Colorectal Cancer:
Biomarkers and Treatment

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1. No employment, speaker’s bureaus, stock ownership, royalties, patents, etc

2. Unpaid advisory boards with Bayer, Genentech, Pfizer

3. PI or Local PI of clinical trials by Genentech/Roche, GSK, Pfizer, Millenium/Takeda, Bayer, Onconova, Immunomedics, and NIH/CTEP.

4. I serve on DSMB’s for OncoMed, Immunomedics.
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CRC: Biomarkers and Treatment

Outline:

1. Introduction: Colorectal Cancer
2. Desirable biomarker qualities
3. Biomarkers in colorectal cancer
4. Future directions
Colorectal Cancer (CRC) Facts

- 3rd most common cancer in the U.S.
- 5-year survival ~11% in the metastatic setting
- Current options for metastatic CRC (mCRC)
  - 5-FU/leucovorin, capecitabine, oxaliplatin, irinotecan, bevacizumab, ziv-aflibercept (approved 8/12), regorafenib (9/12), ramucirumab (4/2015), TAS-102 (9/2015)
  - Cetuximab or panitumumab if KRAS/NRAS wild-type (both approved in first line metastatic setting)
- Numerous gaps in understanding the disease
  - e.g., why don’t biologics work in adjuvant setting?
  - What are predictive biomarkers for VEGFR inhibitors?
- Additional treatment options are needed
- Additional prevention options are needed
- Most treatment decisions not based on biomarkers

-General Themes in mCRC Treatment

- Chemotherapy backbones appear to be interchangeable (FOLFOX vs CAPOX vs FOLFIRI)
  - There may be differences in combinations with biologics

- Some patients with stage IV disease are cured using multi-disciplinary approaches (surgery, chemo, etc)

- Combination therapy is generally well-tolerated, but sequential therapy is also reasonable

- Biologics have added incremental (and somewhat disappointing) benefit

- Era of personalized therapy began with KRAS
# 11 Drugs for Colorectal Cancer

<table>
<thead>
<tr>
<th>“Cytotoxics”</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</td>
<td>-&gt; oral 5-FU pro-drug</td>
</tr>
<tr>
<td>3. TAS-102</td>
<td>-&gt; 5-FU drug with metabolism inhibitor</td>
</tr>
<tr>
<td>4. irinotecan</td>
<td>-&gt; topoisomerase I inhibitor</td>
</tr>
<tr>
<td>5. oxaliplatin</td>
<td>-&gt; 3rd generation platinum</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>“Biologics/Targeted”</th>
<th>Mechanism</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. cetuximab</td>
<td>-&gt; antibody against EGFR</td>
</tr>
<tr>
<td>2. panitumumab</td>
<td>-&gt; antibody against EGFR</td>
</tr>
<tr>
<td>3. bevacizumab</td>
<td>-&gt; antibody against VEGF</td>
</tr>
<tr>
<td>4. ziv-aflibercept</td>
<td>-&gt; dummy VEGF receptor</td>
</tr>
<tr>
<td>5. regorafenib</td>
<td>-&gt; tyrosine kinase inhibitor</td>
</tr>
<tr>
<td>6. ramucirumab</td>
<td>-&gt; antibody against VEGFR2</td>
</tr>
</tbody>
</table>

**VEGF** = Vascular Endothelial Growth Factor; **EGFR** = Epidermal Growth Factor Receptor
## How are we going to pay for this?

### Chemotherapy for Colorectal Cancer (2 weeks)

<table>
<thead>
<tr>
<th>Drug</th>
<th>Cost</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-FU (500 mg/m²)</td>
<td>$6</td>
</tr>
<tr>
<td>leucovorin (500 mg/m²)</td>
<td>$85</td>
</tr>
<tr>
<td>capecitabine (2000 mg/m²/day)</td>
<td>$3,250 / $1,250</td>
</tr>
<tr>
<td>irinotecan (180 mg/m²) / generic</td>
<td>$2,300 / $480</td>
</tr>
<tr>
<td>oxaliplatin (85 mg/m²) / generic</td>
<td>$4,190 / $590</td>
</tr>
<tr>
<td>bevacizumab (5 mg/kg)</td>
<td>$2,560</td>
</tr>
<tr>
<td>cetuximab (250 mg/m²)</td>
<td>$5,120</td>
</tr>
<tr>
<td>panitumumab (6 mg/kg)</td>
<td>$4,360</td>
</tr>
<tr>
<td>ziv-Aflibercept (4 mg/kg)</td>
<td>$5,380</td>
</tr>
<tr>
<td>regorafenib (160 mg, 3/1)</td>
<td>$5,650</td>
</tr>
<tr>
<td>ramucirumab (6 mg/kg)</td>
<td>$7,140</td>
</tr>
</tbody>
</table>

### 1997:
6 months of 5-FU/LV costs ~$500

### 2013:
24 months therapy with combinations costs >$300,000
Cancer Genome Atlas - CRC

Genomic changes in 195 primary colorectal cancers.
Hypermutated tumors (top) segregate from others.
TCGA, Nature 2012
- Complex genetic data is simplified by analysis of pathways.
- Again, hypermutated tumors segregate from others.
- Alterations in pathways identified by mutations, deletions, amplifications, or significant up- or down-regulation of genes.

Molecular Clustering: Colorectal

Four consensus subtypes created among 6 published datasets

CRC: Biomarkers and Treatment

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Definitions:

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
Pharmacodiagnostic.

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)
**Multiplex Testing**

**Definition:** measuring multiple analytes on a single assay (as opposed to one analyte at a time)

**Advantages:** “one-stop shopping”; efficient use of tissue; often cost-effective

**Disadvantages:** Frequently includes extra information of uncertain significance, leading to confusion, pressure to try off-label therapies without safety information; “multiple unreported phase I trials”
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Large Molecule VEGF Inhibitors

PIGF
VEGF-B

VEGF-A

VEGF-C, VEGF-D

PIGF = placental growth factor.

Functions

VEGF-R1
(Flt-1)
Migration
Invasion
Survival

VEGF-R2
(KDR/Flk-1)
Proliferation
Survival
Permeability

VEGF-R3
(Flt-4)
Lymphangiogenesis

Aflibercept
(VEGF Trap)

Bevacizumab

Ramucirumab
# Targeting VEGF

## First-Line bevacizumab in mCRC, Phase III Trials

<table>
<thead>
<tr>
<th>Trial Regimen</th>
<th>Response rate (%)</th>
<th>Median OS (mo)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CT</td>
<td>CT + Bev</td>
</tr>
<tr>
<td>AVF2107g IFL (n = 411) vs IFL + bev (n = 402)</td>
<td>35</td>
<td>45</td>
</tr>
<tr>
<td>NO16966 FOLFOX/CAPEOX (n = 701) vs FOLFOX/CAPOX + bev (n = 699)</td>
<td>49</td>
<td>47</td>
</tr>
<tr>
<td>BICC-C: FOLFIRI (n = 144) vs FOLFIRI + bev (n = 57)</td>
<td>47</td>
<td>58</td>
</tr>
<tr>
<td>BICC-C mIFL (n = 141) vs mIFL + bev (n = 60)</td>
<td>43</td>
<td>53</td>
</tr>
<tr>
<td>AVEX (pt &gt; 70 years) Bev + Cape (n = 140) vs Cape (n = 140)</td>
<td>10%</td>
<td>19%</td>
</tr>
</tbody>
</table>

CT, chemotherapy; OS, overall survival; Bev, bevacizumab; cape, capecitabine.

VEGF-Targeted Agents in 2nd mCRC

Modest improvements in PFS and OS, even in patients with prior exposure to bevacizumab.

Hazard Ratios (HR) for OS are 0.81 (bevacizumab), 0.82 (afiblercept), 0.84 (ramucirumab). Remarkably similar.

<table>
<thead>
<tr>
<th></th>
<th>TML</th>
<th>VELOUR*</th>
<th>RAISE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CT (N=410)</td>
<td>CT + bevacizumab (N=409)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HR (P value)</td>
<td>HR (P value)</td>
<td>HR (P value)</td>
</tr>
<tr>
<td>mOS, mos</td>
<td>9.8</td>
<td>11.2</td>
<td>11.7</td>
</tr>
<tr>
<td></td>
<td>0.81; P=0.02</td>
<td>12.5</td>
<td>0.86; P=NR</td>
</tr>
<tr>
<td>mPFS, mos</td>
<td>4.1</td>
<td>5.7</td>
<td>3.9</td>
</tr>
<tr>
<td></td>
<td>0.68; P&lt;0.001</td>
<td>6.7</td>
<td>0.66; P=NR</td>
</tr>
<tr>
<td></td>
<td>0.79; P=0.0005</td>
<td>4.5</td>
<td>5.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.84; P=0.02</td>
</tr>
</tbody>
</table>

PFS = progression-free survival; OS = overall survival
Biomarkers for VEGF Inhibitors

This slide sums up what we know with certainty!
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, 146 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
**RAS Genes and Human Cancers**

### Table 2. Incidence of KRAS Mutations in Three Human Cancers

<table>
<thead>
<tr>
<th></th>
<th>All KRAS</th>
<th>G12C</th>
<th>G12D</th>
<th>G12V</th>
<th>G13D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colorectal</td>
<td>60,000</td>
<td>5,700</td>
<td>25,000</td>
<td>15,700</td>
<td>13,600</td>
</tr>
<tr>
<td>Lung</td>
<td>45,600</td>
<td>23,000</td>
<td>9,200</td>
<td>11,900</td>
<td>1,500</td>
</tr>
<tr>
<td>Pancreas</td>
<td>32,200</td>
<td>1,000</td>
<td>19,500</td>
<td>11,500</td>
<td>200</td>
</tr>
<tr>
<td>Total new cases/year</td>
<td>137,800</td>
<td>29,700</td>
<td>53,700</td>
<td>39,100</td>
<td>15,300</td>
</tr>
</tbody>
</table>

Shown are the numbers of new cancer cases per year in the United States that contain the most frequent KRAS mutant alleles. Data are based on estimated new case incidence values from the National Cancer Institute and primary tumor mutation frequency data from COSMIC v.67.

KRAS mutations are common in CRC; one of few with codon 13.

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

Challenges with targeting RAS

- Numerous feedback loops which can lead to over-activation of these pathways
- Mutant KRAS does not correlate with ERK activation
- Mutant BRAF has different roles in melanoma vs colorectal cancer
- MEK and BRAF monotherapy inhibition has yielded unimpressive results
In reality, many RAS effectors!

Anti-KRAS Strategies

Inhibitors of plasma membrane association

Direct inhibitors of Ras-GTP

Inhibitors of downstream effector signaling

Channing Der PhD. ASCO GI 2012
NCIC CO.17 Trial

- Previously treated metastatic colorectal cancer
- N=572

Overall Survival:

<table>
<thead>
<tr>
<th>Mutant K-ras</th>
<th>Wild-type K-ras</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cetuximab plus best supportive care</td>
<td>76</td>
</tr>
<tr>
<td>Best supportive care alone</td>
<td>60</td>
</tr>
</tbody>
</table>

Overall Survival: Mutant

Not Prognostic! (BSC patients)

Karapetis et al. NEJM 2008; 359(17):1757-1765
PRIME: FOLFOX +/- Pmab
PFS by KRAS Mutation Status

<table>
<thead>
<tr>
<th>KRAS WT</th>
<th>Events</th>
<th>n (%)</th>
<th>Median (95% CI) months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Panitumumab + FOLFOX</td>
<td>199 (61)</td>
<td>9.6 (9.2–11.1)</td>
<td></td>
</tr>
<tr>
<td>FOLFOX</td>
<td>215 (65)</td>
<td>8.0 (7.5–9.3)</td>
<td></td>
</tr>
</tbody>
</table>

HR = 0.80 (95% CI 0.66–0.97)

P Value = .02

<table>
<thead>
<tr>
<th>KRAS MT</th>
<th>Events</th>
<th>n (%)</th>
<th>Median (95% CI) months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Panitumumab + FOLFOX</td>
<td>167 (76)</td>
<td>7.3 (6.3–8.0)</td>
<td></td>
</tr>
<tr>
<td>FOLFOX</td>
<td>157 (72)</td>
<td>8.8 (7.7–9.4)</td>
<td></td>
</tr>
</tbody>
</table>

HR = 1.29 (95% CI 1.04–1.62)

P Value = .02

Overall Survival

<table>
<thead>
<tr>
<th>Subgroup</th>
<th>No.</th>
<th>Hazard Ratio for Death from Any Cause (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Primary analysis</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nonmutated KRAS exon 2</td>
<td>656</td>
<td>0.83 (0.67 - 1.02)</td>
</tr>
<tr>
<td>Mutated KRAS exon 2</td>
<td>440</td>
<td>1.24 (0.98 - 1.57)</td>
</tr>
<tr>
<td><strong>Prospective-retrospective analysis</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nonmutated RAS</td>
<td>512</td>
<td>0.78 (0.62 - 0.99)</td>
</tr>
<tr>
<td>Mutated RAS</td>
<td>548</td>
<td>1.25 (1.02 - 1.55)</td>
</tr>
<tr>
<td>Nonmutated KRAS exon 2 RAS mutated other</td>
<td>108</td>
<td>1.29 (0.79 - 2.10)</td>
</tr>
</tbody>
</table>

Conclusion: NRAS and “other” KRAS mutations behave similarly to KRAS codon 12 and 13. Patients with these mutations should not receive EGFR-targeting mAb’s.

Distribution of mutations in mCRC

- KRAS wt: ~50%
- KRAS mt: ~40%
- New RAS mt: ~10%
- Rare KRAS Mutations
- NRAS Mutations
- RAS wt: ~50%
In KRAS WT patients, the incidence of other Ras mutations is small, **but the numbers add up**. We should not spend hundreds of millions of dollars **harming patients**.
Is Re-Biopsy Necessary?

Answer: No

• >96% concordance between primaries and metastases

• Only 2% clinically relevant

FIRE-3 study design

- mCRC 1st-line therapy KRAS wild-type
- Randomize 1:1

**FOLFIRI + cetuximab**
- Cetuximab: 400 mg/m² i.v. 120min initial dose
- 250 mg/m² i.v. 60min q 1w

**FOLFIRI + bevacizumab**
- Bevacizumab: 5 mg/kg i.v. 30-90min q 2w

FOLFIRI q2w: 5-FU: 400 mg/m² (i.v. bolus);
- Folinic acid: 400mg/m²
- Irinotecan: 180 mg/m²
- 5-FU: 2,400 mg/m² (i.v. 46h)

- **Primary endpoint: Overall response rate (RECIST 1.0)**
- **Amendment in 10/08** to include only KRAS wild-type patients
- 150 active centers in Germany and Austria

Events
n/N (%)  Median (months)  95% CI
FOLFIRI + Cetuximab  91/171  (53.2%)  33.1  24.5 – 39.4
FOLFIRI + Bevacizumab  110/171  (64.3%)  25.6  22.7 – 28.6

HR 0.70 (95% CI: 0.53 – 0.92)
p (log-rank)= 0.011

Δ = 7.5 months

Unexplained difference in survival AFTER cessation of study treatment


* KRAS and NRAS exon 2, 3 and 4 wild-type
CALGB/SWOG 80405: Which biologic 1st line?

N = 1140

1° Endpoint: Overall Survival

mCRC 1st-line

KRAS wild type (codons 12,13)

STRATA: FOLFOX/FOLFIRI
Prior adjuvant
Prior XRT

FOLFIRI or FOLFOX
MD choice

Chemo + cetuximab

Chemo + bevacizumab

Venook, ASCO 2014
CALGB/SWOG 80405: Progression-Free Survival
(*Investigator Determined*)

<table>
<thead>
<tr>
<th>Arm</th>
<th>N (Events)</th>
<th>PFS (m) Median</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemo + bev</td>
<td>559 (498)</td>
<td>10.8</td>
<td>9.7-11.4</td>
</tr>
<tr>
<td>Chemo + cetux</td>
<td>578 (499)</td>
<td>10.4</td>
<td>9.6-11.3</td>
</tr>
</tbody>
</table>

P=0.55
HR 1.04 (0.91 - 1.17)
CALGB/SWOG 80405: Overall Survival

<table>
<thead>
<tr>
<th>Arm</th>
<th>N (Events)</th>
<th>OS (m) Median</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemo + cetux</td>
<td>578 (375)</td>
<td>29.9</td>
<td>27.0-32.9</td>
</tr>
<tr>
<td>Chemo + bev</td>
<td>559 (371)</td>
<td>29.0</td>
<td>25.7-31.2</td>
</tr>
</tbody>
</table>

P=0.34
HR 0.925 (0.78-1.09)

Will conclusion change when other KRAS, NRAS is analyzed?
Does Expanded RAS Make A Difference in 80405?

BRAF Background

- Overall, approximately 8% of all tumors have a BRAF mutation; in CRC it ranges from 5-10%.

- The predominant mutation, similar to melanoma, is a single-base substitution of valine by glutamic acid at position 600 (V600E) within the activation segment.

- Signals through MEK/ERK activation pathway.

- BRAF mutation is an early event in CRC and there is a high concordance between primary and metastatic tissue.

- Associated with:
  - R-sided tumors; high grade
  - Older age, female
  - MSI-high (due to epigenetic mechanisms)
  - Serrated (as opposed to tubular) adenoma pathway.
Due to the confounding effect of MSI status in BRAF MT patients, Ogino proposed this strategy for classification. Must split, rather than lump, BRAF MT patients.
V600E (500-fold greater kinase activity) is most common mutation. BRAF and KRAS mutations appear to be mutually exclusive.
RAS and BRAF do not overlap

KRAS/NRAS and BRAF are usually mutually exclusive; but PIK3CA, others are not. **Do not need to test for BRAF and NRAS in a KRAS mutated tumor.**
“CRYSTAL” Trial: 1\textsuperscript{st} line chemo/cetuximab

BRAF mutation is a negative prognostic factor

Van Cutsem et al, JCO 2011

© 2011 by American Society of Clinical Oncology
BRAF Inhibitor Monotherapy Is Disappointing

Preclinical studies show lack of benefit from single-agent vemurafenib, and BRAF knock-down, in CRC cell lines.

Phase I study of vemurafenib in BRAF mutant CRC is not as effective as seen in melanoma (one response).

Primary Resistance BRAF Inhibition in mCRC

Resistance may be mediated by activation of EGFR pathway through inhibition of CDC25C, which usually inhibits EGFR activation.
Targeting BRAF with Combinations

Update from ASCO 2014 and 2015: Hope!

Small Molecule Combinations
- **BRAF+MEK** (Corcoran) with 12% response rate

**EGFR mAb combinations**
- **BRAF+MEK+EGFR** (Bendell) with **40%** response rate (this arm will be in FOCUS4 trial in UK)
- **BRAF+EGFR** (van Geel), with **29%** response rate
- **BRAF+EGFR+Irino** (Hong), with **50%** response rate
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)
PI3K Mutations

(Samuels, Science 2004)

- 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)

Functionally important:
- Nontruncating
- Nonsynonymous
- Conserved residues
- Higher PI3K activity
Signaling Pathway Target: PI3K

**Ligands**

- PI3K inhibitors (XL147, GDC-0941, PX-866, SF1126, BEZ235)
- AKT inhibitors (MK-2206, GSK2141795, SR13668, XL418, GSK690693)
- mTOR inhibitors (sirolimus, temsirolimus, everolimus, AP23573, AZD8055, OSI-027, palomid 529)

**Growth Factor Receptor**

- PI3K
  - p85
  - p110
- PIP
  - PIP2
  - PIP3
- PTEN

**Blocking the Pathway**
PIK3CA mutations and aspirin

Mutant = aspirin benefit

wildtype

Possible Mechanism?

PI3K signal transduction pathways regulate COX-2 expression and PGE2 synthesis.

PD = PD098059 (MAPK inhibitor)
LY = LY294002 (PI3K inhibitor)

Di Popolo et al, Oncogene 2000
Microsatellite Unstable Tumors
Micosatellite Unstable Tumors

Germline: “HNPCC” or Lynch Syndrome
- Due to mutations in one of the mismatch repair genes: MLH1, MSH2, MSH6, PMS2, and/or EPCAM
- Increased lifetime risk of colorectal, endometrial, stomach, ovarian, urothelial, and other cancers

Acquired MSI
- Most due to hypermethylation of the MLH1 promoter and epigenetic silencing of MLH1
- Can also have “double somatic” MSI caused by mutations in MMR genes

Two methods for testing
- **PCR-based** microsatellite instability (MSI) testing to identify variation in genomic repeats
- Immunohistochemistry (IHC) for loss of expression of one or more of the MMR proteins
Type, Density, and Location of Immune Cells Within Human Colorectal Tumors Predict Clinical Outcome

1960 - 1964

Immunosurveillance Hypothesis
Lack of lymphocytes = poor prognosis
MSI-H carries a better prognosis

Forrest plot of studies on MSI-H subgroup

MSI-H patients do better!

**Immune Checkpoint Inhibitors in CRC**

**BMS-936550**
- **RR:** 0/18 CRC

**Tremelimumab**
- **RR:** 1/45 CRC
  - (response duration 15m)

**Nivolumab**
- **RR:** 1/14 CRC
  - (response duration >21m, MSI-H pt)
- **RR:** 0/19 CRC

---

**References**

   - *Original Article*
   - Safety and Activity of Anti–PD-L1 Antibody in Patients with Advanced Cancer
   - BMS-936550
   - **RR:** 0/18 CRC

2. *Journal of Clinical Oncology*
   - Phase I Study of Single-Agent Anti–Programmed Death-1 (MDX-1106) in Refractory Solid Tumors: Safety, Clinical Activity, Pharmacodynamics, and Immunologic Correlates
   - Tremelimumab
   - **RR:** 1/45 CRC
   - (response duration 15m)

3. *Journal of Clinical Oncology*
   - Phase II Study of the Anti-Cytotoxic T-Lymphocyte–Associated Antigen 4 Monoclonal Antibody, Tremelimumab, in Patients With Refractory Metastatic Colorectal Cancer
   - Tremelimumab
   - **RR:** 1/45 CRC
   - (response duration 15m)

   - Safety, Activity, and Immune Correlates of Anti–PD-1 Antibody in Cancer
   - Nivolumab
   - **RR:** 0/19 CRC
Activity of PD-1 (pembrolizumab) in MSI-H tumors

**Table 2. Objective Responses According to RECIST Criteria.**

<table>
<thead>
<tr>
<th>Type of Response</th>
<th>Mismatch Repair–Deficient Colorectal Cancer (N = 10)</th>
<th>Mismatch Repair–Proficient Colorectal Cancer (N = 18)</th>
<th>Mismatch Repair–Deficient Noncolorectal Cancer (N = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete response — no. (%)</td>
<td>0</td>
<td>0</td>
<td>1 (14)*</td>
</tr>
<tr>
<td>Partial response — no. (%)</td>
<td>4 (40)</td>
<td>0</td>
<td>4 (57)†</td>
</tr>
<tr>
<td>Stable disease at week 12 — no. (%)</td>
<td>5 (50)</td>
<td>2 (11)</td>
<td>0</td>
</tr>
<tr>
<td>Progressive disease — no. (%)</td>
<td>1 (10)</td>
<td>11 (61)</td>
<td>2 (29)</td>
</tr>
<tr>
<td>Could not be evaluated — no. (%)</td>
<td>0</td>
<td>5 (28)</td>
<td>0</td>
</tr>
<tr>
<td>Objective response rate (95% CI) — %</td>
<td>40 (12–74)</td>
<td>0 (0–19)</td>
<td>71 (29–96)</td>
</tr>
<tr>
<td>Disease control rate (95% CI) — %</td>
<td>90 (55–100)</td>
<td>11 (1–35)</td>
<td>71 (29–96)</td>
</tr>
<tr>
<td>Median duration of response — wk</td>
<td>Not reached</td>
<td>NA‖</td>
<td>Not reached</td>
</tr>
<tr>
<td>Median time to response (range) — wk</td>
<td>28 (13–35)</td>
<td>NA‖</td>
<td>12 (10–13)</td>
</tr>
</tbody>
</table>

* The patient had a partial response at 12 weeks, which then became a complete response at 20 weeks.
† One patient had a partial response at 12 weeks.
‡ Patients could not be evaluated if they did not undergo a scan at 12 weeks because of clinical progression.
§ The rate of disease control was defined as the percentage of patients who had a complete response, partial response, or stable disease for 12 weeks or more.
‖ The median time to response was not applicable (NA) because no responses were observed among patients with mismatch repair–proficient colorectal cancer.

Le, NEJM 2015
Responses in MSI-H subgroup

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)

Le, NEJM 2015
IHC showed PD-L1 expression differences
Mutational load correlates with response

Le, NEJM 2015
Ongoing Anti-PD1 or Anti-PDL1 Clinical Trials with MSI-high CRC Subsets

- NCT01876511: Phase 2 study of **MK-3475** in patients with microsatellite unstable (MSI) tumors
  - MSI-high CRC, MSS CRC, MSI-high non-CRC
- NCT02060188: A study of **nivolumab and nivolumab plus ipilimumab** in recurrent and metastatic colon cancer (CheckMate 142)
  - MSI-high CRC, MSS CRC
- NCT02227667: Evaluate the Efficacy of **MEDI4736** in Immunological Subsets of Advanced Colorectal Cancer
  - MSI-high CRC, TIL high CRC
- NCT02404411: phase I/II study of **PDR001** in patients with advanced malignancies
  - MSI-high CRC, other tumors
- NCT01633970: A phase 1b study of **MPDL3280A** (an engineered anti-PDL1 antibody) in combination with bevacizumab and/or chemotherapy in patients with advanced or metastatic solid tumors
  - MSI-high CRC, other tumors
- A phase 1, open-label study of **GSK3174998** administered alone and in combination with anticancer agents including **Pembrolizumab** in subjects with selected advanced solid tumors
  - MSI-high CRC, other tumors

Overman, MDACC
TAS102: RECURSE

Combination of two agents:
- **Trifluridine** (FTD), a nucleoside analog activated by thymidine kinase
- **Tipiracil hydrochloride** (TPI), a thymidine phosphorylase inhibitor which inhibits metabolism of trifluridine; also has **anti-angiogenic properties** via PDGF inhibition.

RECURSE trial
- Global phase III trial conducted in 13 countries at 114 centres
- mCRC refractory to all standard therapies (including EGFR-targeting mAb for KRAS WT patients)
- Randomized 2:1 to **TAS-102** (534 patients), 35 mg/m2 BID on Days 1-5 and 8-12 of each 28-day cycle, or **placebo** (266 patients)
- The primary endpoint was **overall survival**.

Yoshino, ESMO 2014, #0022
TAS-102: Phase II

Randomized pII study in Japan
N=169, randomized 2:1

Improvement in:
OS (HR=0.56) 9.0 v. 6.6 mos

Yoshino, Lancet Oncol 2012
TAS-102 RE COURSE: PFS

HR = 0.48, p<0.001 (log rank)

Mayer, NEJM 2015
TAS-102 RECOVERY: OS

HR = 0.68, p<0.001

Mayer, NEJM 2015
# TAS-102 RECORSE: Toxicity

<table>
<thead>
<tr>
<th>Event</th>
<th>TAS-102 (N=533)</th>
<th>Placebo (N=265)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Any Grade</td>
<td>Grade ≥3</td>
</tr>
<tr>
<td>Nausea</td>
<td>258 (48)</td>
<td>10 (2)</td>
</tr>
<tr>
<td>Vomiting</td>
<td>148 (28)</td>
<td>11 (2)</td>
</tr>
<tr>
<td>Decreased appetite</td>
<td>208 (39)</td>
<td>19 (4)</td>
</tr>
<tr>
<td>Fatigue</td>
<td>188 (35)</td>
<td>21 (4)</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>170 (32)</td>
<td>16 (3)</td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>113 (21)</td>
<td>13 (2)</td>
</tr>
<tr>
<td>Fever</td>
<td>99 (19)</td>
<td>7 (1)</td>
</tr>
<tr>
<td>Asthenia</td>
<td>97 (18)</td>
<td>18 (3)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Events associated with fluoropyrimidine treatment — no. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Febrile neutropenia</td>
</tr>
<tr>
<td>Stomatitis</td>
</tr>
<tr>
<td>Hand–foot syndrome</td>
</tr>
<tr>
<td>Cardiac ischemia:‡</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Laboratory abnormalities — no./total no. (%)‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neutropenia</td>
</tr>
<tr>
<td>Leukopenia</td>
</tr>
<tr>
<td>Anemia</td>
</tr>
<tr>
<td>Thrombocytopenia</td>
</tr>
</tbody>
</table>

Mayer, NEJM 2015
Metastatic Colorectal Cancer

Outline:
1. Introduction: Colorectal Cancer
2. Desirable biomarker qualities
3. Biomarkers in colorectal cancer
4. Future directions
Drug Development Paradigm

Biomarker Discovery:
- Dissecting cancer systems biology

Biomarker Validation:
- Unraveling Achilles' heels of cancer

Clinical Trial:
- Translating cancer genome insights to guide individualized therapies

To identify predictive biomarkers by integrating multi-layered genomic data:
- Gene mutation
- Translocation & CNV
- Gene Expression Analysis

Colo205 (SEN) vs. LS174T (RES)
- KRAS Mutational Analysis
- IGFR1 FISH Analysis
- Gene Expression Analysis

Prediction & Validation on direct human cancer explants

Genomic signature

"Knock-down" experiments on up-regulated genes in RES:
- siRNA silencing
- miRNA silencing

To identify drug-able resistant pathways:

SEN RES

Pathway analysis:
- To identify active pathways in the resistant cases, which can be used in combination with the targeted therapy

Pathway signature

RKO: resistant to IGFR1 and MEK inhibitors show synergy in the combination

Combinatorial Index

New therapies

Combination of targeted therapies
Explant Model: Principles (1)

• 60-80% “take” rate in pancreas and colorectal cancer
• Genetically stable (but not perfect)
• Typically do not metastasize; most publications use subQ placement, some orthotopic.
• Difficult to study immunologic phenomena (nude mice)
• Biologic bias in “take rate”, in that patients whose tumors can be successfully explanted have poorer prognosis (e.g., colorectal CA often KRAS MT)
Explant Model: Principles (2)

- Typically too slow-growing to dictate treatment in real time.
- Issues of stroma (mouse vs human) often ignored.

Human COT-1 (red) versus mouse COT-1 (green) in colorectal explant shows that stromal elements are MOUSE.
WNT pathway associated with resistance to MEKi

Spreafico, CCR 2013
MEK Inhibitors in CRC

3/5 R CRC Explants had > 50% increase in FZD2 post-treatment.

Spreatfico, CCR 2013
Combination MEK/WNT

Clinical trial currently enrolling initial cohort with this combination (CTEP/UM1).

Spreafico, CCR 2013
How are current trial designs being influenced by molecular testing?
CURRENT DESIGN

Progression on First-line Treatment of Metastatic Colorectal Cancer

Consent patient to screen

Analysis of metastatic tumor specimen

Consent patient to sub-study

Marker Defined Sub-Groups (potential options)

- MSI
- BRAF
- PIK3CA
- PTEN
- AKT
- HER2
- All RAS WT

Targeted therapy Control arm*
Targeted therapy Control arm*
Targeted therapy Control arm*
Targeted therapy Control arm*
Targeted therapy Control arm*
Targeted therapy Control arm*

Some Issues:
When to register the patient
When to acquire tissue
Potential for delay in RX
Definition of “second-line”

*Standard chemotherapy-containing regimen
**CURRENT ASSIGN “PROGRAM”**

Progression on First-line Treatment of Metastatic Colorectal Cancer

Use Standard of Care Markers

Analysis of tumor specimen

**Current Goals of the ASSIGN Therapeutics Committee**

1. Continue discussions among academic centers, NCI, FOCR, advocates
2. Monitor the best science for molecular subsets and translational opportunities
3. Facilitate molecularly-driven trials, whether in ASSIGN or not
4. Continue to lay the groundwork for a large molecularly driven CRC study

**I/C= Irinotecan + Cetuximab**

- **BRAF**
  - S1406, Kopetz
  - In development
  - Use Standard of Care Markers

- **MSI**
  - Overman
  - Analysis of tumor specimen

- **Her2**
  - Raghav, Fakih
  - “MATCH” collaborations

- **Chemotherapy**
  - Trastuzumab + Pertuzumab

- **Immunotherapy**
  - I/C + vemurafenib

- **Open**
  - “MATCH” collaborations

**“MATCH” collaborations**

- Raghav, Fakih
- I/C
Enrollment on “Master Protocol”

- Highly prevalent molecular subtypes need several studies
- Need treatment for unselected subtypes
How Clinical Practice Changed: 2015

- **Check RAS status** (CLIA-certified lab) on all advanced colorectal cancer patients
  - KRAS/KRAS codons 12, 13, 61, 146
  - Avoid EGFR-targeting mAb’s in RAS mutants
  - Jury is still out with BRAF (poor prognosis is certain)

- **TAS-102** is FDA approved for 3\(^{rd}/4^{th}\) line mCRC setting.

- **Ramucirumab** is approved for 2\(^{nd}\) line setting (FOLFIRI).

- Conflicting data on whether there is a survival benefit for first-line use of EGFR mAb, versus bevacizumab. FIRE-3 says yes, C80405 says no.

- Try to find a PD-1 or PD-L1 clinical trial for your MSI-H patients; and combination study for BRAF MT patients.
Thank you!

University of Colorado Cancer Center

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