Lynch syndrome: immunology and immunotherapy

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Chairman, Department of Clinical Genetics
Fox Chase Cancer Center
Philadelphia PA
Saturday April 18th 10:15-10:45 AM
• Immune system, MSI, and hereditary mismatch repair deficiency (Lynch Syndrome)

• Current immune-directed therapies in MSI-H tumors and in Lynch Syndrome-associated tumors

• Immuno-prevention in Lynch Syndrome
• **Mismatch repair deficiency (dMMR)**
  
  – A component of the MMR gene family (MLH1, MSH2, MSH6, PMS2, EPCAM) is not working appropriately, hampering normal MMR

• **MSI or MSI-H**
  
  – A result of dMMR, where multiple mono- and di-nucleotide repeat regions of the DNA in coding and non-coding regions of the DNA demonstrate expansions or contractions
Immunoreactivity and dMMR

Revised Bethesda Guidelines
--Published 2004
--Focused on MSI-H tumors
--Tumor infiltrating lymphocytes (TILs)
--Crohn’s-like lymphocytic reaction
--Expansive, cohesive borders

Colon Cancer Family Registry
--1098 colorectal tumors
--MSPath Predictor
  --TILs          OR 9.1 (5.9-14.1)
  --Proximal     OR 4.7 (3.1-7.3)
  --Mucinous     OR 2.8 (1.7-4.8)

Mismatch repair deficiency (dMMR)

Somatic (sdMMR)
- **MLH1-promoter methylation**
  - 12-17% colorectal tumors
  - Diploid tumors (75%), CIMP
  - Bi-allelic MLH1 promoter methylation
  - BRAF V600E mutations
  - Serrated adenomas
  - Rare!!! hereditary inheritance
- **Bi-allelic MMR inactivation**
  - Bi-allelic MMR mutations
  - Mutation + LOH

Hereditary (hdMMR)
- **Lynch syndrome (LS)**
  - Autosomal dominant
  - Mutations in MLH1, MSH2, MSH6, PMS2, EPCAM
  - 1/279 prevalence
  - De novo mutations are rare
  - CMMRD—recessive (PMS2)
- **Non-Lynch hdMMR**
  - Biallelic MUTYH
    - RARE cause of MSI

Germline or somatic POL mutations

Kane MF et al. Methylation of the hMLH1 promoter correlates with lack of expression of hMLH1 in sporadic colon tumors and mismatch repair defective human tumor cells lines. Cancer Research 1779;57:808
• Immune system, MSI, and hereditary mismatch repair deficiency (Lynch Syndrome)

• Current immune-directed therapies in MSI-H tumors and in Lynch Syndrome-associated tumors

• Immuno-prevention in Lynch Syndrome
Impact of dMMR

• **#1: Decreased repair of DNA base mis-pairings and insertion-deletion loops due to DNA polymerase “slippage” in microsatellites during S–phase**
  - Increased base pair insertions and deletions and point mutations
  - Increased total mutational load in tumor (TMB)—quantification
  - Frameshift peptides and epitopes (quantification vs quality) and protein misfolding

• **#2: Inactivation of genes relevant in neoplastic transformation of cells**
  - Multi-pathway inactivation leading to neoplasia
  - Increased genomic instability

• **#3: Mutations modulate responsiveness of tumors to immune system surveillance and to current immunotherapies**
  - Other mutations that increase response to immunotherapy (PBRM1)
  - Mutations that decrease the response to immunotherapy
dMMR leads to frameshifts in DNA and increased TMB

Genomic impact of dMMR is widespread and disease specific

– Mutational Signature 6


www.seltarbase.org
...but NOT random

Coding microsatellites experience less MSI in the setting of dMMR


dMMR driven MSI is heterogeneous by target, anatomy, and gene affected

Pinheiro M et al. Target gene mutational pattern in Lynch syndrome CRC according to tumor location and germline mutation. BJC 2015;113:686
TMB predicts immune response to anti-PD-1/L1 therapy

Both quality and quantity are important

Frameshifts cause protein mis-folding and generate neo-antigens

- Small indels in coding microsatellites lead to frame-shift peptides (FSP) and protein folding issues
- FSP presented on MHC Class 1 molecules and drive adaptive immune response
- Inflammatory microenvironment rich in prostaglandins and cytokines

Willis JA et al. Immune activation in Mismatch-repair deficient carcinogenesis: More than just mutation rare
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  – Multi-pathway inactivation contributing to neoplasia
  – Increased genomic instability with loss of MMR

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  – Mutations that decrease the response to immunotherapy
dMMR inactivates genes relevant in neoplasia

Table 2. Mutational frequency of the target gene microsatellite sequences according to tumour location in the Lynch syndrome MSI-H test (Porto series)

<table>
<thead>
<tr>
<th>Gene</th>
<th>Total (%)</th>
<th>Proximal colon (%)</th>
<th>Distal colon (%)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACVR2A</td>
<td>70/77 (90.9)</td>
<td>46/51 (90.2)</td>
<td>24/26 (92.3)</td>
<td>1</td>
</tr>
<tr>
<td>TGFB2</td>
<td>69/77 (89.6)</td>
<td>46/51 (90.2)</td>
<td>23/26 (88.5)</td>
<td>1</td>
</tr>
<tr>
<td>EGFR</td>
<td>68/76a (88.3)</td>
<td>44/50 (88.0)</td>
<td>24/26 (92.3)</td>
<td>0.708</td>
</tr>
<tr>
<td>BMPR2A11</td>
<td>57/76* (75.0)</td>
<td>36/50 (72.0)</td>
<td>21/26 (80.8)</td>
<td>0.578</td>
</tr>
<tr>
<td>E2F4</td>
<td>40/75b (53.3)</td>
<td>30/50 (60.0)</td>
<td>10/25 (40.0)</td>
<td>0.141</td>
</tr>
<tr>
<td>MSH3</td>
<td>38/77 (49.4)</td>
<td>20/51 (39.2)</td>
<td>18/26 (69.2)</td>
<td>0.017</td>
</tr>
<tr>
<td>BAX</td>
<td>34/77 (44.2)</td>
<td>21/51 (41.2)</td>
<td>13/26 (50.0)</td>
<td>0.478</td>
</tr>
<tr>
<td>TCF7L2</td>
<td>32/77 (41.6)</td>
<td>19/51 (37.3)</td>
<td>13/26 (50.0)</td>
<td>0.333</td>
</tr>
<tr>
<td>BMPR2A7</td>
<td>27/77 (35.1)</td>
<td>13/51 (25.5)</td>
<td>14/26 (53.8)</td>
<td>0.022</td>
</tr>
<tr>
<td>PRDM2</td>
<td>22/77 (28.6)</td>
<td>15/51 (29.4)</td>
<td>7/26 (26.9)</td>
<td>1</td>
</tr>
<tr>
<td>MSH6</td>
<td>19/77 (24.7)</td>
<td>10/51 (19.6)</td>
<td>9/26 (34.6)</td>
<td>0.170</td>
</tr>
<tr>
<td>IGF2R</td>
<td>16/77 (20.8)</td>
<td>9/51 (17.6)</td>
<td>7/26 (26.9)</td>
<td>0.382</td>
</tr>
<tr>
<td>B2M</td>
<td>7/77 (9.1)</td>
<td>6/51 (11.8)</td>
<td>1/26 (3.8)</td>
<td>0.412</td>
</tr>
<tr>
<td>APC</td>
<td>6/77 (7.8)</td>
<td>6/51 (11.8)</td>
<td>0/26 (0.0)</td>
<td>0.091</td>
</tr>
<tr>
<td>PTEN</td>
<td>6/77 (7.8)</td>
<td>0/51 (0.0)</td>
<td>6/26 (23.1)</td>
<td>0.001</td>
</tr>
<tr>
<td>AXIN2</td>
<td>3/77 (3.9)</td>
<td>1/51 (2.0)</td>
<td>2/26 (7.7)</td>
<td>0.262</td>
</tr>
</tbody>
</table>

Abbreviations: CRC = colorectal carcinoma; MSI = microsatellite instability. We did not detect mutations in BMPR1A microsatellite sequences. P<0.05 are indicated in bold.
aOne proximal CRC case was not analysed for this gene.
bTwo cases (one proximal and one distal CRC) were not analysed for this gene.

Inactivated by FS
- TGF-B superfamily
- MMR
- BCL2 pathway
- WNT family
- Tumor suppressors

Genes upregulated in LS tumors
- Antigen presentation
- Apoptosis
- NK cell cytotoxicity
- T cell activation

Pinheiro M et al. Target gene mutational pattern in Lynch syndrome CRC according to tumor location and germline mutation. BJC 2015;113:686
DNA repair domino effect

Kobayashi H et al. Oncology Reports 2013
Impact of dMMR

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  – Increased base pair insertions and deletions
  – Increased total mutational load in tumor (TMB)—quantification
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• #2: Inactivation of genes relevant in neoplastic transformation of cells
  – Multi-pathway inactivation contributing to neoplasia
  – Increased overall genomic instability

• #3: Mutations modulate responsiveness of tumors to immune system surveillance and to current immunotherapies
  – Other mutations that increase response to immunotherapy (PBRM1)
  – Mutations that decrease the response to immunotherapy
dMMR modulates immune system interactions with tumors through antigen presentation

**MHC CLASS I**

- **B2M mutations**
  - 30% dMMR tumors have B2M mutations
  - B2M contains 4 coding microsatellites
  - Frameshifts lead to lack of HLA-Class 1 on tumor
  - Insensitivity to CD8+ T-cell recognition killing
  - MORE frequent in LS
- **TAP1 and TAP2**
  - These mutations can inactivate transport of antigens for presentation on MHC Class 1
  - LESS common in LS

**MHC Class II**

- **RFX5 and CIITA mutations**
  - lead to lack of MHC Class II expression; often in tumors with CD4+ T-cell infiltrate

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Willis JA et al. Immune activation in Mismatch-repair deficient carcinogenesis: More than just mutation rare
dMMR modulates immune system interactions

**dMMR tumors show higher expression of PD-1 and PD-L1**

**PD-1/PD-L1 downregulate T-cells and induce T-cell exhaustion**

**Lynch syndrome tumors**

-- Upregulation of genes
  - Antigen processing/presentation
  - Apoptosis
  - NK cell mediated cytotoxicity
  - T-cell activation

-- Loss of B2M
-- Upregulation of PD-L1

Llosa NJ et al. The vigorous immune microenvironment of MSI CRC is balanced by counter-inhibitory checkpoints. Cancer Discov 2014;5
Walkowska J et al. Immunoprofiles of CRC from LS. Oncoimmunology 2019;8(1)
Outline

• Immune system, MSI, and hereditary mismatch repair deficiency (Lynch Syndrome)

• Current immune-directed therapies in MSI-H tumors and in Lynch Syndrome-associated tumors

• Immuno-prevention in Lynch Syndrome
Anti-PD-1/L1 therapies are effective in treatment of dMMR CRC and non-CRC

Responses begin early and deepen over time

Even pancreatic cancers showed substantial responses to ICB

No clear trial evidence hdMMR (Lynch) and sdMMR respond differently

Le DT et al. Science 28(357): Aug 2017
Dual checkpoint blockade improves DCR but not CR rate

<table>
<thead>
<tr>
<th>Metastatic MSI-H CRC treated with nivolumab</th>
<th>Metastatic MSI-H CRC treated with nivolumab and ipilimumab</th>
</tr>
</thead>
<tbody>
<tr>
<td>• DCR 64% at 12 wks</td>
<td>• DCR 80% at 12 wks</td>
</tr>
<tr>
<td>– 3% CR</td>
<td>– 3% CR</td>
</tr>
<tr>
<td>– 30% PR</td>
<td>– 51% PR</td>
</tr>
<tr>
<td>– 34% SD</td>
<td>– 31% SD</td>
</tr>
<tr>
<td>• Toxicity</td>
<td>• Toxicity</td>
</tr>
<tr>
<td>– 70% drug-related AEs</td>
<td>– 73% drug-related AEs</td>
</tr>
</tbody>
</table>

Overman MJ et al Lancet 2017, JCO 2018
### Table 2: HLA, APM and β2m expression in RST and HNPCC colon cancer

<table>
<thead>
<tr>
<th>Variable</th>
<th>Lynch syndrome (N = 141)**</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD3</td>
<td>HR</td>
</tr>
<tr>
<td>Low vs. high</td>
<td>3.4</td>
</tr>
<tr>
<td>CD8</td>
<td></td>
</tr>
<tr>
<td>Low vs. high</td>
<td>2.0</td>
</tr>
<tr>
<td>CD68</td>
<td></td>
</tr>
<tr>
<td>Intermediate vs. high</td>
<td>3.4</td>
</tr>
<tr>
<td>Low vs. high</td>
<td>6.5</td>
</tr>
<tr>
<td>B2M</td>
<td></td>
</tr>
<tr>
<td>Normal vs. loss</td>
<td>3.5</td>
</tr>
</tbody>
</table>

### Above: LS associated tumors versus sporadic MSI-H tumors:
- Less loss of MHC 1 expression
- Less APM loss (TAP1/2, etc)
- More B2M mutations that deactivate MHC 1 antigen presentation

### Left: Predictors of survival in LS treated with immunotherapy
- High CD3+ and CD8+ T cells
- High CD68+ T cells
- Loss of B2M

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Walkowska J et al. Immunoprofiles of CRC from LS. Oncoimmunology 2019;8(1)
B2M mutations occur more commonly in LS-associated dMMR tumors.

PD-L1 expression is generally correlated with better OS to anti-PD-L1 therapy.

Dierssen JW et al. HNPCC versus sporadic MSI 2007 BMC Cancer 7;33
Walkowska J et al. Immunoprofiles of CRC from LS. Oncoimmunology 2019;8(1)
# TRIALS TO WATCH

<table>
<thead>
<tr>
<th>NAME</th>
<th>PHASE</th>
<th>AGENTS</th>
<th>STATUS</th>
</tr>
</thead>
<tbody>
<tr>
<td>FRONT-LINE METASTATIC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Keynote-177</td>
<td>III</td>
<td>Pembrolizumab</td>
<td>Accrual complete</td>
</tr>
<tr>
<td>Checkmate 8HW</td>
<td>III</td>
<td>Nivo or Nivo/Ipi or other</td>
<td>Accruing</td>
</tr>
<tr>
<td>NOUS-209</td>
<td>I</td>
<td>NOUS-209+Pembrolizumab</td>
<td>Accruing</td>
</tr>
<tr>
<td>ADJUVANT</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ATOMIC</td>
<td>Adjuvant</td>
<td>FOLFOX+Atezolizumab</td>
<td>Accruing</td>
</tr>
</tbody>
</table>
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• Immune system, MSI, and hereditary mismatch repair deficiency (Lynch Syndrome)

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Cancer free LS patients have detectable FSP and T-cell reactivity

- Unaffected MMR germline mutation carriers make FSP
- Autoimmunization
- FSP reactive T cells are found in peripheral blood of LS patients with and without cancer and in tumors

dMMR in pre-neoplastic lesions


Only a fraction of MMR deficient crypts become cancer—importance of immune editing!!!
Progression of LS crypts to cancer

**LS CRYPTS:** Early MSI, some MSS

**LS POLYPS:** 50% MSI (Early vs New) 50% MSS

40% APC, 50% RNF43, DNA repair, MHC/B2M

**LS CANCER:** 100% MSI (Early MSI vs Newer MSI vs Late MSI)

Immune activation and editing

Autoimmunization
Aspirin and CRC

• Aspirin and COX-1/2 inhibitors have preventive benefit in CRC
  – Prevents adenomas
  – Reduces CRC incidence and morality
  – Reduce polyp number and size in FAP

• Aspirin improves survival in CRC, but efficacy is modulated by the immune checkpoint pathway
  – PD-L1 expression dampens ASA benefits

Hamada T. et al ASA use and CRC survival according to tumor CD274 (PD-L1) expression status. JCO 2017;35(16): 1836
Aspirin and Resistant Starch: CAPP2 consortium

Colorectal cancer

Other Lynch cancers

ABOVE: Resistant starch decreased crypt length

LEFT: Among those who were able to take ASPIRIN continuously for 2 years, ASPIRIN decreased the risk of CRC and other LS cancers


CAPP3: A randomised double blind dose non-inferiority trial of a daily dose of 600mg versus 300mg versus 100mg of enteric coated aspirin as a cancer preventive in carriers of a germline pathological mismatch repair gene defect, Lynch Syndrome. Project 3 in the Cancer Prevention Programme (CaPP3).

PI: Dr. John Burn

ClinicalTrials.gov Identifier: NCT02497820

**Primary outcome:** The number of new primary mismatch repair deficient cancers at 5 years and beyond among participants who remain on prescribed treatment for a minimum of 2 years.

**Study population/size:** 3,000 patients
Vaccine therapies

Frame shift vaccine for cancer prevention in Lynch syndrome

Kloor, von Knebel Doeberitz (Heidelberg)

Vaccine directed to 3 FSP:

Safety study completed in healthy population and in patients with MSI-H CRC.

Frame shift vaccine to enhance the efficacy of anti-PD-1/L1 therapy

NOUS-209-01 trial (NCT04041310)

First in human, “off the shelf” vaccine

MSI-H gastric, gastro-esophageal, and CRC + pembrolizumab

Goal: induce CD8+/CD4+ responses

TAFB(-1): NTQIKALMRGLKKTILKKAGIGMCVKVSSIFFINKKQP
AIM2(-1): HSTIKVIKAKKHREVKRTNSSLV
ASTE(-1): EIFLPKGRSNSKKGGRRNRIAPAVRTEGEPLHPSV

Willis JA et al. Immune activation in Mismatch-repair deficient carcinogenesis: More than just mutation rare (refs)
Differential response to immunotherapy associated with microbiome

F. nucleatum is an invasive anaerobe human gut bacterium
   --enriched in tumor tissue
   --persists in metastatic lesions
   --impairs T-cell mediated anti-tumor response
   --promotes tumor cell proliferation

Hamada T et al (CIR 2018)
NHS/HPFS sample (n=1041 out of 170,000+)
F. nucleatum+ vs - in MSI-H tumors a/with:
   -Fewer TILs
     31% vs 41%, p=0.05
   -Intratumor periglandular reaction
     42% vs 26%, p=0.002

Thank you