated by a balance of activating and inhibitory ‘checkpoint’ signals.

Neoplastic cells protect themselves from immune attack by dysregulating these checkpoints.

Therapeutic targeting of these checkpoints restores the immune response and promote destruction of the neoplastic cells.
Programmed death-ligand 1 (PD-L1; CD274; B7 homologue (H)1) is a transmembrane protein expressed on immune, vascular endothelial and epithelial cells.

Programmed death-1 (PD-1) is its receptor, expressed on immune cells, especially activated T lymphocytes.

Binding of the two inhibits proliferation of CD8+ T lymphocytes, effectively halting their destructive potential.
PD-L1 expression and prognosis in NSCLC

Correlates of PD-L1 expression

- Immune cell infiltration
- Squamous differentiation
- Mutational burden (>200)
- Smoking history
- Previous irradiation
- Previous chemotherapy
The requirements
The requirements

- Extent and/or strength of expression of PD-L1 by tumour cells (TCs) and/or infiltrating immune cells (ICs) should relate to, and therefore predict, sensitivity of the tumour to anti-PD-1 or PD-L1 immune modulators
- Technique should be robust, with demonstration of PD-L1 expression consistent and reproducible between laboratories
- Interpretation should be consistent, with assessment of PD-L1 expression objective and reproducible between observers
The problems
# Diagnostic tests: anti-PD-L1 antibodies

<table>
<thead>
<tr>
<th></th>
<th>Pembrolizumab Merck, Sharp &amp; Dohme</th>
<th>Nivolumab Bristol-Myers Squibb</th>
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<tr>
<td><strong>TARGET</strong></td>
<td>PD-1</td>
<td>PD-1</td>
<td>PD-L1</td>
<td>PD-L1</td>
</tr>
<tr>
<td><strong>DETECTION</strong></td>
<td>Dako 22C3</td>
<td>Dako 28-8</td>
<td>Roche SP142</td>
<td>Roche SP263</td>
</tr>
<tr>
<td><strong>RELEVANT EXPRESSION</strong></td>
<td>Surface of tumour cells</td>
<td>Surface of tumour cells</td>
<td>Surface of tumour cells and immune cells</td>
<td>Surface of tumour cells</td>
</tr>
<tr>
<td><strong>CRITERIA FOR ‘POSITIVITY’</strong></td>
<td>≥ 1% or ≥ 50% expression</td>
<td>≥1% expression</td>
<td>TC expression 0-3: &lt;1, 1-4, 5-49, ≥50; % of tumour infiltrated by PD-L1+ve ICs 0-3: &lt;1, 1-4, 5-9, ≥10</td>
<td>≥25% expression</td>
</tr>
</tbody>
</table>
Inter-observer concordance comparable for all tests, but higher when assessing TC expression (~90%; kappa ~0.5) than when assessing IC expression (~20%; kappa ~0.2)

Dako 22C3 and 28-8 closely similar: strong expression on TCs, but delineate few ICs

Ventana SP142: strong expression on ICs, but delineate few TCs

Ventana SP263: strong expression on both TCs and ICs

- Scheel HA et al., Modern Pathology 2016, 1-8; Gaule et al., ASCO 2016, abstract 9040; Gatalica et al., ASCO 2016, abstract 11548
# Diagnostic tests: anti–PD–L1 diagnostics

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<td>Roche SP263</td>
</tr>
<tr>
<td><strong>Relevant</strong></td>
<td>Surface of tumour cells</td>
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<td>Surface of tumour cells and immune cells</td>
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Interpretation
Kim et al., AACR 2015, abstract 570

- Expression of PD-L1 assessed using Dako 22C3 antibody in paired tumour specimens from 90 patients with resected, recurrent NSCLC
- Median interval between first and second specimens 21 months, 97% and 53% respectively pulmonary, 89% and 63% surgical
- PD-L1 expression identical in 39%, higher in 32% and lower in 29%; 20% of positive specimens had ‘become’ negative; 12% of negative cases had ‘become’ positive
Spatial heterogeneity

Variance predominantly between fields of view (mm scale) rather than between blocks (cm scale): Gaule *et al.* ASCO 2016, abstract 9040
Spatial heterogeneity
Spatial heterogeneity

DAKO 22C3
Why bother with a diagnostic test?
Why bother with a diagnostic test?
Is PD-L1 the best biomarker?
The inter-relationships between the different anti-PD-L1 diagnostic tests are becoming clearer and important similarities and differences are emerging.

Expression of PD-L1 by tumour cells is more easily assessed and reproducible between observers than is expression by immune cells.

Though essentially pragmatic and despite their limitations, the current companion diagnostics are effective if applied and interpreted with care by those experienced in the pathology of pulmonary tumours.

Alternative biomarkers predicting sensitivity of NSCLCs to anti-PD1/PD-L1 immune modulators might emerge in the near future, but none is imminent.
Questions!