Prognostic and Predictive Tests in Colorectal Cancer

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December 2010
Conflict of Interest:

1. No speaker’s bureaus, stock ownership, royalties, etc
2. Unpaid Scientific Advisory Boards with Genentech, AstraZeneca
3. Principal investigator of laboratory projects sponsored by Pfizer and Roche
Colorectal Cancer
Predictive / Prognostic Tests

Outline:
1. Brief background
2. Overview of colorectal cancer treatment
3. Predictive tests
4. Prognostic tests
Men 290,000
Women 270,000

Colorectal cancer represents 2nd leading cause of death

Available at: http://www.cancer.org.
# 7 Drugs for Colorectal Cancer

## “Cytotoxics”

<table>
<thead>
<tr>
<th>Drug</th>
<th>Mechanism</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. 5-Fluorouracil (5-FU)</td>
<td>-&gt; pyrimidine analog</td>
</tr>
<tr>
<td>2. Capecitabine (Xeloda)</td>
<td>-&gt; oral 5-FU pro-drug</td>
</tr>
<tr>
<td>3. Irinotecan (Camptosar)</td>
<td>-&gt; topoisomerase I inhibitor</td>
</tr>
<tr>
<td>4. Oxaliplatin (Eloxatin)</td>
<td>-&gt; 3rd generation platinum</td>
</tr>
</tbody>
</table>

## “Biologics”

<table>
<thead>
<tr>
<th>Drug</th>
<th>Mechanism</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Cetuximab (Erbitux)</td>
<td>-&gt; antibody against EGFR</td>
</tr>
<tr>
<td></td>
<td>Epidermal Growth Factor Receptor</td>
</tr>
<tr>
<td>2. Panitumumab (Vectibix)</td>
<td>-&gt; antibody against EGFR</td>
</tr>
<tr>
<td>3. Bevacizumab (Avastin)</td>
<td>-&gt; antibody against VEGF</td>
</tr>
<tr>
<td></td>
<td>Vascular Endothelial Growth Factor</td>
</tr>
</tbody>
</table>
Chemotherapy for Colorectal Cancer

Combination Regimens: Terminology

**FOLF-** Leucovorin (FOLinic acid) / infusional 5-FU
FOLF-OX + oxaliplatin (Eloxatin)
FOLF-IRI + irinotecan (Camptosar)

FOLFOX = infusional 5-FU/LV (two days) + oxaliplatin
FOLFIRI = infusional 5-FU/LV + irinotecan

**CAP-** Capecitabine (Xeloda)
CAP-OX = XELOX = capecitabine + oxaliplatin
CAP-IRI = XELIRI = capecitabine + irinotecan
Chemotherapy for Colorectal Cancer

“Infusional” versus “Bolus”

Bolus – rapid administration

5-FU
LV
weekly

Infusional – slow, sustained administration

5-FU
LV
Q2 weeks
5-FU infusion
History of Treatment for Colorectal Cancer

- **~1960**: 5-FU is a cornerstone of first-line therapy; bolus/infusion
- **~1985**: Addition of LV (biomodulator) to 5-FU bolus regimens
- **1998**: Irinotecan as single agent approved as second-line
- **2000**: Irinotecan approved as first-line in CRC (bolus IFL)
- **2001**: Capecitabine approved as first-line in CRC in selected pts
- **2002**: Oxaliplatin approved as second-line agent (FOLFOX)
- **2004**: Oxaliplatin approved as first-line agent in infusional regimen
- **2004**: Approval of Cetuximab (Erbitux) & Bevacizumab (Avastin)
- **2006**: Approval of Panitumumab (Vectibix)
- **2008**: KRAS mutations predict lack of benefit of EGFR mAb’s
- **2008**: Negative studies (harm) with “double biologics”
- **2010**: Negative studies for adjuvant cetuximab, bevacizumab
Chemotherapy for CRC

• **Marked Improvements in the last decade**
  
  – **Six new drugs since 1998**
    
    (cytotoxics: irinotecan, oxaliplatin, capecitabine)
    (biologics: cetuximab, bevacizumab, panitumumab)
  
  – **Overall survival has doubled** compared to 5-FU alone
    
    • 19-24 month median survival in modern trials
  
  – **Response rates in large trials as high as 60%**
    
    • CRYSRTAL ¹ (KRAS WT, FOLFIRI/Cetx – 59%)
    • OPUS ² (KRAS WT, FOLFOX/Cetx – 61%)
    • GONO ³ (FOLFOXIRI – 66%)
  
  – **KRAS testing** opened era of “targeted” patients

¹ Van Cutsem, ASCO 2008; ² Bokemeyer, JCO 2009; ³ Falcone, JCO 2007
Incremental Survival Advantage in First-Line Metastatic Colorectal Cancer

- No active drug: ~4-6 mo
- 5-FU/LV: 12-14 mo
- IFL: ~15-16 mo
- FOLFOX4: ~20 mo
- IFL + bevacizumab: 20.3 mo
- FOLFOX/FOLFIRI: 21.5 mo
- FOLFOX/FOLFIRI + double biologics: ~?

Are we hitting a wall with current drugs?
Colorectal Cancer
Predictive / Prognostic Tests

Outline:
1. Brief background
2. Overview of colorectal cancer treatment
3. Predictive tests
4. Prognostic tests
**Therapy for Advanced Colorectal Cancer:**

**Response rates and survival**

<table>
<thead>
<tr>
<th>First Line</th>
<th>Second Line</th>
<th>Third Line</th>
</tr>
</thead>
<tbody>
<tr>
<td>- FOLFOX or</td>
<td>- FOLFOX or</td>
<td>- Irinotecan + Cetuximab (KRAS)</td>
</tr>
<tr>
<td>- CAPOX or</td>
<td>- FOLIRI or</td>
<td>- Cetuximab (KRAS)</td>
</tr>
<tr>
<td>- FOLFIRI</td>
<td>- Irinotecan alone</td>
<td>- Panitumumab</td>
</tr>
<tr>
<td>+/- Bevacizumab</td>
<td>+/- Bevacizumab</td>
<td></td>
</tr>
<tr>
<td>+/- Cetx/Pmab (KRAS)</td>
<td>- Irinotecan/Cetuximab</td>
<td>(KRAS)</td>
</tr>
</tbody>
</table>

**Response Rates in Randomized Trials:**

- 30-60%
- 5-15%
- 10-20%

**Survival Benefit in Randomized Trials:**

- Yes
- Yes
- Yes
Studies of Cytotoxics for Advanced Colorectal Cancer #1: Tournigand Trial (GERCOR)

Randomized trial of 220 patients comparing the sequence of FOLFOX and FOLFIRI.

Untreated advanced colorectal cancer (N=220)

Arm A: FOLFIRI -> FOLFOX

Arm B: FOLFOX -> FOLFIRI

First-line therapy continued until progression, at which time the second-line therapy was instituted.
Doesn’t matter whether you start with FOLFOX or FOLFIRI

There are specific reasons why one regimen may be chosen over another for an individual patient.

Tournigand, *JCO* 2004

Fig 4. Overall survival curves. FOLFIRI, folinic acid, fluorouracil, and irinotecan; FOLFOX6, folinic acid, fluorouracil, and oxaliplatin.
Studies of Cytotoxics for Advanced Colorectal Cancer
• Substituting capecitabine for infusional 5-FU

#2 NO16966 (Cassidy, ESMO 2006)

Randomized phase III trial of 2035 patients comparing FOLFOX vs XELOX (with or without bevacizumab) in first-line colorectal CA

Previously untreated metastatic colorectal cancer
n=2034

FOLFOX = 5-FU. LV, oxaliplatin
n=1017

XELOX = capecitabine, oxaliplatin
n=1017
XELOX and FOLFOX appear equivalent
NO16966 (Cassidy, ESMO 2006)

HR=1.04 [97.5% CI 0.93–1.16]
Upper limit ≤ 1.23
(non-inferiority margin)

“Targeted Therapy”, or “Biologics” in Colorectal Cancer

1) Cetuximab
   (Erbitux™; monoclonal antibody against EGFR)

2) Panitumumab
   (Vectibix™; monoclonal antibody against EGFR)

3) Bevacizumab
   (Avastin™; monoclonal antibody against VEGF)

EGFR = Epidermal Growth Factor Receptor

VEGF = Vascular Endothelial Growth Factor
The HER Family

HER = Human Epidermal growth factor Receptor

growth factor binding sets off a signaling cascade in the cell to stimulate cancer cell growth

Ligands:

Transmembrane

Tyrosine kinase

Ligand binding

Receptors:

\[
\begin{align*}
\text{erb-b1} & \quad \text{EGFR} & \quad \text{HER1} \\
\text{neu} & \quad \text{Erb-b2} & \quad \text{HER2} \\
\text{Erb-b3} & \quad \text{HER3} \\
\text{Erb-b4} & \quad \text{HER4}
\end{align*}
\]
In contrast, “small molecule” tyrosine kinase inhibitor likely cause compensatory over-expression of receptors.

ERBITUX package insert, February 2004
Studies of Biologics for Advanced Colorectal Cancer
#1: “Bond” Trial (Cunningham, NEJM 2004)

Randomized phase II trial of 329 patients with irinotecan-refractory disease comparing cetuximab with cetuximab/irinotecan.

EMR 62202-007

Irinotecan-refractory advanced CRC

N=329 (randomized 2:1)

EGFR ≥1+ IHC

Arm A: Cetuximab* + irinotecan → PD

Arm B: Cetuximab* → PD
Optional crossover to Arm A

*400 mg/m² wk 1 (loading), then 250 mg/m² qweek.
Cetuximab With and Without Irinotecan in HER1/EGFR-Positive Irinotecan-Refractory Metastatic Colorectal Cancer: Time to Progression

Cunningham, NEJM 2004

Note: overall survival curves were nearly identical.
Did staining for EGFR matter?  

NO!

Positive

Faint/Barely

1+

21%

Weak to Moderate

2+

25%

Strong

3+

23%

Negative

0

Response Rates

Courtesy of DakoCytomation, 2004
Unique side effects:

- Patient on EGFR inhibitor similar to cetuximab or panitumumab

- Also carefully monitor magnesium with EGFR mAb’s

Herbst et al, JCO 2002
Blood Vessels and Tumor Growth

• Solid tumors cannot grow beyond 1 to 2 mm³ without an increase in blood supply via new vessel formation¹

• “Angiogenesis” is thus required for tumor growth and metastasis¹

• Inhibition of tumor angiogenesis leads to tumor cell growth arrest, death of tumor cells, and in some cases, tumor regression²

Tumor angiogenesis is stimulated… New vessels then facilitate tumor growth.

Courtesy of Novartis Oncology
Studies of Biologics for Advanced Colorectal Cancer

#2 Bevacizumab Trial (Hurwitz, NEJM 2004)

Randomized phase III trial of 813 patients comparing chemo with or without bevacizumab (Avastin)

“AVF2107”

Previously untreated metastatic colorectal cancer

N=813

Arm A: IFL + Bevacizumab*

Arm B: IFL + Placebo

IFL = Irinotecan / 5-FU / Leucovorin
Bevacizumab (5 mg/kg q2 weeks) added to IFL significantly improved overall survival by 4.5 months.

Hurwitz, NEJM 2005
Studies of Biologics for Advanced Colorectal Cancer
• Substituting capecitabine for infusional 5-FU
• Adding bevacizumab to oxaliplatin regimens 1\textsuperscript{st} line

#3 NO16966 (Cassidy, ESMO 2006)

Randomized phase III trial of 2035 patients comparing FOLFOX vs XELOX with or without bevacizumab (Avastin) in first-line colorectal CA

- Previously untreated metastatic colorectal cancer
  - n=2035

- FOLFOX = 5-FU. LV, oxaliplatin
- XELOX = capecitabine, oxaliplatin
Survival Advantage for Bevacizumab NO16966 Pooled Analysis (Cassidy, ESMO 2006)

- **FOLFOX + Placebo/XELOX + Placebo, n=701; 547 events**
- **FOLFOX + Bev/XELOX + Bev, n=699; 513 events**

HR=0.83 [97.5% CI 0.72–0.95] (ITT)

*P*=0.0023

Colorectal Cancer: Chemotherapy

Key Trials: Biologics

“Dual Biologic” strategy of combining VEGF- (bevacizumab) and EGFR-targeting antibodies (panitumumab or cetuximab) with chemotherapy”

(surely 5 drugs must be better than 4)
Studies of Biologics for Advanced Colorectal Cancer

#4 PACCE Trial

Randomized phase III trial of 1000 patients comparing chemo/Bev with or without panitumumab (Vectibix)

Previously untreated metastatic colorectal cancer
N=1000

5-FU/Oxali/Bev
N=800
+ panitumumab
(alone)

5-FU/Irino/Bev
N=200
+ panitumumab
(alone)

Hecht, World GI 2007
#4 PACCE Trial
Progression-Free Survival

Hecht, World GI 2007

## Patients at risk:

<table>
<thead>
<tr>
<th>Treatment</th>
<th>N</th>
<th>0</th>
<th>5</th>
<th>10</th>
<th>15</th>
<th>20</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pmab+bev/Ox-CT</td>
<td>413</td>
<td>267</td>
<td>92</td>
<td>21</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>bev/Ox-CT</td>
<td>410</td>
<td>298</td>
<td>96</td>
<td>21</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

## # PFS events

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Count (%)</th>
<th>Median (95%CI), mos</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pmab+bev/Ox-CT</td>
<td>206 (50)</td>
<td>9.0 (8.5-10.4)</td>
</tr>
<tr>
<td>bev/Ox-CT</td>
<td>172 (42)</td>
<td>10.5 (9.7-11.6)</td>
</tr>
</tbody>
</table>

HR = 1.29 (95% CI: 1.05-1.58)

Control group did better!
CAIRO2 and PACCE

CAIRO2 design was similar to PACCE, except with capecitabine-based regimens

Primary endpoint was progression-free survival

Previously untreated metastatic colorectal cancer

N=755

CAPOX/Bev

CAPOX/Bev + Cetuximab
CAIRO2 similar to PACCE
Progression-Free Survival

Progression-free survival

Arm A (without cetuximab) 10.7 months (9.7-12.5)
Arm B (with cetuximab) 9.6 months (8.5-10.7)

Hazard ratio for progression 1.21
p value 0.018

Again, control arm did better!
Therapy for Advanced Colorectal Cancer: CONCLUSIONS

1) Mainstays of therapy are:
   -“conventional chemo”: 5-FU (with leucovorin), capecitabine, irinotecan, oxaliplatin
       -“biologics”: bevacizumab, cetuximab, panitumumab

2) Infusional regimens (FOLFOX, FOLFIRI) and capecitabine/oxaliplatin (CAPOX) are standard of care

3) Bevacizumab (Avastin) is FDA-approved for use with first-line and second-line (Bev naïve) regimens.

4) Cetuximab (Erbitux) and Panitumumab (Vectibix) are FDA-approved for chemoresistant disease in KRAS wildtype pts.

5) Double biologics should not be used outside of a clinical trial
Other Chemotherapy Strategies

“Stop and Go” = No decrement in Survival

OPTIMOX1 (n=620): Tournigand, JCO 2006

- FOLFOX4
- FOLFOX7 → 5-FU → FOLFOX7

OPTIMOX2 (n=202): Maindrault-Goebel, ASCO 2006

- FOLFOX7 → 5-FU → FOLFOX7
- FOLFOX7 → Observation → Progression → FOLFOX7

GISCAD (n=336): Labianca, ACSO 2006

- FOLFIRI
- FOLFIRI → Break → FOLFIRI → Break
## Chemotherapy for Colorectal Cancer

Can we afford this?  >$7.5 Billion Dollars in the US!

<table>
<thead>
<tr>
<th>Drug</th>
<th>1995 Cost</th>
<th>2004 Cost</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-FU (500 mg/m²)</td>
<td>$9</td>
<td></td>
</tr>
<tr>
<td>Leucovorin (500 mg/m²)</td>
<td>$61</td>
<td></td>
</tr>
<tr>
<td>Capecitabine (2000 mg/m²/day)</td>
<td>$853</td>
<td></td>
</tr>
<tr>
<td>Irinotecan (180 mg/m²) -&gt; generic</td>
<td>($2,608)</td>
<td>($2,983)</td>
</tr>
<tr>
<td>Oxaliplatin (85 mg/m²) -&gt; generic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bevacizumab (5 mg/kg)</td>
<td>$2,750</td>
<td></td>
</tr>
<tr>
<td>Cetuximab (250 mg/m²)</td>
<td>$5,760</td>
<td></td>
</tr>
<tr>
<td>Panitumumab (6 mg/kg)</td>
<td>$3,460</td>
<td></td>
</tr>
</tbody>
</table>

1995: 6 months of 5-FU/LV costs ~$500

2004: 20 months therapy with combinations costs >$250,000

L. Saltz, ASCO 2004
Colorectal Cancer
Predictive / Prognostic Tests

Outline:
1. Brief background
2. Overview of colorectal cancer treatment
3. Predictive tests
4. Prognostic tests
Definitions (1):

1. **Predictive Marker**: factor associated with response or benefit to therapy
   - **positive** (her2neu and trastuzumab)
   - **negative** (KRAS and EGFR mAb’s)

2. **Prognostic Marker**: characteristic at diagnosis associated with a clinical outcome such as survival
   - **Strict definition** – with no treatment
Desirable Qualities of Assays (1)

1. **Objective:** not subject to an observer’s interpretation (e.g., 1+ versus 2+ staining, immunohistochemistry)

2. **Sensitive:** small amounts of tumor (e.g., PCR-based reactions), and/or low % of tumor cells in specimen

3. **Early oncogenic event:** re-biopsies of metastatic sites not needed
Pharmacodiagnosics

Desirable Qualities of Assays (2)

4. **Consistent:** not variable between various metastatic sites (again, early event)

5. **Biologically plausible:** functional ramifications in important pathways

6. **Unaffected by tissue processing:** snap-freezing not needed; time in formalin not crucial (for archival tissue)
Colorectal Diagnostics: What Was State of Art in 2008? (two years ago)

**CEA** – recommended for staging, post-operative, monitoring response

**DNA ploidy or proliferation assays** – not recommended

**p53, TS, DPD, TP** – insufficient data

**Ras** – insufficient data

**MSI** – not recommended

**18q- or DCC** - not recommended

**FDA approved tests for colorectal cancer (not widely used):**
- **EGFR Immunohistochemistry** (Dakocytomation PharmDx)
- **UGT1A1 Invader Assay** (irinotecan glucuronidation) - toxicity
- **Circulating tumor cells** (CellSearch test) - prognosis
RAS Genes and Proteins

- The three RAS genes encode highly homologous proteins: HRAS, NRAS, KRAS 4A and KRAS 4B (alternative splicing)\(^1\)

- GTP/GDP-binding proteins (21 kDa) located at inner surface of the plasma membrane; signal transducers

- Somatic point mutations of RAS genes occur in about 30% of all cancers\(^1\)

- Mutations result in amino acid substitutions at codons 12, 13, 61 which favor GTP-bound, active state.

- KRAS mutation is an early event in polyp progression\(^2\); high concordance between primary and metastases\(^3\)

\(^1\)Schubbert, Nat Rev Cancer 2007; \(^2\)Fearon and Vogelstein, Cell 1990; \(^3\)Santini, Oncologist 2008
- First 85 amino acids are identical in the four isoforms (HRAS, NRAS, KRAS4A, KRAS4B), and include:
  - “P-loop” (aa 10-16) bind phosphate of GDP and GTP
  - “Switch I” and “Switch II” mediate binding to regulators and effectors
  - C terminal end has hypervariable region which specifies membrane localization through post-translational modifications such as farnesylation

EGFR Signalling: Activation of RAS/RAF/MEK or PTEN/PI3K/AKT Pathways Can Mediate Resistance to EGFR Blockade

Nearly all mutations occur in codon 12 and 13

## Single-Arm Study Results: Response

<table>
<thead>
<tr>
<th>Study</th>
<th>Treatment</th>
<th>Total Pts</th>
<th>MT</th>
<th>WT</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Benvenuti</strong></td>
<td>P or C or C + CT</td>
<td>48</td>
<td>1 (6)</td>
<td>10 (31)</td>
</tr>
<tr>
<td>2007</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>De Roock</strong></td>
<td>C ± CT</td>
<td>113</td>
<td>0 (0)</td>
<td>27 (41)</td>
</tr>
<tr>
<td>2008</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Finocchiaro</strong></td>
<td>C ± CT</td>
<td>81</td>
<td>2 (6)</td>
<td>13 (26)</td>
</tr>
<tr>
<td>2007</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Di Fiore</strong></td>
<td>C + CT</td>
<td>59</td>
<td>0 (0)</td>
<td>13 (28)</td>
</tr>
<tr>
<td>2007</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Khambata</strong></td>
<td>C</td>
<td>80</td>
<td>0 (0)</td>
<td>5 (10)</td>
</tr>
<tr>
<td>2007</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Lievre</strong></td>
<td>C CT</td>
<td>114</td>
<td>0 (0)</td>
<td>34 (44)</td>
</tr>
<tr>
<td>2008</td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

C = cetuximab; P = panitumumab; CT = chemotherapy
## Randomized Trial Results

### Median PFS (Cetux- or Pmab- containing arms)

<table>
<thead>
<tr>
<th>Study</th>
<th>Treatment</th>
<th>Total Pts</th>
<th>MT</th>
<th>WT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amado 2008</td>
<td>P versus BSC (3rd line)</td>
<td>427</td>
<td>7.4 wks</td>
<td>12.3 wks</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>HR 0.99</td>
<td>HR 0.45</td>
</tr>
<tr>
<td>Karapetis 2008</td>
<td>C versus BSC (no X-over)</td>
<td>394</td>
<td>1.9 mos</td>
<td>3.7 mos</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>HR 0.40</td>
<td></td>
</tr>
<tr>
<td>Van Cutsem 2008</td>
<td>FOLFIRI +/- C (1st line)</td>
<td>540</td>
<td>7.6 mos</td>
<td>9.9 mos</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>HR 1.07</td>
<td>HR 0.68</td>
</tr>
<tr>
<td>Bokemeyer 2008</td>
<td>FOLFOX +/- C (1st line)</td>
<td>233</td>
<td>5.5 mos</td>
<td>7.7 mos</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>HR 1.83</td>
<td>HR 0.57</td>
</tr>
</tbody>
</table>

C = cetuximab; P = panitumumab; BSC = best supportive care
Amgen “408” Trial

- Patients had to be “EGFR positive,” defined as ≥1% tumor cells staining by IHC
- Pretreated with 5-FU, oxaliplatin, irinotecan

Previously treated metastatic colorectal cancer
N=463

Panitumumab 6 mg/kg Q2 weeks

Best Supportive Care

Optional Crossover

PFS by treatment overall

Resulted in FDA approval of Panitumumab for use in 3rd line setting

PFS by treatment within KRAS groups

KRas and Panitumumab

Note: only seven different Ras mutations seen

Waterfall plot shows responses (tumor shrinkage) were confined to Ras WT patients

NCIC CO.17 Trial

-Published in the NEJM since this was the only study to show an OS difference (no cross-over)

- Pretreated with 5-FU, oxaliplatin, irinotecan

Previously treated metastatic colorectal cancer
N=572

Cetuximab 250 mg/m² weekly (1st dose 400)

Best Supportive Care

Karapetis et al. NEJM 2008; 359(17):1757-1765
Overall Survival: Mutant

Not Prognostic! (BSC patients)

Karapetis et al. NEJM 2008; 359(17):1757-1765
Is K-Ras Predictive for cytotoxic chemo?  Probably Not

<table>
<thead>
<tr>
<th></th>
<th>KRAS WT</th>
<th></th>
<th>KRAS MT</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cetuximab +FOLFIRI</td>
<td>FOLFIRI</td>
<td>Cetuximab +FOLFIRI</td>
<td>FOLFIRI</td>
</tr>
<tr>
<td>n</td>
<td>172</td>
<td></td>
<td>176</td>
<td></td>
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<tr>
<td></td>
<td>105</td>
<td></td>
<td>87</td>
<td></td>
</tr>
<tr>
<td>ORR (%)</td>
<td>59</td>
<td></td>
<td>43</td>
<td></td>
</tr>
<tr>
<td></td>
<td>36</td>
<td></td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>p-value</td>
<td>0.0025</td>
<td></td>
<td>0.46</td>
<td></td>
</tr>
<tr>
<td>mPFS (mos)</td>
<td>9.9</td>
<td></td>
<td>8.7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>7.6</td>
<td></td>
<td>8.1</td>
<td></td>
</tr>
<tr>
<td>HR</td>
<td>0.68</td>
<td></td>
<td>1.07</td>
<td></td>
</tr>
<tr>
<td>p-value</td>
<td>0.017</td>
<td></td>
<td>0.47</td>
<td></td>
</tr>
</tbody>
</table>

FOLFOX HR=1.404; p=0.1655
mPFS FOLFOX wild-type (n=73): 7.2 months
mPFS FOLFOX mutant (n=47): 8.6 months

CRYSTAL Van Cutsem, ASCO 2008

OPUS Bokemeyer, ASCO 2008
Do EGFR antibodies harm KRAS MT?  
Yes, especially combined with bevacizumab (VEGF antibody)

Progression-Free Survival and Hazard Ratios of EGFR Ab Arms

<table>
<thead>
<tr>
<th>Study</th>
<th>Treatment</th>
<th>Total Patients</th>
<th>MT</th>
<th>WT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tol NEJM 2009</td>
<td>CAPOX/Bev +/- C</td>
<td>755</td>
<td><strong>8.1 mos (worst)</strong></td>
<td>10.5 mos</td>
</tr>
<tr>
<td>Hecht JCO 2009</td>
<td>5-FU/OX/Bev +/- P</td>
<td>823</td>
<td>10.4 mos HR=1.25</td>
<td>9.8 mos HR 1.36</td>
</tr>
<tr>
<td>Hecht JCO 2009</td>
<td>5-FU/IRI/Bev +/- P</td>
<td>230</td>
<td>8.3 mos HR 1.19</td>
<td>10.0 mos HR 1.50</td>
</tr>
<tr>
<td>Bokemeyer JCO 2009</td>
<td>FOLFOX +/- C</td>
<td>344</td>
<td><strong>5.5 mos HR 1.83</strong></td>
<td>7.7 mos HR 0.57</td>
</tr>
</tbody>
</table>

CAP = capecitabine; OX = oxaliplatin; IRI = irinotecan; Bev = bevacizumab; C = cetuximab; P = panitumumab
### What is the best KRAS test? Unknown

**KRAS Mutation Analysis Methods**

<table>
<thead>
<tr>
<th>Method</th>
<th>Principle</th>
<th>Sensitivity (MT/WT; %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Direct sequencing</td>
<td>Non-mutation-specific determination of test case nucleotide sequence and comparison with normal sequence</td>
<td>20-50</td>
</tr>
<tr>
<td>Restriction fragmentation length polymorphism, confirmed by direct sequencing</td>
<td>Mutation presence induces or eliminates specific sites where DNA-targeting enzymes insert cuts in DNA</td>
<td>0.10</td>
</tr>
<tr>
<td>Allele specific probe</td>
<td>Polymerase chain reaction/selective detection</td>
<td>10</td>
</tr>
<tr>
<td>High resolution melting analysis, confirmed by direct sequencing</td>
<td>Sequences with mutations hybridize at different, fixed temperatures</td>
<td>5</td>
</tr>
<tr>
<td>Amplification refractory mutation system</td>
<td>Mutation specific polymerase chain reaction/detection</td>
<td>1</td>
</tr>
</tbody>
</table>

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Jimeno, Messersmith, Hirsch, Franklin, and Eckhardt, JCO 2009
50 CRC samples analyzed using sections vs blocks; various testing methods. ARMS/Scorpion on cores from blocks appears most sensitive (in press)
PI3K Mutations
(Samuels, Science 2004)

High Frequency of Mutations of the PIK3CA Gene in Human Cancers

Yardena Samuels,1 Zhenghe Wang,1 Alberto Bardelli,1 Natalie Silliman,1 Janine Ptak,1 Steve Szabo,1 Hai Yan,2 Adi Gazdar,3 Steven M. Powell,4 Gregory J. Riggins,1 James K. V. Willson,5 Sanford Markowitz,5 Kenneth W. Kinzler,1 Bert Vogelstein,1 Victor E. Velculescu1*

Functionally important:
- Nontruncating
- Nonsynonymous
- Conserved residues
- Higher PI3K activity

- Colorectal and gastric cancers frequently harbor mutations.
- Not found in 76 polyps (except two >5cm tubulovillous adenomas)
- Co-existent with KRAS and BRAF mutations (distinct pathway)
PI3K Pathway as a Target

PI3K (phosphoinositide 3-kinases) is a family of lipid kinases which activate a signal transduction cascade promoting cancer growth and survival.

Discovered in 1980’s, probably the most commonly activated pathway in human cancers.

Multiple PI3K effectors (via phospholipids)
- AKT (AK-transforming)
- Non-AKT
  - BTK (Bruton tyrosine kinase)
  - SGK’s (serum/glucocorticoid kinases)
  - Tec (nonreceptor tyrosine kinase)
How strong is PI3K data?
Retrospective clinical cases; conflicting.

<table>
<thead>
<tr>
<th>Report</th>
<th>Drugs</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lievre</td>
<td>C</td>
<td>7% with PI3K mut’n</td>
</tr>
<tr>
<td>Can Res 2006, n=30</td>
<td>(97% chemo)</td>
<td>no effect</td>
</tr>
<tr>
<td>Sartore-Bianchi</td>
<td>C and P</td>
<td>13.6% with PI3K mut’n</td>
</tr>
<tr>
<td>Can Res 2009, n=110</td>
<td>(67% chemo)</td>
<td>lower RR, survival</td>
</tr>
<tr>
<td>Perrone</td>
<td>C</td>
<td>13% with PI3K mut’n</td>
</tr>
<tr>
<td>Ann Oncol 2009, n=32</td>
<td>(100% chemo)</td>
<td>lower RR, survival</td>
</tr>
<tr>
<td>De Roock (#1896)</td>
<td>C and P</td>
<td>12% with PI3K mut’n</td>
</tr>
<tr>
<td>AACR 2009, n=200</td>
<td></td>
<td>no effect</td>
</tr>
<tr>
<td>Di Nicolantonio</td>
<td>C and P</td>
<td>13% with PI3K mut’n</td>
</tr>
<tr>
<td>AACR 2009, n=132</td>
<td></td>
<td>lower RR, survival</td>
</tr>
</tbody>
</table>
PI3K as a predictive marker
Prenen, Clinic Can Res 2009, n=200, 12% PIK3CA MT

- Used sequenome MALDI-TOF MassArray system
- No relationship between PIK3CA and KRAS
- No relationship between PIK3CA and response, survival

Note: PIK3CA mutations have been associated with resistance to trastuzumab in breast cancer (possible increased dependence on her2/her3 dimerization). Burns, Cancer Cell 2007
PI3K testing ready for the clinic?  No

- Need confirmation from randomized trials.

- Mutation-based biomarkers and assays are
  - more definitive / quantitative than other diagnostics (e.g., IHC)
  - less likely to be affected by tissue processing / storage (important!)

- but... unknowns include correlation between primary and metastases; effects of cytotoxic chemotherapy; etc
# How strong is PTEN data?

Retrospective clinical cases only; conflicting.

<table>
<thead>
<tr>
<th>Report</th>
<th>Drugs</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frattini</td>
<td>C</td>
<td>41% with loss of PTEN</td>
</tr>
<tr>
<td>BJC 2007, n=27</td>
<td>(67% chemo)</td>
<td>no responses</td>
</tr>
<tr>
<td>Loupakis</td>
<td>C</td>
<td>42% with loss of PTEN</td>
</tr>
<tr>
<td>ASCO 2008, n=102</td>
<td>(100% chemo)</td>
<td>lower RR and PFS</td>
</tr>
<tr>
<td>Razis</td>
<td>C</td>
<td>14% with loss PTEN</td>
</tr>
<tr>
<td>BMC Can 2008, n=72</td>
<td>(98% chemo)</td>
<td>higher RR and TTP</td>
</tr>
<tr>
<td>Di Nicolantonio</td>
<td>C and P</td>
<td>31% with loss PTEN</td>
</tr>
<tr>
<td>AACR 2009, n=132</td>
<td></td>
<td>lower RR and PFS</td>
</tr>
</tbody>
</table>

RR = response rate; PFS = Progression-free survival; TTP = time to progression
# PTEN Assay Variability

<table>
<thead>
<tr>
<th>Report</th>
<th>PTEN Assay and Scoring</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Frattini</strong></td>
<td>IHC; “dramatic reduction or absence” in &gt;50% cells</td>
</tr>
<tr>
<td>BJC 2007, n=27</td>
<td></td>
</tr>
<tr>
<td><strong>Loupakis</strong></td>
<td>IHC; score = percentage x intensity</td>
</tr>
<tr>
<td>ASCO 2008, n=102</td>
<td>Score ≥ 4 = “PTEN positive”</td>
</tr>
<tr>
<td><strong>Razis</strong></td>
<td>FISH analysis</td>
</tr>
<tr>
<td>BMC Can 2008, n=72</td>
<td>Ratio 0.85 – 1.15 = “normal”</td>
</tr>
<tr>
<td><strong>Di Nicolantonio</strong></td>
<td>? (Await publication)</td>
</tr>
<tr>
<td>AACR 2009, n=132</td>
<td></td>
</tr>
</tbody>
</table>

IHC = immunohistochemistry; FISH = fluorescence in situ hybridization
PTEN ready for clinic? No

- Different assays (IHC vs FISH), different antibodies, different cut-offs

- Correlation between primary and metastases appears poor (only 60%\textsuperscript{1})

- PTEN loss appears to carry a poor prognosis in colorectal cancer\textsuperscript{2}

- Effects of tissue processing (delays before formalin; formalin fixation time; storage conditions) not fully characterized

\textsuperscript{1}Loupakis, ASCO 2008; \textsuperscript{2}Sawai, BMC GI 2008
Complication of Overlapping Markers

KRAS and BRAF are usually mutually exclusive; but PIK3CA, NRAS, others are not!

DeRoock, Lancet Oncol 2010
Additive Prediction

Response rate increase as markers are added (retrospective database)

DeRoock, Lancet Oncol 2010
Are all KRAS mutations the same?

- KRAS mut’ns usually in codons 12 and 13
- Biology may be different for various mutations
- 17% have codon 13 mut’ns

In this pooled analysis (n=529) of 7 clinical trials and off-study patients treated with cetuximab, G13D patients fared as well as KRAS wild-type patients. (D = aspartic acid)

©2010 American Medical Association
Epiregulin and Amphiregulin

EGFR ligands found to be associated with response to cetuximab treatment in profiling efforts (Khambata-Ford, JCO 2007)

\[ N = 110 \]
All had pretreatment biopsies

- Technical feasibility of obtaining mRNA from archival tissue was a major challenge.
Cetuximab-treated patients whose tumors had high epiregulin mRNA (along with KRAS WT) had **longest survival.**
Colorectal Cancer Predictive / Prognostic Tests

Outline:
1. Brief background
2. Overview of colorectal cancer treatment
3. Predictive tests
4. Prognostic tests
V600E (500-fold greater kinase activity) is most common mutation. BRAF and KRAS mutations appear to be mutually exclusive.
Retrospective BRAF data?  Predictive Clinical cases were consistent.

<table>
<thead>
<tr>
<th>Report</th>
<th>Drugs</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benvenuti</td>
<td>C and P</td>
<td>12% had BRAF mut’n</td>
</tr>
<tr>
<td>Can Res 2007, n=48</td>
<td>(23% chemo)</td>
<td>lower RR, survival</td>
</tr>
<tr>
<td>Di Nicolantonio</td>
<td>C and P</td>
<td>10% had BRAF mut’n</td>
</tr>
<tr>
<td>JCO 2008, n=113</td>
<td>(45% chemo)</td>
<td>lower RR, survival</td>
</tr>
<tr>
<td>Laurent-Puig</td>
<td>C</td>
<td>3% had BRAF mut’n</td>
</tr>
<tr>
<td>AACR 2009, n=133</td>
<td></td>
<td>shorter survival</td>
</tr>
<tr>
<td>Di Nicolantonio</td>
<td>C and P</td>
<td>8% with BRAF mut’n</td>
</tr>
<tr>
<td>AACR 2009, n=132</td>
<td></td>
<td>lower RR, survival</td>
</tr>
</tbody>
</table>

Note: BRAF testing only performed on KRAS WT samples in some studies.
Prospective trial BRAF data? Prognostic “CRYSTAL”

- First-line metastatic colorectal cancer
- “EGFR positive” defined as ≥1% tumor cells staining IHC
- Primary endpoint: Progression-free survival (PFS)
- Secondary endpoints: RR, OS, QoL, safety

Previously untreated metastatic colorectal cancer
N=1198

Van Cutsem et al, NEJM 2009
## BRAF mutation and prognosis

<table>
<thead>
<tr>
<th></th>
<th>KRAS wt/BRAF wt (n=566)</th>
<th>KRAS wt/BRAF mt (n=59)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FOLFIRI (n=289)</td>
<td>Cetuximab +FOLFIRI (n=277)</td>
</tr>
<tr>
<td>HR [95% CI] p-value(^a)</td>
<td>0.830 [0.687–1.004]</td>
<td>0.0549</td>
</tr>
<tr>
<td>Median PFS mo [95% CI]</td>
<td>8.8 [7.6–9.4]</td>
<td>10.9 [9.4–11.8]</td>
</tr>
<tr>
<td>HR [95% CI] p-value(^a)</td>
<td>0.679 [0.533–0.864]</td>
<td>0.0016</td>
</tr>
<tr>
<td>OR rate (%) [95% CI]</td>
<td>42.6 [36.8–48.5]</td>
<td>61.0 [55.0–66.8]</td>
</tr>
<tr>
<td>p-value(^b)</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\)Stratified log-rank test; \(^b\)Cochran-Mantel-Haenszel test

CI, confidence interval; OR, best overall response; OS, overall survival; PFS, progression-free survival; mo, months; mt, mutant; wt, wild-type
BRAF mutation and prognosis
“CAIRO2” (also first-line)

Previously untreated metastatic colorectal cancer
N=755

<table>
<thead>
<tr>
<th>Treatment</th>
<th>PFS</th>
<th>OS</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAPOX/Bev</td>
<td>10.6 m</td>
<td>22.4 m</td>
</tr>
<tr>
<td>(p=0.30)</td>
<td></td>
<td>(p=0.64)</td>
</tr>
<tr>
<td>CAPOX/Bev + Cetuximab</td>
<td>10.5 m</td>
<td>21.8 m</td>
</tr>
<tr>
<td>(as with CRYSTAL, increase of 10% of RR with cetx)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

As with other “double biologic” studies, **no benefit** to adding EGFR-targeting monoclonal antibody, even in KRAS WT population.

Tol et al, NEJM 2009
BRAF mutation and prognosis

“CAIRO2” (also first-line)

- BRAF testing performed in 516 tumors (of 755)
- BRAF mutation detected in 45 tumors (8.7%), mutually exclusive with KRAS
- BRAF was highly negatively prognostic, regardless of treatment (not predictive)

<table>
<thead>
<tr>
<th></th>
<th>WT</th>
<th>BRAF MT</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PFS</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chemo + Bev</td>
<td>12.2</td>
<td>5.9 (p=0.003)</td>
</tr>
<tr>
<td>Chemo + Bev + Cetx</td>
<td>10.4</td>
<td>6.6 (p=0.01)</td>
</tr>
<tr>
<td><strong>OS</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chemo + Bev</td>
<td>24.6</td>
<td>15.0 (p=0.002)</td>
</tr>
<tr>
<td>Chemo + Bev + Cetx</td>
<td>21.5</td>
<td>15.2 (p=0.001)</td>
</tr>
</tbody>
</table>

Tol et al, NEJM 2009 (Letter to Editor)
BRAF mutation and prognosis

“CAIRO2” (also first-line)

Punt, ASCO GI 2010. BRAF Mutated groups in blue.
Is PI3K Prognostic?
Probably, at least in KRAS WT
- Ogino et al (Fuchs), JCO 2009. Nurses Health Study (121,700) and Health Professional Follow-Up Study (51,5000).
- Analysis of 450 stage I-III tumors, 18% harbored PIK3CA mutation (co-exist with KRAS, BRAF)
- Among KRAS WT tumors, PIK3CA mutation was associated with increase in CRC-specific mortality (HR = 3.8, 1.56 – 9.27). No effect in patients with MT tumors.
- Note that CpG island methylation phenotype (CIMP), microsatellite instability (MSI), KRAS, BRAF, p53, and long interspersed nucleotide element-1 (LINE-1) methylation also tested.
Is PI3K Prognostic?

However, some data is conflicting
- Jehan et al, J. Pathology 2009, examined PIK3CA (chromosome 3q26) gene amplification (FISH) in 448 colorectal tumors, adenomas, normal mucosa.
  - PIK3CA/centromere ratio >2.0 was defined as amplified; found in 38% of cancers. No association with PIK3CA mutations. Seen in adenomas (early event).
  - Among treated patients (n=220), PIK3CA gene amplification was found to have a favorable prognosis.
Other non-EGFR Prognostic Factors

Too many to name, most unconfirmed…

**Favorable**
- Microsatellite instability (MSI)
- Cyclin D1  
  (n=602, Ogino, Clin Can Res 2009)

**Unfavorable**
- Loss of Heterozygosity (LOH) 18q
- Circulating Tumor Cells (CTC’s)
- MicroRNA miR-21  
  (n=198, Schetter, Clin Can Res 2009)
Conclusions for Colorectal Cancer (1)

- The era of stacking drugs $(A + B + C)$ in colorectal cancer, and seeing benefit, is over. Billions of dollars unintentionally wasted from 2004-2009 covering drugs and drug combos that do not benefit, or even harm, patients.

- KRAS testing is now a standard predictive test to determine whether patients should received EGFR-targeting antibodies. Only a subset of KRAS wildtype patients benefit, however, and other positive/negative biomarkers needed.

- Other biomarkers such as BRAF, PIK3CA, PTEN not yet ready for routine clinical use. BRAF mutations appear to carry a negative prognosis.

- We need to rethink tissue processing, whereby a multitude of useful information is destroyed by formalin in the name of preserving 16th century technology (the microscope).
Conclusions for Colorectal Cancer (2)

- Large, multinational databases will likely be required to tease out drug effects in rare subsets.

- Novel therapies have been hampered by the logjam of Coke vs Pepsi studies which have dominated the field for the last decade. Future studies can be biomarker-driven when there are clear pathway alterations in patient subsets.

- University of Colorado Cancer Center supports a highly specialized, multi-disciplinary GI cancer program. Progress in GI cancer research will continue to require close collaboration between surgery and other disciplines.
Gastrointestinal (GI) Oncology Program Team

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Colin Weekes, MD/PhD
Stephen Leong, MD
Madeleine Kane, MD/PhD
Mary Kay Schultz, NP (also phase I)
Anne Leyba, NP (also phase I)
Chris Thompson (fellow)
Arvind Dasari, MD (fellow)

Study Coordinators
Brittany Hines, RN - Team Mgr
Nikki Ayodejey, RN
Catherine Wood, BS
Phase I Team

Scheduling (includes phase I)
Christine Miller, Saraberta Lenz

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GI Surgical Oncology
Martin McCarter, MD  Greg Stiegmann, MD
Nathan Pearlman, MD  Others
Csaba Gajdos, MD

Radiation Oncology
Tracey Schefter, MD  Laurie Gaspar, MD
David Raben, MD  Brian Kavanaugh, MD

Interventional Endoscopy
Raj Shah, MD  Roy Yen, MD
Norio Fukami, MD  Brian Brauer, MD

GI Pathology / Molecular Pathology
Wilbur Franklin, MD  Dara Aisner, MD/PhD
Martine McManus, MD  Max Smith, MD

Hepatology / Liver Transplant Team (Burton, etc)
Interventional Radiology (Ray, Gupta, Durham, etc)
Radiology (Russ, Klingensmith, Serkova, Dodd, etc)

Dieticians, ostomy support, LICSW’s, etc

Nurses
Julie Banahan, RN, OCN
Mary Cunningham, RN, OCN
Gari Jensen, RN, OCN
Surgery / Med Onc Collaboration

Searching for Biomarkers in CRC
Human tumor explant model
(Johns Hopkins, UCCC)
Surgery / Med Onc Collaboration

Searching for Biomarkers in CRC

Human tumor explant model

Using xenografts at the extremes of sensitivity, search for expression profiles. Reduce expression profile to assay that can be implemented in the clinic with limited samples.
Thank you for your attention!

Wells Messersmith, MD, FACP
Associate Professor, Division of Medical Oncology
Director, Gastrointestinal Medical Oncology Program
Deputy Head, Division of Medical Oncology
University of Colorado Cancer Center

Wells.Messersmith@ucdenver.edu