Introduction

- Microsatellite instability and mismatch repair protein deficiency
- KRAS mutations in predicting response to therapy in colorectal carcinoma
- Role of EGFR pathway

Why Do MSI/MMR Analysis?

- Screen for possible HNPCC\(^*\) syndrome
- Prognosis
- Predict response to therapy

\(^*\)Hereditary Nonpolyposis Colorectal Carcinoma

Hereditary Nonpolyposis Colorectal Carcinoma

- Autosomal dominant inherited tendency to develop colorectal cancer
- Other tumors: endometrial cancer, ovarian cancer, gastric cancer, sebaceous carcinoma, urothelial carcinoma of the upper urinary tract
- Cancers generally occur at a significantly younger age than is observed among patients with sporadic tumor of the same type

*Hereditary Nonpolyposis Colorectal Carcinoma
Patterns of CRC Risk

- Sporadic (average risk) (65%–85%)
- Family history (10%–30%)
- Hereditary nonpolyposis colorectal cancer (HNPCC) (5%)
- Familial adenomatous polyposis (FAP) (1%)
- Rare syndromes (<0.1%)
- Germline MSI
- MSS

15% all CRC have MSI

Why Identify HNPCC Patient?

- Implement regular screening colonoscopy
- Implement cancer screening for other potentially affected organs
- Identify other family members with the gene defect to implement early preventive screening

Amsterdam Criteria

- ≥ 3 relatives with HNPCC-associated cancers (colorectal, endometrial, small bowel, ureteral, or renal pelvis), one of whom is a first degree relative
- Cancers involving at least two generations
- ≥ 1 cancers diagnosed before the age of 50

Bethesda Criteria

- Colorectal cancer < 50 years of age
- Synchronous or metachronous colorectal or other HNPCC-associated tumors, regardless of age
- Colorectal cancer with MSI-H morphology in patients under 60 years of age.
- Colorectal cancer with one or more first-degree relatives with HNPCC tumors, one diagnosed under age 50
- Colorectal cancer in two or more first- or second-degree relatives with HNPCC-related tumor, regardless of age
Why Test?

• Most patients who meet criteria for possible HNPCC do not have defective mismatch repair protein genes (70%)
• Those who do not have defective mismatch repair protein genes have lower risk of colorectal cancer and possibly, other cancers and so do not benefit from early screening

How To Test?

• Microsatellite instability (MSI)
• Mismatch repair protein deficiency (MMR)
• Gene sequencing

What Is Tested?

• Cancer tissue for mismatch repair protein deficiency
• Cancer tissue and normal tissue (or peripheral blood) for microsatellite instability
• Precursor lesions (adenomas, atypical endometrial hyperplasia) can be tested, but may yield false negative results

Microsatellite Instability (MSI)

• Five mononucleotide microsatellites (BAT-25, BAT-26, NR-21, NR-24, and MONO-27) (Promega)
• Allelic profiles from the normal and malignant tissue are compared
• MSI-H = 2 or more abnormal profiles
• MSI-L = 1 abnormal profile
• MSS = no abnormal
• MSI-H vs MSI-S/MSI-L
MMR In Hereditary Nonpolyposis Colorectal Cancer Syndrome

- MLH1
- PMS2
- MSH2
- MSH6
MMR* In Hereditary Nonpolyposis Colorectal Cancer Syndrome

- MLH1 (40%)
- MSH2 (40%)
- MSH6 (10%)
- PMS2 (5%)

*Mismatch repair protein deficient = dMMR
Mismatch repair protein proficient = pMMR
MMR/MSI In Colorectal Cancer

- Not all dMMR/MSI-H colorectal carcinoma is associated with HNPCC
- Likelihood depends on personal/family history and MMR protein
- MSH2/MSH6 high likelihood of germline mutation (approx 90%)
- MLH1/PMS2 may be somatic (usually hypermethylation)

MSI-H/dMMR In Sporadic Colorectal Cancer

- Proximal tumor site, high grade, early stage, and diploid
- Favorable outcome
- Most due to inactivation of MLH1 (95%), followed by MSH2 (5%), MSH6 (<1%)
- Most common mechanism of MLH1 gene inactivation among unselected cases (~90 percent of cases) is promoter hypermethylation

Who Is Tested?

- Bethesda criteria*
- Colorectal carcinoma ≤ 50 years
- Multiple sebaceous carcinomas
- Endometrial carcinoma with mixed well differentiated and high grade undifferentiated histology (Stanford)
- Ovarian clear cell carcinoma ≤ 50 (Stanford)

*J Clin Oncol. 2008;26:5783-5788
Why Do MSI/MMR Analysis?

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- Prognosis
- Predict response to therapy

*Hereditary Nonpolyposis Colorectal Carcinoma

MSI Predicts Prognosis

Forest Plot of Hazard Ratios of OS for all Stage II-III CRC associated with MSI

HR = 0.67 (95% CI 0.58-0.78)

Why Do MSI/MMR Analysis?

• Screen for possible HNPCC* syndrome
• Prognosis
  ➢ Predict response to therapy

*Hereditary Nonpolyposis Colorectal Carcinoma

MSI Predicts Response to Chemotherapy

570 tissue specimen from stage II / III pts enrolled in randomized trials of 5FU based chemo

MSI (PCR) testing

95 (16.7%) MSI-H


MSI/MMR Predicts Response To Chemotherapy

341 tumor specimen from stage II / III pts on randomized trials of chemo (5FU) vs. no chemo

MMR/MSI testing

47 (13.8%) dMMR or MSI-H
294 (86.2%) pMMR or MSS/MSI-L

J Clin Oncol 26: 2008 (May 20 suppl; abstr 4008)

MSI/MMR Predicts Response To Chemotherapy

47 (13.8%) dMMR or MSI-H
294 (86.2%) pMMR or MSS/MSI-L

Randomized: 5FU-based vs no chemo

OS (HR = 1.26, p = 0.68)
DFS (HR = 1.41, p = 0.53)

Randomized: 5FU-based vs no chemo

OS (HR = 0.69, p = 0.047)
DFS (HR = 0.59, p = 0.004)

J Clin Oncol 26: 2008 (May 20 suppl; abstr 4008)
Conclusions

• MSI/MMR predicts risk of familial colon cancer
• MSI/MMR predicts prognosis
• MSI/MMR predicts response to chemotherapy in patients with stage II/III CRC

What is KRAS?

• Transforming activities of Harvey and Kirsten murine sarcoma retroviruses contribute to carcinogenesis through a common set of genes, termed RAS.
• The Harvey-sarcoma virus-associated oncogene was termed HRAS and the Kirsten sarcoma virus was called KRAS.
• The KRAS gene, located at 12p12, encodes a protein that is a member of the small GTPase superfamily.


KRAS and Cancer

• Mutant KRAS alleles are found in many human cancers, including approximately 60% pancreas, 30% colon, 6% gastric (also 20% lung, 15% ovarian)
• Much research has been undertaken to define RAS regulators and downstream effectors, although the precise role of KRAS in carcinogenesis is incompletely understood.
The Colorectal KRAS Story

Requires a bit of history

Metastatic Colorectal Cancer: Timeline

1950 - 1975

- Oxaliplatin
- Cetuximab
- Panitumumab
- Capecitabine
- Irinotecan
- 5FU

2000

- Capecitabine
- Irinotecan
- Oxaliplatin
- Cetuximab
- Panitumumab

EGFR Signaling

- PI3K/ AKT/ mTOR
- VEGF
- STAT GRB2/

- K-RAS/BRAF
- MAPK

Survival
Metastasis
Angiogenesis
Survival
Proliferation

Antibodies Against EGFR

http://www.kras-info.com/
**EGFR Inhibitor Therapy in CRC**

- “On February 12, 2004, the U.S. Food and Drug Administration approved cetuximab (Erbitux®, made by Imclone Systems, Inc.), a monoclonal antibody directed against the epidermal growth factor receptor (EGFR). Cetuximab is approved for use, in combination with irinotecan, for the treatment of EGFR-expressing, metastatic colorectal carcinoma in patients who are refractory to irinotecan-based chemotherapy.
- Cetuximab is also approved for use as a single agent for the treatment of EGFR-expressing, recurrent metastatic colorectal carcinoma in patients who are intolerant to irinotecan-based chemotherapy.”

**EGFR Inhibitor Therapy in CRC**

- Only 15-20% response rate
- EGFR status does not predict response (unlike lung)*
- How can we predict responders?


**EGFR Signaling**

![EGFR Signaling Diagram]

- PI3K/AKT/mTOR
  - Survival
  - Metastasis
  - Angiogenesis
- VEGF
- STAT GRB2/
  - Survival
  - Metastasis
- K-RAS/BRAF/MAPK
  - Proliferation

**Priority Report**

**KRAS Mutation Status Is Predictive of Response to Cetuximab Therapy in Colorectal Cancer**

Methods: In this study, tumors from 30 metastatic colorectal cancer patients treated by cetuximab were screened for KRAS mutation by direct sequencing.


KRAS-(1)
Methods:

89 metastatic CRC patients treated with cetuximab were analyzed for KRAS mutations. The association between KRAS mutations and tumor response and survival was analyzed.
Kaplan–Meier Curves for Overall Survival According to Treatment

Kaplan–Meier Curves for Overall Survival According to Treatment

Location of KRAS Mutations

Codons 12 & 13

Cosmic Database: http://www.sanger.ac.uk/genetics/CGP/cosmic/
**ASCO PROVISIONAL CLINICAL OPINION**

“Based on systematic reviews of the relevant literature, all patients with metastatic colorectal carcinoma who are candidates for anti-EGFR antibody therapy should have their tumor tested for KRAS mutations in a CLIA-accredited laboratory. If KRAS mutation in codon 12 or 13 is detected, then patients with metastatic colorectal carcinoma should not receive anti-EGFR antibody therapy as part of their treatment. ”

*J Clin Oncol 2009;27:2091-2096*

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**NCCN Updates (November 3, 2008)**

- “FORT WASHINGTON, PA — The National Comprehensive Cancer Network (NCCN) announces important updates to the NCCN Guidelines on Colon and Rectal Cancers. These changes reflect leading developments in the treatment of patients with colon and rectal cancers and represent the standard of care in oncology in both community and academic settings. “

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**NCCN Updates (November 3, 2008)**

- “New to the NCCN Guidelines is the recommendation that a determination of the KRAS gene status of either the primary tumor or a site of metastasis should be part of the pre-treatment work-up for all patients diagnosed with metastatic colorectal cancer.”
- “Another important update to the NCCN Guidelines is that the epidermal growth factor receptor (EGFR) inhibitors, cetuximab (Erbitux®, Bristol-Myers Squibb Company/imClone Systems Incorporated) and panitumumab ( Vectibix®, Amgen), either as single agents, or, in the case of cetuximab, in combination with other agents, are now recommended only for patients with tumors characterized by the wild-type KRAS gene.”

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**How Do You Test?**

- Formalin fixed paraffin embedded tissue is acceptable
- Primary or metastasis
- Amount (% contribution) of tumor tissue dependent in part on methodology
How Can We Test for KRAS Mutations?

Sequencing:
- Advantages:
  • Comprehensive
- Disadvantages:
  • Difficult interpretation
  • Limited sensitivity: able to detect mutation down to only 15-20% mutant in wild-type background.

How Can We Test for K-RAS Mutations?

- TrimGem Mutector™
  - Method
  • PCR amplification of 120 bp region of DNA containing K-RAS codons 12 and 13
  - Advantages
  • Higher sensitivity: able to detect down to 5% mutant DNA in wild-type background

What About The Other Additional Possible Predictors Of Resistance to EGFR Inhibitor Therapy

- Lack of EGFR amplification/activation
- Loss of PTEN
- BRAF mutation
- Mutation or inactivation of p13K/AKT/mTOR pathway
- Inactivation of VEGF
- Completely separate pathway (i.e., not EGFR dependent)

Conclusions

- MSI/MMR predicts risk of familial colon cancer
- MSI/MMR predicts prognosis
- MSI/MMR predicts response to chemotherapy in patients with stage II CRC
- KRAS mutation predicts response to cetuximab in CRC in patients with advanced CRC
- Likely more to follow…
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