Colorectal Tumour Genomic Instability
DNA Mismatch Repair (MMR) pathway
Sporadic Colorectal Cancer - defective MMR (dMMR)
Lynch Syndrome (>3% all CRCs)
Lynch CRC Precursors
Colorectal tumour classification & genomic instability

Genomic instability:

- **(1) Chromosomal instability (CIN, ~85%)**
  - Changes in chromosome number & structure (aneuploidy)
- **(2) Microsatellite instability (MSI or MIN, ~15%)**
  - Change in length of repetitive sequences (“microsatellites”) e.g. –AAAAAA- or –CACACACA–
  - (CA)$_4$ change to (CA)$_3$ or (CA)$_5$ - instability
  - Due to abnormal DNA mismatch repair (see later)
  - Often “near-diploid” & Sporadic CRC – MLH1 promoter methylation
Chromosomal Instability (Aneuploidy) in a Colorectal Cancer

SW403: an MSS colorectal cancer showing near-triploid pattern & unbalanced translocations
Microsatellite Instability in a Colorectal Cancer

HCT116: MSI+ colon cancer cell line near-diploid with 2 unbalanced translocations (8;16, 17;18)
DNA Mismatch Repair Pathway


DNA Mismatch Repair Pathway

Base mismatch

DNA replication error

DNA Mismatch Repair Pathway

Repetitive sequence – slippage during replication

Insertion/deletion loop (InDel)
DNA Mismatch Repair Pathway

\[ \text{mismatch} \]
DNA Mismatch Repair Pathway

MutS complex
**DNA Mismatch Repair Pathway**

Mismatch: **MSH2** / **MSH6**

1bp InDel: **MSH2** / **MSH6**

2-6bp InDel: **MSH2** / **MSH3**
DNA Mismatch Repair Pathway


MSH2  MSH6  MLH1  PMS2

MutL complex
DNA Mismatch Repair Pathway


MSH2

MSH6

MLH1

PMS2

ADP
DNA Mismatch Repair Pathway

Excision, resynthesis, ligation

Repair
Defective DNA Mismatch Repair Microsatellite Instability (15%)

Serrated Pathway:
Hyperplastic Polyp – serrated adenoma - cancer
Dr Henry Lynch : 1966

- 1966: Lynch: “Cancer family syndrome”
- Hereditary Non-Polyposis Colorectal Cancer Syndrome
Lynch syndrome

- Autosomal Dominant – inherited mutations
- De novo mutations
- Partially penetrant, variably expressed, sex limited and phenocopied
- High risk of cancer: starts 20s – 40s
- Defined by / Due to constitutional (“germline”) pathogenic mutations affecting a DNA mismatch repair (MMR) gene
  
  - MSH2, MLH1, MSH6, PMS2
  
  - (EPCAM, LRRFIP2)

- Risks vary with MMR gene, sex, site, age, environment …
- Prevalence of 1 in ~120 (>3% all CRCs)

Biallelic / recessive (two mutations in the same gene) = Constitutional MisMatch Repair-Disorder (CMMR-D)

https://www.insight-group.org/syndromes/
LS risks

- **Principal**: colon & rectum
- **Major**: endometrium (lower segment) (Cx?)
- **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
- **Sarcoma (?)** ...

Prospective Lynch Syndrome Database (PLSD)

`path_MLH1` risks

https://ehtg.org/collaborative-studies/plsd/
path_MSH2 & path_MSH6 risks

![Graphs showing cumulative risk of cancer for path_MSH2 and path_MSH6 across different age groups for both genders combined and endometrial cancer in females.](Image)
**path_PMS2 risks**

- **Any - both genders combined**
  - **path_PMS2**

- **Any cancer type - male**
  - **path_PMS2**

- **Any cancer type - female**
  - **path_PMS2**

- **Endometrial cancer - female**
  - **path_PMS2**
Relative cumulative incidence (RR) of cancer at 75 y by gene.

<table>
<thead>
<tr>
<th>Organ</th>
<th>Population incidence</th>
<th>MLH1</th>
<th>MSH2</th>
<th>MSH6</th>
<th>PMS2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Small bowel</td>
<td>0.1%</td>
<td>64.7</td>
<td>20.1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Endometrium</td>
<td>1.6%</td>
<td>26.7</td>
<td>35.5</td>
<td>28.9</td>
<td>16.5</td>
</tr>
<tr>
<td>Colon</td>
<td>2.1%</td>
<td>22.3</td>
<td>20.2</td>
<td>6.8</td>
<td>0</td>
</tr>
<tr>
<td>Bile duct and gall bladder</td>
<td>0.2%</td>
<td>18.7</td>
<td>8.6</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Ovary</td>
<td>1.0%</td>
<td>10.1</td>
<td>16.9</td>
<td>13.1</td>
<td>0</td>
</tr>
<tr>
<td>Ureter and kidney</td>
<td>1.3%</td>
<td>3.5</td>
<td>13.7</td>
<td>2.3</td>
<td>0</td>
</tr>
<tr>
<td>Sigmoid and rectum</td>
<td>1.4%</td>
<td>8.4</td>
<td>13</td>
<td>3.3</td>
<td>0</td>
</tr>
<tr>
<td>Brain</td>
<td>0.5%</td>
<td>1.9</td>
<td>10.5</td>
<td>2.9</td>
<td>0</td>
</tr>
<tr>
<td>Stomach</td>
<td>0.8%</td>
<td>8.9</td>
<td>9.7</td>
<td>6.6</td>
<td>0</td>
</tr>
<tr>
<td>Urinary bladder</td>
<td>1.0%</td>
<td>4.1</td>
<td>8.1</td>
<td>8.2</td>
<td>0</td>
</tr>
<tr>
<td>Pancreas</td>
<td>0.8%</td>
<td>7.3</td>
<td>0.6</td>
<td>1.8</td>
<td>0</td>
</tr>
<tr>
<td>Prostate</td>
<td>10.0%</td>
<td>1.7</td>
<td>3.2</td>
<td>1.8</td>
<td>3.8</td>
</tr>
<tr>
<td>Breast</td>
<td>9.4%</td>
<td>1.3</td>
<td>1.2</td>
<td>1.4</td>
<td>6</td>
</tr>
</tbody>
</table>

Significantly increased (p<0.05) RRs in bold. Maximum RR by gene underlined. 95% CIs omitted for clarity.

ALL colorectal cancers in the UK must be tested for LS.

Savings in avoidable cancers, in the surgical and oncology budgets, pay for the costs in pathology, genetics and endoscopy ...
Lynch Syndrome: Diagnostic Tests

- Family history
  - Not bad, but poor sensitivity and specificity
- Constitutional mutation testing
  - Defines LS
- Tests on tumours:
- Microsatellite instability (MSI)
  - optimised for *colonic* cancers
  - Technique fairly well standardized (NEQAS molec)
  - Adenomas and Ca at other sites: reduced sensitivity
  - MSH6 and PMS2 tumours: reduced sensitivity

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

- *BRAF* V600E – in 80-90% sporadic CRC (or *MLH1* promoter methylation – in sporadic Endometrial Cancer)
MMR Immunohistochemistry

MSH2  Mucinous colon cancer  MLH1

Compare adjacent tumour & normal/stroma – beware poor fixation

Loss of MLH1 protein expression
MMR Immunohistochemistry

Loss of MSH2 protein expression
Endometrioid Carcinoma: MMR Immunohistochemistry

MSH2 & MSH6 heterodimeric binding partners
Caecal Carcinoma: MMR Immunohistochemistry

Loss/weak expression of both MLH1 & PMS2 (binding partners)
MMR Immunohistochemistry

Late loss of MMR at adenoma-cancer transition
Colonic Adenoma: MMR Immunohistochemistry

Early loss of MMR in adenoma
Endometrial Cancer MMR IHC – Clonal Loss Pattern

MSH2

Clonal Loss MSH2 / MSH 6

MSH6

MSH2 & MSH6 heterodimeric binding partners

MLH1

PMS2
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
  – ~2-5% patchy / weak expression
  – ~2-5% CRC due to Lynch Syndrome show normal MMR IHC expression or clonal loss pattern
  – Must take part in NEQAS (Alimentary Tract - HNPCC/MMR module – started 2007) for quality control

• Other tests useful – MSI & BRAF
  – MSI to confirm dMMR
  – *BRAF* to distinguish Lynch v Sporadic CRC (for *MLH1*-neg CRC)
  – Use *MLH1* Promoter Methylation for Endometrial cancers Lynch v Sporadic
  – Must take part in NEQAS (MSI Mol Gen module) for quality control
Microsatellite Instability (MSI)

Normal (black)

Tumour (red & blue)

Frayling, Arends et al. (2005) Application of Molecular Diagnostics to Hereditary Non-Polyposis Colorectal Cancer, in Molecular Diagnostics For the Clinical Laboratorian, Eds. Coleman, W.B., Tsongalis, G.J.  Humana Press, Totowa, NJ.
MMR deficiency in normal intestinal mucosa

Courtesy: Dr Matthias Kloor

Possible precursors of CRC in Lynch syndrome

**precursor: adenoma**

- APC mutation -> MMR inactivation

**precursor: MMR-deficient crypt focus**

- MMR inactivation -> B-Catenin mutation
  - (flat)   (immediate invasion)

*blue: MMR-deficient cell clones*
Suggested model of Lynch syndrome colonic carcinogenesis

Courtesy: Dr Matthias Kloor
Immunotherapy for dMMR Cancers

Conclusions

- Mutant mismatch repair genes **MLH1 & MSH2** cause most Lynch Syndrome: investigated by a combined approach:
  - Family History, Immunohistochemistry, Microsatellite Instability -> Mutation detection
  - allows diagnosis/exclusion of Lynch Syndrome, directs mutation detection to specific gene, permits interpretation of mis-sense DNA variants (pathogenic mutations vs polymorphisms)
  - Systematic testing of CRC up to age 70 is cost-effective – UK introduced testing of CRC <50y (<60y Scotland), now working up to testing ALL colorectal cancers (NICE)
  - Manchester Consensus Meeting - systematic testing of ALL endometrial cancers in UK (published 2019 in Genetics In Medicine)
  - Gynae cancers – sentinel events for Lynch Syndrome – screening opportunity
  - LS screening – cancer surveillance programmes, preventative measures, cascade testing of at-risk relatives, influence treatment options (immune checkpoint blockade)
  - Lynch colonic precursors can be **polypoid** (APC mutation early, MMR loss late), or **flat** (MMR loss early, B-Catenin mutation late)
WHO Classification of Tumours ONLINE

Now available at: tumourclassification.iarc.who.int

Access to the following books:

5th edition
- Digestive Tumours
- Breast Tumours

4th edition
- Skin Tumours
- Endocrine Tumours
- Eye Tumours
- Head and Neck Tumours

Special launch rate of 100 Euros

More details from the IARC team – Booth A14, 2nd level, Agora 2 Hall
9am to 5.15pm, from Sunday 8 to Tuesday 10 September
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