Molecular testing in GI cancer

Wade S. Samowitz, M.D.
University of Utah
Disclosures

• Potential royalties in the future related to the Ventana BRAF V600E antibody
THE TEN COMMANDMENTS

I Thou shalt have no other gods before me.

II Thou shalt not make unto thee any graven image, or any likeness of anything that is in heaven above, in earth beneath, or in the water under the earth.

III Thou shalt not take the name of the Lord thy God in vain, for the Lord will not hold him guiltless that taketh his name in vain.

IV Remember the Sabbath day, to keep it holy.

V Honour thy father and thy mother; that thy days may be long upon the land.

VI Thou shalt not kill.

VII Thou shalt not commit adultery.

VIII Thou shalt not steal.

IX Thou shalt not bear false witness against thy neighbour.

X Thou shalt not covet thy neighbour's house, wife, manservant, maidservant, ox, ass, nor anything that is thy neighbour's.

Exodus 20:7-17
Why do you need to know this

• A lot of ignorance out there
• Clinicians will bother you about this
• Even if your lab doesn’t perform these tests
  – Need to know what to order
  – Need to know what it means
Topics

• Mutation detection in solid tumors: general considerations
• Lynch syndrome
• Therapy, especially EGFR pathway
• Future
Mutation detection in fixed tissue: General Considerations

• Solid tumors are different than germline DNA (or even most hematolymphoid samples)
  – Consist of heterogeneous cell types
  – Requires some form of microdissection
  – Need AP/CP coordination

• Garbage in, garbage out
  – Choose best tumor block (highest concentration of tumor)
Slide of a colon cancer with a circled area of colon cancer which will be microdissected
It's a mammoth.

Early microscope
Circled area avoids lymphoid follicle
Excluded lymphoid follicle
Another circled cancer
Higher power; relatively high tumor concentration
Another circled area thin line
Higher power shows numerous neutrophils.
KRAS
34 G>T
30%T
34 G>T
13% T
Topics

• Mutation detection in solid tumors: general considerations
• Lynch syndrome
• Therapy, especially EGFR pathway
• Future
Lynch syndrome (HNPCC)

• Early onset colon cancer
• Right-sided
• Extra-colonic cancers: endometrium, ovary, renal pelvis, ureter, small intestine, stomach, hepatobiliary tract, pancreas
• Muir-Torre: Lynch + sebaceous neoplasms
• Turcot’s: Lynch + brain tumor (GBM) (Hamilton, NEJM, 1995)
Lynch syndrome

• Germline mutations in mismatch repair genes: \textit{MLH1, MSH2, MSH6} or \textit{PMS2}
• Autosomal dominant
• Phenotype not so obvious (unlike FAP, for example)
• Family history not always obvious or available
• Fortunately, we can use the molecular features of the tumor (mismatch repair deficiency) to help in work-up
How do we assess mismatch repair deficiency?

- 1. Microsatellite instability
- 2. IHC for mismatch repair proteins
Microsatellite repeats

• Type of repetitive DNA in which repeat unit is short (1-6 nucleotides)
  – Mononucleotide: AAAAAAAAAAAAA
  – Dinucleotide: CACACACACACACA
• Most in non-coding regions
  – Some exceptions: Mononucleotide repeats such as in TGFBRII
• Often slippage during DNA replication of these repeats
  – Leads to changes in number of repeats
• Usually fixed by mismatch repair apparatus
Microsatellite instability

• Expansion or contraction of microsatellite repeats
  – For example, 10 CA’s to 14 CA’s
• Requires a mistake in replication plus deficiency in mismatch repair
Chromosome 5  Maternal

Chromosome 5  Paternal

10 CA

12 CA
PCR of Normal

120

124

Maternal

Paternal
PCR of MSI

Maternal

118  120  122

Paternal

124  126
Bethesda Consensus Panel

- Two mononucleotide repeats, three dinucleotide repeats
- MSI high: Instability in two or more repeats
- Microsatellite stable (MSS): No instability
- MSI low: Instability in one repeat
  - Controversial
  - Lynch-associated cancers show MSI high, not low
Mononucleotide repeat panel

• Mononucleotide repeats are probably more sensitive and specific for MMR deficiency

• New panel(s) of 5 mononucleotide repeats
  – MSI high: two or more unstable, although typically all (or almost all) repeats are unstable
  – Since instability in even one mononucleotide repeat may indicate MMR deficiency, instability in one repeat is termed “indeterminate” rather than MSI low
How do we assess mismatch repair deficiency?

- 1. Microsatellite instability
- 2. IHC for mismatch repair proteins
IHC interpretation

- MLH1 complexes with PMS2
- MSH2 complexes with MSH6
- The stability of PMS2 and MSH6 depends upon these complexes
- Therefore, if MLH1 is lost, PMS2 is usually lost; if MSH2 is lost, MHS6 is lost.
- Corollary usually not true (MLH1 and MSH2 bind to other proteins as well)
<table>
<thead>
<tr>
<th>IHC Result</th>
<th>Likely Defective Gene</th>
</tr>
</thead>
<tbody>
<tr>
<td>Loss of MLH1, PMS2</td>
<td>MLH1</td>
</tr>
<tr>
<td>Loss of MSH2, MSH6</td>
<td>MSH2</td>
</tr>
<tr>
<td>Isolated Loss of MSH6</td>
<td>MSH6</td>
</tr>
<tr>
<td>Isolated Loss of PMS2</td>
<td>PMS2*</td>
</tr>
</tbody>
</table>

*Germline MLH1 mutations associated with isolated loss of PMS2 have been reported.*
IHC vs. PCR

- Complementary (both miss some mmr def)
- MSH6 negative tumors can be stable by PCR (not as much of an issue with mononucleotide repeat panels)
- Missense mutations may be normal by IHC
- IHC can be hard to interpret
- IHC can guide subsequent mismatch repair gene testing
MMR IHC: Problems in interpretation

- Staining variability (use internal controls)
- “Clonal” MSH6 loss due to MSH6 coding mononucleotide repeat (Shia, Modern Path 2013)
- Decreased MSH6 staining after chemoradiation (Bao, Am J Surg Pathol, 2010)
- Decreased staining intensity rather than complete loss
  - If marked suggest MSI by PCR
MSS tumor MLH1
MSS tumor MLH1
Clonal MSH6 loss

- Caused by instability in the MSH6 coding mononucleotide repeat in certain parts of the tumor (but not in others)
- Typically occurs in a tumor which is mismatch repair deficient due to an alteration besides MSH6
  - Sporadic mmr deficiency
  - Germline mutation in another gene
MMR deficient colorectal cancer: two clinical contexts

• Lynch/HNPCC
  – Germline mutation in one of the mismatch repair genes (MLH1, MSH2, MSH6, or PMS2)

• 10-15% Sporadic colorectal cancer
  – Acquired hypermethylation of MLH1 promoter
  – IHC: MLH1/PMS2 loss (same as Lynch syndrome due to germline MLH1 mutation)
Lynch syndrome work-up

• Is tumor mismatch repair deficient?
• Is mismatch repair deficient tumor sporadic or Lynch syndrome?
IHC loss of MLH1/PMS2: Sporadic or Lynch? Why care?

- Sporadic mmr deficient tumors are more common than Lynch; MLH1/PMS2 loss is the most common abnormal IHC result
- If it is sporadic
  - Don’t need to sequence MLH1 in the germline
  - Don’t need to follow-up patient as Lynch syndrome or evaluate family members
IHC loss of MLH1/PMS2: Sporadic or Lynch?

• Approximately 50% of sporadic mmr deficient colorectal cancers have BRAF V600E mutation; extremely rare in Lynch syndrome
  – Molecular test or antibody specific to V600E
    • Potentially a mostly IHC work-up (Toon et al, AJSP 2013)
• BRAF mutations are uncommon in extracolonic sporadic mmr deficient tumors (e.g. endometrial cancers)
• Most sporadic mmr deficient tumors of any site have MLH1 methylation; rarely seen in Lynch syndrome
What about the BRAF antibody?

- Molecular test is still the gold standard: most objective and definitive
- Still need molecular testing (MLH1 methylation) for Lynch work-up of many tumors
  - BRAF mutations are not common in extra-colonic mismatch repair deficient tumors (like endometrium)
  - 50% of sporadic mmr def colorectal cancers are BRAF wild type, and many of these are MLH1 methylated
Colorectal cancer Lynch Syndrome Test Algorithm

Consider Lynch syndrome

MMR deficiency by: Immunohistochemical stains or PCR

Positive for MMR deficiency

Normal MLH1 staining and abnormal for MSH2, MSH6, or PMS2

Seq&Del/Dup MSH2, MSH6, PMS2

Alteration present

Probable sporadic colorectal cancer

Negative for MMR deficiency

Probably not Lynch syndrome

Abnormal MLH1 staining

Test for BRAF V600E mutation, MLH1 methylation

Alteration absent (wild-type)

Seq &Del/Dup MLH1

Alteration present

Probable sporadic colorectal cancer
Do acquired mutations in mismatch repair genes occur?

- Previous literature suggested any abnormal IHC profile besides loss of MLH1/PMS2 was Lynch
- Also, MLH1/PMS2 loss without BRAF mutation or MLH1 methylation was Lynch
- New reports suggest substantial proportion of these are due to acquired mutations in MMR genes (Haroldsdottir et al, Gastroenterology, 2014).
- Implications for reporting of IHC results
Which tumors should be tested?

- Revised Bethesda guidelines
  - More than just age and histology, e.g. personal and family history of Lynch syndrome tumors
  - Estimated to miss nearly 30% of Lynch
- Colon cancer under 70 (Jerusalem criteria)
  - Misses 10% of Lynch
- Universal screening
Work with other clinicians!

• Make sure your findings are seen, understood and acted upon

• Work with genetic counselors
  – Appropriate follow-up and genetic testing are more likely (Heald et al, 2013)
  – Depending upon how likely they think Lynch is, might do MSI by PCR if IHC is normal, might still sequence genes even if BRAF is mutated or MLH1 is methylated
Should you test adenomas?

- Not all Lynch-associated adenomas are MMR deficient
  - 50% in recent study were not MMR deficient (Yurgelun, Cancer Prev Res, 2012)
  - Incidence of mmr deficiency related to size
    - Large polyps (8-10 mm or more) much more likely to be mmr deficient
- Therefore, the lack of mmr deficiency in an adenoma is not as strong a criterion to exclude Lynch syndrome as the lack of instability in a cancer
- However, the presence of mmr deficiency in an adenoma is probably more specific for Lynch
What about next generation sequencing?

• Someday cost of germline sequencing of all four genes (and many other inherited colon cancer genes) may be less than the cost of tissue testing, but
  – IHC alone is very cheap and will exclude most cases
  – IHC profile may help with interpretation of variants of unknown significance (VUS)
Topics

• Mutation detection in solid tumors: general considerations
• Lynch syndrome
• Therapy, especially EGFR pathway
• Future
Therapy

• MSI
  – 5FU may not be effective (may even be harmful) if tumor is unstable (Ribic, NEJM, 2003)
  – May not be relevant to new therapies which include oxaliplatin
  – MSI status sometimes used as part of decision on whether to treat Stage II disease (in addition to gene expression profiling, which predicts recurrence in Stage II and III)
Personalized (precision) medicine

• Not one size fits all, but targeted therapy based upon mutational profile of each tumor
• Need to evaluate molecular targets in each tumor type
Precision medicine for colorectal cancer

• EGFR pathway is activated (but EGFR is not mutated) in colorectal cancer

• Cetuximab is an antibody that binds to EGFR, blocking activation by ligands like epidermal growth factor

• A mutation downstream of EGFR that activates the pathway makes this blocking irrelevant

• Bad to give a toxic and expensive drug if it won’t work
EGFR pathway inhibition

• EGFR inhibitors used in Stage IV cancers
• Original studies: EGFR inhibition ineffective if mutation in codon 12 or 13 of KRAS
• Subsequently extended to codons 12, 13, 61, 117 or 146 of KRAS and NRAS
• Codon 1047 PIK3CA mutations, loss of PTEN
• BRAF may be prognostic marker (bad) rather than predictive of therapy response
Topics

• Mutation detection in solid tumors: general considerations
• Lynch syndrome
• Therapy, especially EGFR pathway
• Future
Next generation sequencing

• Cost effective way to test multiple genes
  – And relevant genes (which code for targetable protein products) will continue to grow, making multiple single gene tests even more expensive

• Targeted NGS approach requires very little input DNA (10 ng) and provides fast turnaround time
Pulmonary adenocarcinoma
EGFR Exon 19: c.2236_2250del, p.E746_750del