Predictive molecular markers in Colorectal Cancer

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How to apply average group derived data to an individual patient

• Stratify findings according to patient’s individual characteristics
• Can the intervention be approximated in a non-study setting
• Quantify benefit and harm
• Incorporate individual preferences
Adjuvant chemotherapy for colon cancer Stage II
What is the benefit ??

Tumor Markers

• Prognostic markers define natural history
• Predictive markers define response to treatment

• Some markers may be both: e.g.: MSI, bcr/abl, ras, Her-2-neu…
CRC: Tumor Markers

- Non-molecular markers
- Molecular markers

Tumor Markers

Non Molecular Markers:

A. Pathology
1. Tumor Size
2. Grade
3. Ploidy
4. S-phase
5. Lymphangitic Spread
6. Local Invasion
7. Nodes: N+/-, number
8. CEA, Ca19.9., Serum Albumin, FCM..

B. Clinical
1. Age/Sex
2. Karnofsky
3. Imaging: stage
4. Co-morbidities
5. Surgeon’s competence
6. Smoking
Molecular Markers

Molecular markers are to individualize treatment

Pharmacogenomics:
study of tumoral genome
prediction of efficacy, e.g.: TS, MTHFR..

Pharmacogenetics:
study of host genome
prediction of toxicity, e.g.: DPD, UGT1A1
MOLECULAR MARKERS IN CRC

- MSI
- LOH 18q
- TGFβ
- TS polymorphism
- Thymidine phosphorylase
- Dihydropyrimidine dehydrogenase
- VEGF
- p53/bcl-2 (bax)
- β-catenin
- Catherins
- EGFR
- ras mutation
- Braf
- p21
- Ca cells in BM
- Microarray
- ......

p53/bcl-2 (bax)

- Disruption ~ poor outcome, especially if liver metastases
- IHC: good, bad, no effect
- Nuclear p53 predicts recurrence
- P53> MSI as predictor in stage III,
  Westra J., JCO, 2005, 23:5635
RAS

- Increase in function
- Overexpression ~survival in rectal cancer (53% vs 12%)
- Response to therapy: cetuximab, panitumumab

Biomarkers for treatment: FOCUS trial

- 1313 pts. screened for biomarkers TOPO1, MLH1, HSH2, p53, ERCC1, MGMT, Cox 2, germ line polymorphism in GSTP1, HBCB1, XRCC1, ERCC2, UGT1A1
- only independent factor: Topo-1
- prognostic:
  - low Topo-1 → good prognosis
  - high Topo-1 → poor prognosis
- predictive:
  - high Topo1 → benefit from upfront combination

Braun et al, J Clin Oncol 2008
**Predictive Biomarkers: Associations Between Molecular Biomarkers and Treatment Impact Upon Time to Treatment Failure**

<table>
<thead>
<tr>
<th>Protein, tumor IHC</th>
<th>n</th>
<th>p for interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>ERCC1</td>
<td>1197</td>
<td>0.5</td>
</tr>
<tr>
<td>Topo1</td>
<td>822</td>
<td>0.03</td>
</tr>
<tr>
<td>MLH1/MSH2</td>
<td>931</td>
<td>0.2</td>
</tr>
<tr>
<td>P53</td>
<td>773</td>
<td>0.4</td>
</tr>
<tr>
<td>MGMT</td>
<td>1125</td>
<td>0.7</td>
</tr>
<tr>
<td>Cox-2</td>
<td>1047</td>
<td>0.8</td>
</tr>
</tbody>
</table>

DNA, germline, polymorphism

<table>
<thead>
<tr>
<th>Gene</th>
<th>n</th>
<th>p for interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>GST-P1</td>
<td>1002</td>
<td>0.5</td>
</tr>
<tr>
<td>ABCB</td>
<td>999</td>
<td>0.6</td>
</tr>
<tr>
<td>XRCC1</td>
<td>988</td>
<td>0.9</td>
</tr>
<tr>
<td>ERCC2</td>
<td>982</td>
<td>0.7</td>
</tr>
<tr>
<td>UGT1A1</td>
<td>915</td>
<td>0.4</td>
</tr>
</tbody>
</table>

M. Baum JCO 2008, 26:2690

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**Gene Expression Profiles in Dukes’B Colon Cancer**  Wang Y. et al. JCO 2004,22:1564

- 74 Dukes B Patients, 31 relapses/3 years
- Affymetrix GeneChip: 22000 transcripts
- 23 gene signature predicts recurrence
- Accuracy of 78% (28/36 patients)
- Odd Ratio: 13, p = 0.0001
- Subgroup to be treated?
- Subgroup not to be treated: the others?
## Cox Multivariate Analysis

<table>
<thead>
<tr>
<th>Factor</th>
<th>HR*</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.99</td>
<td>0.6</td>
</tr>
<tr>
<td>T Stage</td>
<td>1.94</td>
<td>0.27</td>
</tr>
<tr>
<td>Grade</td>
<td>1.2</td>
<td>0.81</td>
</tr>
<tr>
<td>Tumor Size</td>
<td>0.99</td>
<td>0.98</td>
</tr>
<tr>
<td>23-gene signature</td>
<td>0.17</td>
<td>0.001</td>
</tr>
</tbody>
</table>


## Microarrays In Colorectal Studies

23 Gene signature that predicts recurrence of Dukes’ B colon Cancer
- Signature validated in 36 independent patients
- Overall performance accuracy of 78%
- Patients with a high-predicted risk of recurrence (13 Fold) could be upstaged to receive adjuvant therapy

Stage II colon cancer prognosis prediction by tumor gene expression profile (1)

A. Barrier et al. JCO 2006, 24: 4685

50 patients, Stage II, no adjuvant treatment:
- 25 metastasized
- 25 disease-free at 5 years

30 gene microarray: accuracy 80%
  sensitivity 75%
  specificity 85%

Calculations show that with 30 genes, increasing the n of patients, predictability improves

Stage II colon cancer prognosis prediction by tumor gene expression profile (2)

A. Barrier et al. JCO 2006, 24: 4685

- All 30 genes do not have the same value: stable group of 12, variable group of 18
- What would be the performance by reduction to these 12 genes?
- 10/30 genes encode ribosomal proteins, all overexpressed in patients who remained disease-free
- 5 of these 10 had the lowest p value (< 0.0001), implied in cell cycle regulation, apoptosis and DNA repair
- Comparison to 23 gene set, very similar predictability, differences mainly due to classification of patients (Wang study, status after 3y)
High Risk Stage II Colon Cancer

- Evidence of bowel obstruction, perforation, adherence to or invasion of adjacent organs, tumor perforation.
- LOH: retrospective: 18q loss 54% vs 93% survival
- MSI & LOH prognostic markers. Are they predictive?
- ECOG 5202: Randomisation according to MSI/LOH comparing FOLFOX+ Avastin vs no Rp.
- NSABP: randomisation Folfox +/- Avastin and retrospective analysis

Commercially available

Oncotype DX
Two types of colorectal cancer

- Tumour LOH + (85%)
  - hyperploid
  - Allelic losses on chromosomes 17p, 18q, 5q, 8p, 22q
  - Frequent tumour suppressor gene mutations: p53 and APC
  - Frequent KRAS2 and PIK3CA gene mutations
  - Mainly in distal colon

- Chromosomal instability

- Tumour MSI + (15%)
  - diploid
  - No allelic losses on chromosome 17p, 18q, 5q
  - Infrequent tumour suppressor gene mutations: p53 and APC
  - Mutations of TGFβ receptor type II, BAX, TCF4, Caspase 5, HNF1α
  - Frequent oncogene mutation BRAF and PIK3CA
    - hMSH2, hMLH1, hMSH6, gene alterations
  - Mainly in proximal colon

- Genetic instability

Directly Targeting the Cancer Cell: Disadvantages

- Major heterogeneity across and between histologies[1]
- Biologically/genetically unstable target
- Acquired genetic instability
  - Increases with progression/stage/pretreatment
  - Limits efficacy of treatment
- Homeostatic response/selection of resistant clones (acquired and de novo resistance)

Attraction of Targeting the Microenvironment

- Genetically stable substrate
  - Less amenable to mutation/acquired resistance
- Common final pathways possible
  - Less heterogeneity of target
  - Relatively predictable response of tissues to cancer
  - Addresses poorly understood and most lethal hallmark of malignancy: metastasis
- Plethora of potential novel targets

VEGF

- Vascular endothelial growth factor (VEGF) restores the oxygen supply to tissues when blood circulation is inadequate
- VEGF's normal function: create new blood vessels
  - during embryonic development,
  - menstrual cycle
  - after injury,
  - in muscle following exercise
  - to bypass blocked vessels.
VEGF

- When VEGF is overexpressed, it can contribute to disease.
- Solid cancers cannot grow beyond a limited size without an adequate blood supply.
- Cancers that can express VEGF are able to grow and metastasize.
- Drugs such as bevacizumab may inhibit VEGF and control or slow those diseases.

NSABP C-08 Study Design

- Stage II/III colon cancer (n=2700)
- Duration of treatment: 24 weeks
- Primary endpoint: disease-free survival

Wolmark N. ASCO 2009
Allegra C. J Clin Oncol 2010
NSABP C-08: DFS UPDATED RESULTS

DFS estimate

<table>
<thead>
<tr>
<th></th>
<th>mFOLFOX6 (n=1,338)</th>
<th>mFOLFOX6 + Avastin (n=1,335)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR</td>
<td>0.93 (95% CI: 0.81–1.080); p=0.34</td>
<td></td>
</tr>
</tbody>
</table>

Number at risk

<table>
<thead>
<tr>
<th></th>
<th>mFOLFOX6</th>
<th>mFOLFOX6 + Avastin</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 months</td>
<td>1,180</td>
<td>1,240</td>
</tr>
<tr>
<td>1 year</td>
<td>1,036</td>
<td>1,086</td>
</tr>
<tr>
<td>2 years</td>
<td>952</td>
<td>991</td>
</tr>
<tr>
<td>3 years</td>
<td>798</td>
<td>819</td>
</tr>
<tr>
<td>4 years</td>
<td>182</td>
<td>173</td>
</tr>
</tbody>
</table>

*Median follow-up 56 months

Allegra, et al. ASCO 2011 (abstract 3508)

NSABP C-08: OS UPDATED RESULTS

OS estimate

<table>
<thead>
<tr>
<th></th>
<th>mFOLFOX6 (n=1,341)</th>
<th>mFOLFOX6 + Bevacizumab (n=1,337)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR</td>
<td>0.96 (95% CI: 0.79–1.15); p=0.64</td>
<td></td>
</tr>
</tbody>
</table>

Number at risk

<table>
<thead>
<tr>
<th></th>
<th>mFOLFOX6</th>
<th>mFOLFOX6 + Avastin</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 months</td>
<td>1,268</td>
<td>1,289</td>
</tr>
<tr>
<td>1 year</td>
<td>1,205</td>
<td>1,233</td>
</tr>
<tr>
<td>2 years</td>
<td>1,135</td>
<td>1,163</td>
</tr>
<tr>
<td>3 years</td>
<td>942</td>
<td>950</td>
</tr>
<tr>
<td>4 years</td>
<td>204</td>
<td>204</td>
</tr>
</tbody>
</table>

Allegra, et al. ASCO 2011 (abstract 3508)
AVANT Study Design

Surgery for high-risk stage II or stage III colon cancer (N=3451)

FOLFOX4

Observation

Follow-up

FOLFOX4 + bevacizumab

Bevacizumab monotherapy

Follow-up

Bev 5 mg/kg q2w

Bev 7.5 mg/kg q3w

24 weeks

XELOX + bevacizumab

Bevacizumab monotherapy

Follow-up

Bev 7.5 mg/kg q3w

24 weeks

AVANT: DFS (ITT Stage III)

(3-Year Minimum Follow-Up)

Andre T ASCO 2011 abstr 3509

Event-free rate

Time (months)

Number at risk

FOLFOX4 955 890 823 779 740 708 600 451 282 121 32 0 0

FOLFOX4 + Bev 960 921 868 791 728 685 586 436 280 123 33 1 0

XELOX + Bev 952 900 865 784 722 668 560 415 268 110 28 0 0

HR

95% CI

FOLFOX4 (N=955)

1.17

(0.98, 1.39)

FOLFOX4 + Bev (N=960)

1.07

(0.90, 1.28)

XELOX + Bev (N=952)

1.0

(0.98, 1.39)
AVANT: Interim OS (ITT Stage III)
Median duration of follow-up 48 months

Event-free rate

<table>
<thead>
<tr>
<th></th>
<th>FOLFOX4 (N=955)</th>
<th>FOLFOX4 + Bev (N=960)</th>
<th>XELOX + Bev (N=952)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Events</td>
<td>115 (12%)</td>
<td>151 (16%)</td>
<td>145 (15%)</td>
</tr>
<tr>
<td>HR</td>
<td>1.03 (0.99, 1.62)</td>
<td>1.27 (0.99, 1.62)</td>
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</tbody>
</table>

Number at risk

<table>
<thead>
<tr>
<th></th>
<th>FOLFOX4</th>
<th>FOLFOX4 + Bev</th>
<th>XELOX + Bev</th>
</tr>
</thead>
<tbody>
<tr>
<td>955</td>
<td>914</td>
<td>899</td>
<td>884</td>
</tr>
<tr>
<td>950</td>
<td>942</td>
<td>889</td>
<td>835</td>
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<tr>
<td>952</td>
<td>920</td>
<td>894</td>
<td>840</td>
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<td>908</td>
<td>840</td>
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<td>952</td>
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</tr>
<tr>
<td>952</td>
<td>920</td>
<td>908</td>
<td>840</td>
</tr>
</tbody>
</table>

AVANT: Summary, Conclusions and implications

- Addition of beva to FOLFOX4 or XELOX did not prolong DFS in adjuvant treatment of stage III colon cancer
  - chemotherapy alone arm was favoured numerically

- Bevacizumab treatment effect was not constant over time
  - transient favourable effect can be seen within 1 year, which is in-line with NSABP C-08

- Immature OS data suggest a potential detriment. Follow up will continue until at least June 2012, for 5 years minimum follow up for analysis of OS
  - A potential rebound effect after discontinuation of beva cannot be excluded
  - As an alternative, beva may impair our ability to detect relapses by conventional imaging
**VELOUR Study Design**

**Randomize**

- **Metastatic Colorectal Cancer**

Stratification factors:
- ECOG PS (0 vs 1 vs 2)
- Prior bevacizumab (Y/N)

**600**

**1:1**

Disease Progression → Death

**600**

**Placebo IV, day 1**
+ **FOLFIRI** q2 weeks

**Aflibercept 4 mg/kg IV, day 1**
+ **FOLFIRI** q2 weeks

**DMC review every 6 months**

Modified from Van Cutsem ESMO WGICC 2011

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**VELOUR: EFFICACY ITT Population**

**OVERALL SURVIVAL**

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>Aflibercept/FOLFIRI</th>
<th>Median (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>362</td>
<td>392</td>
<td>12</td>
</tr>
<tr>
<td>Aflibercept/FOLFIRI</td>
<td>390</td>
<td>402</td>
<td>17</td>
</tr>
</tbody>
</table>

**Logrank y = 0.0032**

**PFS**

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>Aflibercept/FOLFIRI</th>
<th>Median (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>418</td>
<td>430</td>
<td>4.57</td>
</tr>
<tr>
<td>Aflibercept/FOLFIRI</td>
<td>463</td>
<td>476</td>
<td>6.98</td>
</tr>
</tbody>
</table>

**Logrank y = 0.00007**

Modified from Van Cutsem ESMO WGICC 2011
### VELOUR Response Rate, Independent Review Committee

<table>
<thead>
<tr>
<th>Evaluable population*, %</th>
<th>Placebo</th>
<th>Aflibercept</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Best Overall Response</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Complete response</td>
<td>0.4</td>
<td>0</td>
</tr>
<tr>
<td>Partial response</td>
<td>10.8</td>
<td>19.8</td>
</tr>
<tr>
<td>Stable disease</td>
<td>64.9</td>
<td>65.9</td>
</tr>
<tr>
<td>Progressive disease</td>
<td>21.5</td>
<td>10.4</td>
</tr>
<tr>
<td>Not evaluable</td>
<td>2.5</td>
<td>4.0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Overall Response Rate</strong></th>
<th>Placebo</th>
<th>Aflibercept</th>
</tr>
</thead>
<tbody>
<tr>
<td>(CR or PR)</td>
<td>11.1</td>
<td>19.8</td>
</tr>
<tr>
<td>95% CI</td>
<td>8.5 to 13.8</td>
<td>16.4 to 23.2</td>
</tr>
<tr>
<td>p</td>
<td>0.0001**</td>
<td></td>
</tr>
</tbody>
</table>

*Evaluable population: patients with measurable target lesions that have agreed for third party review

** Stratified, Cochran Mantel test

Modified from Van Cutsem ESMO WGICC 2011

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### Conclusions

- **Adding aflibercept to FOLFIRI in mCRC previously treated with an oxaliplatin based regimen provided clinically significant benefits:**
  - Improvement in overall survival (HR=0.817 [95.34%CI, 0.713-0.937], p = 0.0032)
  - Improvement in PFS (HR=0.758 [99.99%CI, 0.578-0.995], p=0.00007)
  - Improvement in overall RR (11.1% vs 19.8%, p=0.0001)

- **The safety profile of aflibercept was acceptable and consistent with known anti-VEGF adverse effects.** Adding aflibercept increases the specific CT related toxicity in the combination arm: neutropenic complications and diarrhea/stomatitis.

Modified from Van Cutsem ESMO WGICC 2011
Do we currently have biomarkers for patient selection for angiogenesis inhibitors in mCRC?

- Preliminary studies have suggested a correlation between activity of bevacizumab and some biologic characteristics, including circulating VEGF levels, VEGF and/or VEGFRs gene polymorphisms.

- Early occurrence of hypertension has been suggested to correlate with bevacizumab activity.

- A panel of circulating cytokines has been suggested to correlate to bevacizumab activity.

- However, no validated biomarker is currently available for selecting patients to treat with bevacizumab.

Results from the MAX trial

OS: low expression of VEGF-D is predictive for bevacizumab benefits

<table>
<thead>
<tr>
<th>VEGF-D</th>
<th>Median Overall Survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>0,1</td>
<td>C: 18.9 months, CB + CBM: Not reached</td>
</tr>
<tr>
<td>2+</td>
<td>C: 20.6 months, CB + CBM: 21.6 months</td>
</tr>
<tr>
<td>3+</td>
<td>C: 24.5 months, CB + CBM: 19.4 months</td>
</tr>
</tbody>
</table>

C = cape  
CB CBM = cape beva mito

Modified from Ciardiello ESMO WGICC 2011  
Weickhardt A ASCO 2011 abstr 3531
Cancer Is More Complex Than Ever Thought

- Systematic analysis of genetic alterations in human breast and colorectal cancers (N = 22)
- 13,023 genes analyzed
- Average of 90 mutated genes per case
  - Only a subset (average of 11 per case) thought to be relevant to neoplastic process

• Genomics: the genotype
• Genes are transcribed into proteins which can undergo essential functional changes (proteomics)
• 1.1% of genome consists of exons coding for proteins
• 24% introns
• 75% without any known function in RNA transcription or protein translation
• >1% of genes are involved in oncogenesis
• SNPs: 1.4Mio in genome; 60.000 in coding regions

• Are we overwhelmed?

Take Home Messages

• What markers to follow: Stage II and others
  LOH, MSI: wait for results (ECOG & NSABP)
• TS, TP & DPD: routine before treatment?
• KRAS mutation established for cetuximab and panitumumab
• Other molecular markers as of now have to be considered as a complement to clinical and pathological data.
• Proteomics are likely to improve predictability
TGF-β in Cancer: Tumorigenesis

EPIGENETICS: Drug Targets for Cancer Therapy

The field of epigenetics relates to the regulation of gene expression that occurs independent of gene sequence.

Transcription: Open chromatin, acetylation
Repression: Compact chromatin, methylation

Drug Targets to inhibit: Histone deacetylase, DNA methyltransferase
Thank You
### Table 5 Influence of KRAS status on efficacy of cetuximab plus FOLFIRI

<table>
<thead>
<tr>
<th>Outcome</th>
<th>KRAS Wildtype</th>
<th>KRAS Mutant</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ORR (%)</td>
<td>56%</td>
<td>47%</td>
<td>0.004</td>
</tr>
<tr>
<td>mPFS (months)</td>
<td>12.6</td>
<td>9.5</td>
<td>0.004</td>
</tr>
<tr>
<td>mOS (months)</td>
<td>16.4</td>
<td>13.9</td>
<td>0.004</td>
</tr>
<tr>
<td>CR rate (%)</td>
<td>28%</td>
<td>17%</td>
<td>0.004</td>
</tr>
<tr>
<td>Absolute change (%)</td>
<td>42%</td>
<td>32%</td>
<td>0.004</td>
</tr>
</tbody>
</table>

“Predictions are difficult, particularly in regard to the future”

Chinese Proverb, 409 B.C.