Molecular Aspects of Colorectal Cancer for the Practicing Surgical Pathologist

Murray Resnick MD PhD
Vice Chief of Pathology, Director Anatomical Pathology
Rhode Island and The Miriam Hospital
Professor of Pathology
Alpert School of Medicine, Brown University
KEY TOPICS

• Importance of Differentiating Microsatellite Stable from Instable Tumors Emphasizing IHC Methods
• Prognostic/Predictive Biomarkers in CRC Emphasizing MSI
• Next Generation Sequencing and Liquid Biopsy
Chromosomal Instability (CIN)  
Microsatellite Stable (MSS)

- Sporadic (85%)
- FAP (<1%)

Acquired

- APC, p53  
- DCC, KRAS  
- Others

Germline

- APC

Microsatellite Instability (MSI)

- Sporadic (12-15%)
- Lynch Syndrome (3-4%)

Acquired

- BRAF V600E  
- MLH1 methylation

Germline

- MMR
  - (MLH1, PMS2  
  - MSH2, MSH6  
  - EPCAM)
Why is MSI Colon Cancer Important?

- Identification of Lynch syndrome patients
- MSI status has prognostic significance and predictive significance for therapy
- MSI status is central to many of the molecular classification systems of CRC

- Pathologists often are first to identify MSI and are key in communicating forward
Lynch Syndrome (HNPCC)

- Most common hereditary CRC syndrome (3-4% of all CRC)
- Autosomal dominant
- Extracolonic (endometrium, ovary, renal pelvis, stomach, others) cancers common
- Colonic screening leads to decreased CRC and death!!!
- Screening of relatives critical
- Difficult to recognize clinically (family history not always obvious or available)
- Lynch CRCs arise from traditional adenomas
Sporadic MSI Tumors

- 10-15% of all colon cancer
- Phenotypically similar to LS tumors
- Right sided, older females
- Commonly arise from sessile serrated adenomas
- MLH1 deficient with wild type BRAF and MLH1 promoter hypermethylation arise from traditional adenomas

(Farchoukh et al AJSP 2016)
Morphological Aspects of MSI Tumors

• **Gross**
  - Primarily right colon, bulky, less likely to have nodal or distal metastases

• **Micro**
  - Increased TILs, Crohn’s like reaction
  - Poorly differentiated (*High grade*), medullary pattern
  - Signet ring and mucinous differentiation
  - Histologic heterogeneity

**MSI**

- Microsatellites are short nucleotide repeat sequences prone to replication errors by DNA polymerase.
- Slippage causes insertion-deletion loops.

### Microsatellite Replication

<table>
<thead>
<tr>
<th>Normal</th>
<th>Abnormal (Mismatch repair defect)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GCACACACACACCT CGTGCTGTGGAGA</td>
<td>GCACACACCT CGTGCTGTGGAGA</td>
</tr>
<tr>
<td>GCACACACACACCT CGTGCTGTGGAGA</td>
<td>C A A C ACCT</td>
</tr>
<tr>
<td>GCACACACACACCT CGTGCTGTGGAGA</td>
<td>Variable allele size</td>
</tr>
</tbody>
</table>

Adapted from Gruber SB, Kohlmann W. The genetics of hereditary nonpolyposis colorectal cancer. J Natl Comp Cancer Net. 2003;1:137-44.

- Mismatch repair (MMR) proteins correct this.
- Errors result in varying lengths of these sequences.
- If not corrected, second round of replication incorporates mutation → frameshift mutations → non functional protein.
MSI is defined by the presence of new bands of tumor DNA not present in PCR components of normal DNA.
Typically analyze 5 microsatellite markers:

- **MSI-H** (2 or more identified by PCR) - dMMR
- **MSI-L** (1 identified by PCR) - pMMR
- **MSS** (0 identified by PCR) - pMMR
MMR Proteins

- Two complexes MLH1/PMS2 and MSH2/MSH6
- Stability of PMS2 and MSH6 depends upon these complexes
- Loss of MLH1 leads to loss of PMS2, loss of MSH2 leads to loss of MSH6
- MLH1 and MSH2 are stable without complex (isolated loss of PMS2 or MSH6)
Universal MSI Testing of CRCs

- **Universal testing all CRCs** (Consensus Statement of US Multi-Society Task Force on CRC, EGAPP and other organizations)

- **Universal testing all CRCs <70 and testing of >70 who meet Bethesda Guidelines** slightly more cost effective option (35% fewer MMR tests than universal testing) (NCCN)

- **Both are cost effective and have equal sensitivities for identifying LS**

- **Either MSI PCR or IHC MMR is valid**
Principles of Lynch Syndrome/MSI Initial Screen

• **Is tumor mismatch repair (MMR) deficient?**
  - Directly by IHC of MMR proteins
  - Indirectly by measuring MSI status by PCR
  - Directly/Indirectly using Next Generation Sequencing

• **Is MMR deficiency indicative of LS or is this sporadic?**
## IHC vs MSI PCR Testing

<table>
<thead>
<tr>
<th>IHC</th>
<th>MSI-PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Relatively cheap</td>
<td>• More expensive</td>
</tr>
<tr>
<td>• Rapid TAT</td>
<td>• 2-5 days</td>
</tr>
<tr>
<td>• Small amount of tumor and normal tissue</td>
<td>• Need at least 40% tumor tissue</td>
</tr>
<tr>
<td>• Indirectly predicts MSI-H</td>
<td>• Separates MSS, MSI-H, MSI-L</td>
</tr>
<tr>
<td>• Results suggest MMR gene</td>
<td></td>
</tr>
<tr>
<td>• May be difficult to interpret</td>
<td></td>
</tr>
</tbody>
</table>
IHC Testing of MMR Proteins

MLH-1

PMS-2
<table>
<thead>
<tr>
<th>IHC Pattern</th>
<th>Rate of results</th>
<th>Clinical</th>
</tr>
</thead>
<tbody>
<tr>
<td>All proteins intact</td>
<td>80-85%</td>
<td>most likely not LS</td>
</tr>
<tr>
<td>MLH1- /PMS2-</td>
<td>10%</td>
<td>Sporadic (BRAF mutation/MLH1 promotor methylation) (80%)</td>
</tr>
<tr>
<td>MSH2- /MSH6-</td>
<td>3%</td>
<td>likely germline LS MSH2</td>
</tr>
<tr>
<td>MSH6-</td>
<td>1%</td>
<td>likely germline LS MSH6</td>
</tr>
<tr>
<td>PMS2-</td>
<td>1%</td>
<td>likely germline LS PMS2</td>
</tr>
</tbody>
</table>
MLH1 and PMS2 Absent (10% of CRC)

- Sporadic (80%)
- Lynch (20%)

**MLH1** hypermethylation (80%)

**BRAF** mutation (70%)

**MLH1** germline mutation
**BRAF Mutations**

- Threonine Kinase involved in the ras/raf/mek/erk pathway
**BRAF Mutations**

- Mutated in 15% of all CRC and 70% of MSI tumors with MLH1 loss
- Virtually exclusive for Lynch (rare reports in LS)
- Exon 15, point mutation in V600E (primarily)
- Has **prognostic (poor)** and possibly therapeutic significance
- **BRAF** mutations are uncommon in extracolonic tumors (endometrium)
MLH1 Promotor Methylation

- Perform for *BRAF* negative MLH1/PMS2 deficient tumors (30%)
- Others go directly to *MLH1* promotor methylation (may be more cost effective but less readily available)

If *BRAF* is not mutated and MLH1 promotor is not methylated refer to mutational analysis of MLH1
Isolated PMS2 Loss
(5% of LS)

• *PMS2* germline mutation

or rarely

• *MLH1* missense mutation resulting in non-functional, antigenically reactive protein

Refer to *PMS2* (and *MLH1*) mutational analysis
MSH2 and MSH6 Absent (35% of LS)

- Likely Lynch due to germline mutation of *MSH2*

Refer to *MSH2* mutational analysis

If genetic testing for *MSH2* is normal refer to *EPCAM* (2% of LS)
MSH2 Loss Due to *EPCAM* Gene Mutations

- *EPCAM* gene is upstream from *MSH2*, mutation leads to *MSH2* inactivation via CpG island methylation

- 25% of cases where MSH2 negative by IHC but germline *MSH2* mutations not found (2-3% overall LS)
Isolated MSH6 loss
(<5% of LS)

- Isolated MSH6 loss - likely *MSH6* mutation rarely *MSH2* mutation

- Patients with *MSH6* mutations may lack evidence of MSI by PCR or loss of MSH6 by IHC

- Patients with MSH6 loss by IHC may lack evidence of MSH6 mutations by genetic testing

Refer to *MSH6* mutational analysis
Correlation Between MMR Loss and Germline Mutation Identification

<table>
<thead>
<tr>
<th>Gene Combination</th>
<th>Frequency</th>
<th>Mutation Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>MLH1/PMS2</td>
<td>33%</td>
<td>MLH1 mutation</td>
</tr>
<tr>
<td>PMS2</td>
<td>56%</td>
<td>PMS2 mutation</td>
</tr>
<tr>
<td>MSH2/MSH6</td>
<td>66%</td>
<td>MSH2 mutation</td>
</tr>
<tr>
<td>MSH6</td>
<td>24%</td>
<td>MSH6 mutation</td>
</tr>
</tbody>
</table>

(Hampel et al J Clin Onc 2008)

Reasons for MMR Deficient Germline Testing Negative

1. Two somatic MMR mutations 66%
2. LOH one allele, somatic mutation other 9%
3. False positive initial screen 20%

(Haroldsdotter et al Gastroenterology 2014)
Rare and Unusual Molecular Scenarios

- MLH1 loss due to constitutional \textit{MLH1} hypermethylation of one allele in germline (\textit{MLH1} hypermethylation will be detectable in adjacent normal tissue) 0.6\% of LS
  (Niessen \textit{et al} Genes Chrom Cancer 2009)

- \textit{MSH2} rearrangement (inversion) not detectable by commonly used panels (Rhees \textit{et al} Fam Cancer 2014)

- Biallelic somatic mutations in MMR genes (\textit{MLH1}, \textit{MSH2})
  (Sourrouille \textit{et al} Fam Cancer 2013, Mesenkamp \textit{et al} Gastro 2014)

- Constituitional MMR deficiency, biallelic germline mutation in one of four genes, very rare, high risk malignancies in childhood, CNS and colorectal, IHC negative in tumor and normal tissue
  (Wimmer \textit{et al} J Med Gen 2014)

- Numerous others…..
Practical Issues Related to MMR IHC Staining
IHC Staining Heterogeneity
(Focal Weak Staining)

• Compare intensity to internal control (germinal center or basal normal epithelium)
  - Stain is adequate only if internal control positive

• If weaker (most common for MLH1) look at partner protein
• If PMS2 loss may suggest *MLH1* mutation
• If PMS2, MSH2 or MSH6 weak likely related to somatic downregulation
• Focal/weak staining may be do to regional hypoxia, poor tissue fixation or older tissue blocks

• MOST consider 10% positivity as a cutoff
Aberrant Staining Patterns

- Cytoplasmic staining, dot-like nuclear or nucleolar staining (MLH1), perinuclear staining likely all technical and are regarded as negative

Pai et al, AJSP 2016
Should We Be Performing IHC For MMR On Biopsy Or Resection Material?

• Biopsy preferable
  - If Lynch patient may undergo total colectomy, prophylactic hysterectomy/oophorectomy.
  - If rectal tumor preop chemoradiotherapy may completely ablate tumor.
Should We Stain Adenomas?

• Two large studies addressing testing of adenomas in Lynch patients (Pino et al JMD 2009, Walsh et al Mod Path 2012)

  Association between HG dysplasia, distal location, villous architecture and loss of staining, adenoma size over 10mm almost significant

• Not recommended as a screening tool for general population or for population fulfilling Revised Bethesda Guidelines !!!!

• In known LS families if tumor is not available and only large adenoma is, testing is appropriate, However Beware of False Negatives! (MSI late event)
CRC biopsy

- MSI test
  - MSS, MSI-L
    - No Further Testing
  - MSI-H
    - No loss of MMR proteins
      - BRAF and/or promotor methylation test
        - BRAF mutated or promotor methylated
          - Refer to Genetic Counseling, Consider Germline Testing
        - No BRAF mutation or promotor methylation
          - MSS, MSI-L
            - No loss of MMR proteins
              - Loss of MLH1/PMS2
                - Loss of other MMR
                  - No Further Testing

- IHC test
  - No Further Testing
Pathologist’s Role in MSI Testing Follow-Up

• **Issue report explaining results** and contact clinician to discuss whenever LS is a possibility, timeliness important

• **Clinician needs to refer patient to geneticist**
  
  *We cannot reflexively order genetic testing, patient needs to consent*

• **Convincing patient to undergo genetic counseling is the largest barrier!!**

If clinical suspicion of LS is high consider ordering germline testing regardless of IHC and MSI testing results!!
Molecular Prognostic and Predictive Markers for CRC:
State of the Art 2017 and Future Visions
Molecular Prognostic and Predictive Markers

Which tumors need to be tested?
Stage II (III) colon cancers (mostly prognosis)
Stage IV (III) tumors (mostly predictive)
CRC Prognostic/Predictive Biomarkers

- Multitude of studies based on:
  - Protein expression by IHC
  - Molecular mutational profile, MSI
  - Expression array mRNA, miRNA profile
  - Serum proteomic and DNA markers
  - Others…….
ASCP/CAP/AMP/ASCO Biomarker Guidelines 2017

• Strongly recommends **MSI** testing of all stage II and above, screen for LS and prognosis, (and predictive)

• Strongly recommends **BRAF (V600E)** testing for stage II and above for prognostic stratification (BRAF status in the decision tree for EGFR targeted therapy is optional and still controversial)

• Strongly recommends **KRAS and NRAS** genotyping in all patients with metastatic CRC being considered for EGFR targeted therapy
MSI as a Prognostic Marker

- MSI Stage II have a more favorable outcome
  (Ribic et al NEJM 2003, Sargent et al JCO 2010)
- MSI Stage III slightly more favorable
  (Roth et al JNCI 2012, Sinicrope et al JCO 2013)

- BRAF Mutation attenuates MSI survival benefit

WHO recommends poorly differentiated MSI-H tumors Should Not be graded as high grade !!
BRAF Mutation as a Prognostic Marker

- BRAF mutation is a poor prognostic factor for stage III and IV MSI and MSS tumors.
  (reviewed by Sinicrope FA et al Clin Gastroenterol Hepatol 2016)
MSI as a Predictive Marker

• Predicts poor response to 5FU therapy
  (Sargent et al JCO 2010)

• Addition of oxaliplatin (FOLFOX) negates adverse effects of MSI
  (Andre et al JCO 2015)

• Immune Checkpoint Therapy
The Immune Landscape of MSI Tumors

Tumor Infiltrating Lymphocytes (TILs)

T-cells are predominantly cytotoxic and activated in MSI Tumors

What attracts tumor infiltrating lymphocytes?

- Immunogenic neopeptides generated by abundant mutations

Shia et al, Modern Pathology, 2016
Why are MSI tumors (which are strongly immunogenic) not rejected by the host?
Checkpoint inhibitors

CTLA4-CD80 - Ipilimumab MELANOMA 2011

Nature Reviews Cancer 12, 252-264
Immune Checkpoint Proteins
PD1-PDL1-1

2A. T CELL INTERACTION with CANCER CELL

2B. ACTION of ANTI-PD-1 DRUG
Programmed Cell Death 1 (PD-1) and Its Ligand (PD-L1) in Common Cancers and Their Correlation with Molecular Cancer Type

Zoran Gatalica¹, Carrie Snyder², Todd Maney¹, Anatole Ghazalpour¹, Daniel A. Holterman¹, Nianqing Xiao¹, Peggy Overberg¹, Inga Rose¹, Gargi D. Basu¹, Semir Vranic³, Henry T. Lynch², Daniel D. Von Hoff⁴, and Omid Hamid⁵

Table 3. PD-1 and PD-L1 expression in colorectal carcinomas in relationship to the microsatellite instability status

<table>
<thead>
<tr>
<th>Colon cancer subtypes (n = 87)</th>
<th>PD-1 expression/hpf (TILs; % and range)</th>
<th>PD-L1 (tumor cells; %)</th>
<th>Concurrent PD-1/PD-L1 expression (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MSS colon cancers (n = 60)</td>
<td>39% (1–11)</td>
<td>13%</td>
<td>4%</td>
</tr>
<tr>
<td>MSI-H colon cancers (n = 27)</td>
<td>77% (1–20)¹</td>
<td>38%¹</td>
<td>32%¹</td>
</tr>
</tbody>
</table>

Abbreviation: hpf, high-power fields.
⁵Significantly higher.
PD-1 Blockade in Tumors with Mismatch-Repair Deficiency


Clinical Responses to Pembrolizumab Treatment.

A Biochemical Response
- Mismatch repair–proficient colorectal cancer
- Mismatch repair–deficient colorectal cancer
- Mismatch repair–deficient noncolorectal cancer

Change in Tumor Marker Level (%)

B Radiographic Response
- Mismatch repair–proficient colorectal cancer
- Mismatch repair–deficient colorectal cancer
- Mismatch repair–deficient noncolorectal cancer

Change from Baseline in the Sum of Longest Diameters (%)

20% increase (progressive disease)
30% decrease (partial response)
Predictive Markers: EGFR Signaling Pathway

- EGFR pathway overexpressed in over 80% of CRC
- EGF and other ligands activates KRAS and other signaling pathways leading to cellular proliferation, angiogenesis, migration and survival
RAS activating mutations predict poor response to anti-EGFR therapy

- **KRAS mutations also predict poor response to VEGF inhibitors.**

- **KRAS and NRAS mutations are also moderately poor prognostic factors for stage III and IV tumors.**
ASCP/CAP/AMP/ASCO Biomarker Guidelines 2017

- Strongly recommends **MSI** testing of all stage II and above, screen for LS and prognosis, *(and predictive)*
- Strongly recommends **BRAF (V600E)** testing for stage II and above for prognostic stratification *(BRAF status in the decision tree for EGFR targeted therapy is optional and still controversial)*
- Strongly recommends **KRAS and NRAS** genotyping in all patients with metastatic CRC being considered for EGFR targeted therapy
Anti-EGFR MAB

EGFR

Alpelisib

PI3K

AKT

BRAF

RAS

CRAF

MEK

ERK

Proliferation
Survival
Invasion
Metastasis

Vemurafenib
Dabrafenib
Encorafenib

Trametinib
Binimetinib
Selumetinib

Pohl et al Dig Dis 2016
Future of Mutational Testing for Colorectal Cancer

- Mutations other than RAS, BRAF effect EGFR pathway and response to EGFR targeted therapy
- Novel therapies target these mutations
- CRC is driven by mutations in other pathways as well, some of which are being targeted

Need for larger panels of mutation analysis (NGS)

- CRC with wild type KRAS develop novel mutations rendering anti-EGFR ineffective over time following targeted therapy

Need for continuous monitoring (LIQUID BIOPSY)
Role for Next Generation Sequencing in Colon Cancer Workup

Colon Cancer Mutations By Frequency

Top 20 genes

<table>
<thead>
<tr>
<th>Gene name (frequency)</th>
<th>Scale (samples)</th>
</tr>
</thead>
<tbody>
<tr>
<td>APC (48%)</td>
<td>20000</td>
</tr>
<tr>
<td>TP53 (45%)</td>
<td>10000</td>
</tr>
<tr>
<td>KRAS (35%)</td>
<td>5000</td>
</tr>
<tr>
<td>PIK3CA (13%)</td>
<td>1000</td>
</tr>
<tr>
<td>BRAF (11%)</td>
<td>500</td>
</tr>
<tr>
<td>SMAD4 (11%)</td>
<td>200</td>
</tr>
<tr>
<td>ARID1A (11%)</td>
<td>100</td>
</tr>
<tr>
<td>KMT2C (11%)</td>
<td>50</td>
</tr>
<tr>
<td>ATM (11%)</td>
<td>20</td>
</tr>
<tr>
<td>FBXW7 (10%)</td>
<td>10</td>
</tr>
<tr>
<td>AKAP9 (10%)</td>
<td>5</td>
</tr>
<tr>
<td>KMT2D (10%)</td>
<td>2</td>
</tr>
<tr>
<td>RNF43 (10%)</td>
<td>1</td>
</tr>
<tr>
<td>TCF7L2 (10%)</td>
<td>0.5</td>
</tr>
<tr>
<td>TRRAP (9%)</td>
<td>0.5</td>
</tr>
<tr>
<td>UBR5 (8%)</td>
<td>0.25</td>
</tr>
<tr>
<td>RNF213 (8%)</td>
<td>0.25</td>
</tr>
<tr>
<td>POLE (8%)</td>
<td>0.1</td>
</tr>
<tr>
<td>SPEN (8%)</td>
<td>0.1</td>
</tr>
<tr>
<td>GRIN2A (8%)</td>
<td>0.1</td>
</tr>
</tbody>
</table>

Cosmic Database 2016
Next Generation Sequencing

Rapid, sensitive and relatively inexpensive analysis:

- Multigene Mutation Panels
- Whole Exome, Whole Genome
- Transcriptome

(R Steven et al Biotechniques 2014)
NGS and the Future of Molecular Testing for CRC

- NGS is more sensitive and as prices continue to drop will become more cost-effective than existing mutational screening strategies for all stage II and greater CRC.
- Mutation panels will cover all actionable mutations for targeted therapies.
- Mutational analysis of LS (other polyposis and genetic syndromes) will be incorporated into NGS panels.
- MSI status will be determined by NGS bioinformatic analysis.
Detection of Circulating Tumor DNA in CRC
“Liquid Biopsies”

McDonald et al  Sci Trans Med 2015
Key applications of circulating tumour cells (CTCs) and circulating tumour DNA (ctDNA) as liquid biopsies for precision medicine

- Early detection of cancer (diagnosis)
- Determine risk for metastatic relapse (prognosis)
- Identification of therapeutic targets
- Stratification for treatment decision
- Real-time monitoring of treatment
- Detection of resistance mechanisms
- Early detection of metastatic relapse

Nature Reviews | Gastroenterology & Hepatology

Liquid Biopsy Market Projected at >10 Billion Dollars by 2020 !!!
Circulating Tumor DNA in CRC Patients

- DNA levels in plasma are higher in CRC patients, decrease after therapy and increase again at tumor recurrence (Frattini et al 2005)

Genotyping Cancer Alleles in ctDNA

- KRAS mutant fragments are detected in the blood of patients with KRAS mutant tumors with high specificity and sensitivity (Bettegowda et al 2014)
- Tumor specific KRAS mutations in plasma have prognostic value (Spindler et al 2015)

Monitoring Drug Resistance and Clonal Evolution

- RAS pathway mutations associated to acquired resistance to EGFR specific antibodies detected before disease progression is detected (Diaz et al, Misale et al 2012)
- ctDNA used to track clonal evolution and targeted drug responses in CRC patients (Siravegna et al 2015)
- WGS of plasma of CRC patients treated with anti-EGFR therapy reveals loss of APC chromosomal 5q22 region and amplifications in known genes involved with resistance of EGFR blockade such as MET, ERBB2 and KRAS (Mohan et al 2014)
Standard Biopsy

- Time intensive
- Invasive
- Localized sampling of tissue
- Difficult to obtain in certain locations
- One point in time

Liquid Biopsy

- Quick and easily obtained
- Allows for continuous monitoring
- Better at detecting tumor heterogeneity?
Summary

• MSI status is an important prognostic and predictive marker and is central to many classification schemes of CRC
• With universal MSI testing we are responsible for guiding clinicians for further genetic work-up whenever LS is a consideration
• Current guidelines recommend testing for mutations in BRAF and genes governing the EGF-R pathway, however, the list of mutations to be tested will undoubtedly increase
• NGS will likely replace existing technologies for mutational analysis, MSI work-up and hereditary CRC work-up
• Liquid biopsies detecting ctDNA will allow for real time monitoring of CRC mutational profiles
Thank You
Consensus Molecular Subtypes of CRC

  - Evaluated the results of 6 CRC subtyping algorithms each developed independently using different gene expression data sets and analytical approaches
  - Total of 4151 patients including The Cancer Genome Atlas
  - Data included:
    - Clinical information and prognosis
    - Mutations and copy number analysis
    - Gene expression including miRNA
    - Epigenetics
    - Proteomics
## Proposed Taxonomy of CRC 2015

<table>
<thead>
<tr>
<th>Tumor Subtype</th>
<th>CMS1 MSI Immune</th>
<th>CMS2 Canonical</th>
<th>CMS3 Metabolic</th>
<th>CMS4 Mesenchymal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proportion</td>
<td>14% Serrated</td>
<td>37% Tubular</td>
<td>13% Unknown</td>
<td>23% Serrated</td>
</tr>
<tr>
<td>Precursor</td>
<td>Serrated</td>
<td>Tubular</td>
<td>Mixed MSI</td>
<td>Serrated</td>
</tr>
<tr>
<td>Genetic features</td>
<td>MSI, CIMP high</td>
<td>SCNA high</td>
<td>SCNA low, CIMP low</td>
<td>SCNA high</td>
</tr>
<tr>
<td>Genetic drivers</td>
<td>BRAF mutations</td>
<td>APC</td>
<td>KRAS mutation</td>
<td>Unknown</td>
</tr>
<tr>
<td>Gene expression signature</td>
<td>Immune infiltration and activation</td>
<td>WNT and MYC activation</td>
<td>Metabolic deregulation</td>
<td>Mesenchymal TGF-b activation angiogenesis</td>
</tr>
<tr>
<td>Prognosis</td>
<td>Good survival but poor after relapse</td>
<td>Superior survival rate after relapse</td>
<td>Intermediate Survival</td>
<td>Worse relapse-free and overall survival</td>
</tr>
</tbody>
</table>