

# Prognostic Assays in Colorectal, NSCLC and Breast Cancer

Barbara A. Conley, MD  
Associate Director, Cancer  
Diagnosis Program, DCTD, NCI

No disclosures

# Goals

- Background
  - Analytic validity
  - Clinical validity
- Current Clinical Utility
- Colorectal cancer: BRAF, KRAS, MSI, MLH1 methylation, OncotypeDx colon
- NSCLC: EGFR, ALK, KRAS,
- Breast Cancer: mammaprint, OncotypeDx

# Colorectal cancer

- BRAF
- KRAS
- MSI, MLH1 promoter methylation
- OncotypeDx Colon

# KRAS

- 40% have KRAS mutations; 14% have “other RAS” mutations
- Not associated with prognosis
- Predictive of non-response to EGFR monoclonal Abs (exon 2, codons 12,13; codons 61, 146; exons 3 and 4)
- NRAS (exon 2,3,4) and PIK3CA mutations negatively affect response to EGFR

# Analytic Validity

- Therascreen KRAS RGO PCR Kit approved (Jul 2012 & May 2014) by FDA for use in identification of patients with mCRC for treatment with cetuximab or panitumumab
- Studies published comparing COBAS test to others ...

# Clinical Validity, Clinical Utility

- Crystal (mCRC randomized to FOLFIRI with or without cetuximab)
- PRIME (mCRC randomized to chemo with or without panitumumab)
- OPUS (mCRC randomized to FOLFOX4 with or without cetuximab)
- All confirm benefit of cetuximab only in wt KRAS

# Questions remaining

- RAS mutations other than KRAS codon 2 seem to indicate patients with them have no benefit from EGFRi
- What is the % of alleles with mutations that is important
- Preanalytic variables: % tumor
- Sanger is not as sensitive (20%)
- ? LDTs

# BRAF

- 5-15% have BRAF mutations (V600E)
- Strong negative prognostic factor
- Often associated with MMR deficient somatic tumors
- No particular clinical ramifications at present
- No particular platform or assay recommended by NCCN

# Analytic and Clinical Validity

- BIOMERIEUX THXID BRAF ASSAY KIT
  - Melanoma
  - V600E or K
- Not cleared/approved for CRC
- Prognostic
  - MDACC: mCRC patients with BRAF mutation have shorter PFS
  - CALGB 80903: BRAF mutations have shorter PFS, OS

# MSI: Biology

- Defects in mismatch repair – checkpoint for replication errors introduced at tandem repeats (microsatellites)
- Multiple short DNA repeats, located in the coding regions of select genes
  - Most commonly mutated is TGFBR2 (90%)
- If not repaired, can cause mutations by “slippage” DNA polymerase

# MSI testing: Clinical Implications

- Assessment for Lynch Syndrome
  - Surveillance
- Cancer Treatment
  - MSI-H unlikely to benefit from FU/LV adjuvant chemo
  - ? Irinotecan
  - ? Oxaliplatin
  - New: PARPi; mTORi; Taxanes
- Prognosis
  - Improved in stage II/III

# MSI – Pathology features

- Tumor infiltrating lymphocytes
- Mucinous/signet ring
- Crohn's like lymphocytic reaction
- Medullary growth

# MSI

- DNA repair genes: *MLH1*, *MSH2*, *MSH3*, *MSH6*, *PMS2*, *EPCAM* (hypermethylation *MSH2*)
- Hereditary 3-5% (Lynch syndrome) or sporadic (10-15%)
- Clinical associations: women, older age, inflammatory conditions, right sided,
- CIMP (CpG island methylator phenotype)

# Bethesda Guidelines for testing for MSI revised 2002

- CRC at age < 50
- Synchronous or metachronous LS tumors, regardless of age
- CRC with MSI histology < age 60
- $\geq 1$  first degree relative with LS cancer (one diagnosed < age 50)
- $\geq 2$  first or second degree relatives with LS cancer, regardless of age

# MSI

- Lynch Syndrome
  - Bethesda criteria
  - Up to 50% of LS patients don't meet Bethesda criteria
  - 90% are MSI-H and lack expression of at least 1 MMR protein by IHC

# MSI

- Germline mutations of MMR genes detected in > 50% of patients meeting Bethesda Criteria
- Lifetime Risk of CRC is 80%
- Surveillance lowers risk (also endometrial)
- Screening tumors from patients meeting the Bethesda criteria shown to be cost-effective

# Lynch syndrome screening

- CRC: MLH1, MSH2
  - Colonoscopy age 20-25 or 2-5 years younger than youngest affected patient. Every 1-2 years
- Endometrial, Ovarian
  - Enhanced attention to symptoms
  - Consider TAHBSO on completion of childbearing (MLH1, MSH2, MSH6)

# LS related cancers

- CRC
- Endometrial
- Gastric
- Ovarian
- Pancreas
- Biliary tract
- Small intestine
- Ureter
- Renal Pelvis
- Brain (Turcot)
- Sebaceous gland adenomas and keratoacanthomas as in Muir-Torre

# MSI and Hypermethylation MLH1

- Lynch-like cancers: MMR deficient but no hypermethylation of MLH1 promoter or MMR mutation
- Somatic cancers
  - Serrated pathway of carcinogenesis: CIMP, BRAF mutations
  - BRAF V600E (40-60% of sporadic; 69% of non IHC staining MLH1)

# Initial testing

- Known familial MMR: Sequencing
- No known familial MMR
  - IHC or MSI (10% false negative)
  - Sensitivity 77-89%; specificity 90%
  - Test BRAF mutation in MLH1 negative by IHC; IMPLIES somatic methylation of promoter
  - About 7% of CRC defective in MMR have Lynch syndrome

# MSI testing: functional assay

- PCR tumor and normal
- Bethesda markers: *BAT 25, BAT 26, D5S346, D2S123, D17S250*
- MSI-H: instability in 2 or more
- MSI-L/MSS: instability in one; none
- Not specific for Lynch syndrome
- Might underestimate
  - Sensitivities reported to be 72–89% for *D2S123*, 50–81% for *D17S250* and 50–59% for *D5S346*

# MSI

- Sensitivity
  - 89% MLH1, MSH2
  - < 80% MSH6, PMS2
- Specificity: 90%

# MLH1 promoter methylation

- A region methylation seen in 16% MSS
- C region methylation common in MSI-H
  - 5% in MLH1 mutation carriers
  - Bisulfite conversion f/b real time PCR

# IHC

- Widely available alternative
- Pinpoint which MMR gene
- Heterodimers: obligate – MSH2, MLH1
- Sensitivity for germline mutation with IHC MSH2 and MLH1 is 85%; some false + (mut catalytic domain but intact antigenic domain)
- Sensitivity 4 Ab panel: MLH1, MSH2, MSH6, PMS2 = 94%
- 10% false negative

# Reflex testing: who should undergo Lynch syndrome testing

- Test all newly diagnosed CRC
  - Cost effective (EGAPP)
  - NCCN endorsed
- Test all newly diagnosed < 70 years and older patients that meet Bethesda guidelines
  - Sensitivity 95.1%; specificity 95.5%
  - NCCN endorsed

# Oncotype DX colon

- 7 cancer related genes selected for correlation with recurrence
  - Activated stroma (BGN, INHBA, FAP)
  - Cell cycle (MK167, MYBL2, MYC)
  - Early response/genotypic stress (GADD45B)

# OncotypeDx Colon

- 12 gene recurrence score derived from 1851 patients with stage II, III CRC who participated in NSABP adjuvant studies
- Analytic validation published in 2010
- Intended use: assist in decision for adjuvant therapy

# OncotypeDx Colon: stage II

The Oncologist 2014;19:492-97

- MMR (IHC) and Oncotype Dx
- 221 pts: 141 had T3 MMR proficient tumors
  - 71% were low risk
  - 5% were high risk
  - 25% were MMR deficient
- 45% had changes in treatment plan
  - 33% decreased intensity; 11% increased intensity
  - Chemo recommendations decreased from 52% to 30%

# Oncotype Dx Colon

- Prognostic
- Not predictive
- Higher risk have same relative but potentially higher absolute benefit from chemo (stage II, III A/B)
- Use in conjunction with T stage, MMR status and clinical information

# Non Small Cell Lung Cancer

- EGFR
- ALK
- KRAS

## Category 1 (NCCN)

- EGFR, ALK; recommend multiplex sequencing or FISH
  - Del 19; L858R
  - T790M
  - IHC ALK
- Adequate data these are predictive
- Also prognostic

# EGFR: Clinical Validity and clinical utility

- 2 approved companion diagnostics
- *therascreen® EGFR RGQ PCR Kit*
- *LuxLung3*: Phase 3 trial of afatinib versus chemotherapy as first-line treatment for patients with stage IIB or IV adenocarcinoma of the non-small cell lung cancer (NSCLC) harboring an EGFR mutation (ClinicalTrials.gov number NCT00949650).
- **cobas® EGFR Mutation Test**
- **Phase 3 EURTAC: first line Tarceva® (erlotinib) versus standard platinum-based chemotherapy**

# ALK Clinical Validity and clinical utility

- FDA approved VYSIS break-apart FISH Assay
- Intended use: aid in the selection of previously treated patients with NSCLC for crizotinib treatment
- Phase II study of crizotinib in NSCLC patients with ALK translocation

# KRAS in NSCLC

- Prognostic? No conclusive evidence
- Mutually exclusive with EGFR
- If KRAS mutation, may end additional testing

# Invasive Breast Cancer

- Mammaprint
- Oncotype Dx

# Breast Cancer: Mammaprint

- Agilent GE array: 70 Genes
- Intended use: predict recurrence risk at 5 years
- Analytic and clinical validity: EGAPP and FDA clearance (women < 61 years with stage I, II LN- breast cancer)
- Clinical Utility: MindAct

# Breast Cancer: Oncotype Dx

- Analytical validation
- Intended use: predict 10 year recurrence risk in early stage ER+ breast cancer after surgery
- Clinical validation in 2 retrospective prospective trials (B20, SWOG-8814)
- Waiting for TailoRx and Rxponder
- Who can avoid chemotherapy?
- High score  $\geq 31$ : chemotherapy
- Improved subgrouping?

# Clinical Utility

- EGAPP did not find evidence of clinical utility for either test
  - Predict effect of chemotherapy
  - Used in clinical decision
  - Changed clinical decision
  - Cost effectiveness, budgetary impact
- Awaiting results of clinical trials
  - TailoRx
  - MindAct



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