KRAS testing in the selection of colorectal patients for anti-EGFR targeted therapy

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KU Leuven, Belgium
**Introduction**

EGFR regulates cancer-cell proliferation, apoptosis and tumor-induced neoangiogenesis

anti-EGFR monoclonal antibodies (cetuximab and panitumumab) are available for the treatment of patients with metastatic colorectal cancer (mCRC), however their clinical treatment efficacy is limited to a subset of patients.

- Activating mutations within KRAS can predict resistance to anti-EGFR monoclonal antibodies in mCRC patients.
- Activating mutations in KRAS may result in EGFR-independent intracellular signal transduction activation
- These mutations are found in approximately 40% of tumors of mCRC patients and are almost exclusively detected in codons 12 and 13 of exon 2.

EGFR-independent, constitutive activation of the RAS signaling pathway impairs response to anti-EGFR drugs.

Learning objectives

• To understand the importance and aims of KRAS testing in colorectal cancer (CRC) patients.

• To understand the requirements for technique performance.

• To understand the components necessary to introduce technique into practice management.
1. What is KRAS?

2. Key studies that demonstrate the impact of KRAS on efficacy of EGFR targeting antibodies in colorectal cancer (CRC).

3. How to test for KRAS?
1. What is KRAS?

- KRAS regulates cellular responses including proliferation, survival and differentiation.

- KRAS is a downstream component of the EGFR signaling network that links growth promoting signals from the cell surface to the nucleus.

- KRAS is a member of the RAS protein group of GTP/GDP binding proteins.
  - KRAS acts as a molecular switch, which is functionally characterized by the change from an inactive GDP-binding state to an active GTP-binding state.
  - GTP-bound RAS can interact with more than 20 effector proteins, including RAF, phosphatidylinositol 3-kinase (PI3-K) and Ral guanine nucleotide-dissociation stimulator (RalGDS),

- The switch to a GTP-binding form normally occurs transiently when growth factor receptors, such as the EGFR, are activated (Figure 1). However,
  - When specific mutations within KRAS occur, the resulting KRAS protein can be constitutively activated; that is, it can function independently of upstream growth factor receptor driven signals and remain active.
Figure 1. RAS mediated intracellular signal transduction pathways

Figure 2. The epidermal growth factor (EGF) receptor

- When the KRAS gene is mutated, the KRAS protein (p21 ras) is active regardless of EGFR activation\(^1,2\)

- KRAS gene mutations are an early event and are found in ~40% of tumors in patients with CRC\(^1,3\)

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Figure 3. Oncogenic mutations in KRAS acquired by the tumor

- Figure 3 depicts the KRAS protein structure.

- KRAS mutations affect the GAP binding domain, which is necessary for the transition to the inactive state.

- KRAS mutations cluster in hotspots - the 7 most frequent mutations in codons 12 and 13 (G12 and G13 in figure), comprise nearly 98% of all mutations.

- It is sufficient for a tumor cell to have only one mutated (active) copy of KRAS - the second copy can remain wild type.

- These characteristics are important for the design of mutation detection assays.

Figure courtesy of the authors

### Table 1 | Frequency of KRAS mutations in CRC patients

<table>
<thead>
<tr>
<th>KRAS mutation</th>
<th>Incidence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Amino acid substitution</strong></td>
<td><strong>Nucleotide substitution</strong></td>
</tr>
<tr>
<td><strong>Codon 12 mutations</strong></td>
<td></td>
</tr>
<tr>
<td>Aspartate (G12D)</td>
<td>G35A</td>
</tr>
<tr>
<td>Valine (G12V)</td>
<td>G35T</td>
</tr>
<tr>
<td>Cysteine (G12C)</td>
<td>G34T</td>
</tr>
<tr>
<td>Serine (G12S)</td>
<td>G3A</td>
</tr>
<tr>
<td>Alanine (G12A)</td>
<td>G35C</td>
</tr>
<tr>
<td>Arginine (G12R)</td>
<td>G34C</td>
</tr>
<tr>
<td><strong>Codon 13 mutations</strong></td>
<td></td>
</tr>
<tr>
<td>Aspartate (G13D)</td>
<td>G38A</td>
</tr>
<tr>
<td>Other Mutations†</td>
<td></td>
</tr>
</tbody>
</table>

*Data extracted from the RASCAL II study and data from the Catalogue of Somatic Mutation in Cancer (COSMIC, Sanger Institute). †Rare mutations that also occur in codons 11, 13, 19, 22, 59, 61, and 146 (COSMIC).
2. **Key studies demonstrate the impact of KRAS on the efficacy of EGFR targeting antibodies in colorectal cancer**

- Recent Phase II and III clinical trial data show benefit to mCRC patients from therapy with anti-EGFR monoclonal antibodies, either as monotherapy or combined with chemotherapy, both in first and later lines of therapy.

- Retrospective subset analyses of these data strongly suggest that patients with KRAS mutations detected in codon 12 or 13 do not benefit from this therapy.

- To date, 5 randomized controlled trials (Table 2, next slide) of cetuximab or panitumumab assessing mCRC patient outcomes in relation to KRAS mutational status have been published.

(In addition to the published series, The ‘COIN’ and first (PRIME) and second line panitumumab trials were presented at the ECCO15 and 34th ESMO Multidisciplinary Congress (September, 2009);

### Table 2 | Randomized clinical trial evidence on relationship of KRAS mutation status to efficacy of anti-EGFR monoclonal antibodies in patients with metastatic colorectal cancer, page 1 of 2

<table>
<thead>
<tr>
<th>Study and population</th>
<th>Treatments by arm</th>
<th>Variable</th>
<th>KRAS WT antibody arm</th>
<th>KRAS WT control arm</th>
<th>KRAS Mutated antibody arm</th>
<th>KRAS Mutated control arm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Van Cutsem et al. 2008&lt;sup&gt;10&lt;/sup&gt; CRYS TAL trial of first line therapy</td>
<td>FOLFIRI ± cetuximab</td>
<td>No. of patients</td>
<td>172</td>
<td>176</td>
<td>105</td>
<td>67</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Response rate, %</td>
<td>59.3</td>
<td>43.2</td>
<td>36.2</td>
<td>40.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>95% CI</td>
<td>51.6 to 66.7</td>
<td>35.8 to 50.9</td>
<td>27.0 to 46.2</td>
<td>29.9 to 51.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P</td>
<td>0.0025</td>
<td>0.87</td>
<td>0.46</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Median PFS, months</td>
<td>9.9</td>
<td>8.7</td>
<td>7.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>HR</td>
<td>1.07</td>
<td>0.68</td>
<td>1.07</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>95% CI</td>
<td>0.68 to 1.47</td>
<td>0.46 to 1.62</td>
<td>0.47</td>
<td></td>
</tr>
<tr>
<td>Bokemeyer et al. 2008&lt;sup&gt;3&lt;/sup&gt; OPUS trial of first line therapy</td>
<td>FOLFOX ± cetuximab</td>
<td>No. of patients</td>
<td>61</td>
<td>73</td>
<td>52</td>
<td>47</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Response rate, %</td>
<td>60.7</td>
<td>37.0</td>
<td>32.7</td>
<td>48.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>95% CI</td>
<td>47.3 to 72.9</td>
<td>26.0 to 49.1</td>
<td>20.3 to 47.1</td>
<td>34.1 to 63.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>OR</td>
<td>2.54</td>
<td>0.11</td>
<td>0.106</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>95% CI</td>
<td>1.24 to 5.23</td>
<td>0.011 to 2.54</td>
<td>0.22 to 1.15</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Median PFS, months</td>
<td>7.7</td>
<td>7.2</td>
<td>5.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>HR</td>
<td>0.57</td>
<td>0.016</td>
<td>1.83</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>95% CI</td>
<td>0.016 to 1.83</td>
<td>0.0192</td>
<td>0.0192</td>
<td></td>
</tr>
<tr>
<td>Punt et al. 2008&lt;sup&gt;8&lt;/sup&gt; CAIRO2 trial of first line therapy</td>
<td>(Capecitabine + oxaliplatin + bevacizumab ± cetuximimab</td>
<td>No. of patients</td>
<td>153</td>
<td>152</td>
<td>93</td>
<td>103</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Median PFS, months</td>
<td>10.5</td>
<td>10.7</td>
<td>8.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>P</td>
<td>0.10</td>
<td>0.35</td>
<td>0.43</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Median OS, months</td>
<td>22.2</td>
<td>23.0</td>
<td>19.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>95% CI</td>
<td>0.49</td>
<td>0.35</td>
<td>0.35</td>
<td></td>
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</tbody>
</table>

Allegra CJ et al. J Clin Oncol. 2009 Apr 20;27(12):2091-6
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<tr>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amado et al. 2008¹</td>
<td>Panitumumab vs. best supportive care</td>
<td>No. of patients</td>
<td>124</td>
<td>119</td>
<td>84</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Response rate, %</td>
<td>17</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Median PFS, weeks</td>
<td>12.3</td>
<td>7.3</td>
<td>7.4</td>
<td>7.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HR 95% CI</td>
<td>0.45</td>
<td>0.34 to 0.59</td>
<td>0.99</td>
<td>0.73 to 1.36</td>
</tr>
<tr>
<td>Karapetis et al. 2008⁰</td>
<td>Cetuximab vs. best supportive care</td>
<td>No. of patients</td>
<td>117</td>
<td>113</td>
<td>81</td>
<td>83</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Response rate, %</td>
<td>12.8</td>
<td>1.2</td>
<td>1.2</td>
<td>1.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Median PFS, months</td>
<td>3.7</td>
<td>1.8</td>
<td>1.8</td>
<td>1.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HR 95% CI</td>
<td>0.40</td>
<td>0.30 to 0.54</td>
<td>0.99</td>
<td>0.73 to 1.35</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P 95% CI</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Median OS, months</td>
<td>9.5</td>
<td>4.8</td>
<td>4.5</td>
<td>4.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>OS at 1 year, % HR (death)</td>
<td>28.3</td>
<td>20.1</td>
<td>13.2</td>
<td>19.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>95% CI</td>
<td>0.55</td>
<td>0.41 to 0.74</td>
<td>0.98</td>
<td>0.70 to 1.37</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.89</td>
<td>0.89</td>
</tr>
</tbody>
</table>

Abbreviations: EGFR, epidermal growth factor receptor; WT – wild type; HR – hazard ratio; OR – odds ratio; PFS – progression free survival; FOLFIRI – folinic acid, fluorouracil, and oxaliplatin; CRYSTAL - cetuximab combined with irinotecan in first line therapy for metastatic colorectal cancer; OPUS – oxaliplatin and cetuximab in in first line therapy for mCRC; CAIRO2 – capecitabine, irinotecan and oxaliplatin in advanced colorectal cancer (2)
### Table 3 | Single arm studies of treatment of metastatic CRC with anti-EGFR monoclonal antibodies and KRAS mutational status, Page 1 of 2

<table>
<thead>
<tr>
<th>Study and population</th>
<th>Treatments by arm</th>
<th>Variable</th>
<th>KRAS WT</th>
<th>KRAS Mutated</th>
</tr>
</thead>
</table>
| **Lievre et al. 2008**<sup>8</sup>  
Second line therapy | Cetuximab | No. of patients  
Response rate  
P | 65  
40  
0.001  
31.4  
19.4 to 36  
0.0001  
14.3  
9.4 to 20  
0.026 | 24  
0  
-  
10.1  
8 to 16  
-  
10.1  
6.1 to 13  
- |
| **E Roock et al. 2008**<sup>4</sup> | Cetuximab vs. with irinotecan | No. of patients  
Response rate  
P (cetuximab + irinotecan)  
P (cetuximab alone)  
PFS, weeks  
95% CI  
P | 57  
41  
0.000001  
0.126  
34  
28.5 to 40  
0.016  
12  
4.2 to 20  
0.351  
44.7  
28.4 to 61  
0.003  
27  
8.9 to 45.1  
0.330 | 46  
0  
-  
12  
5.4 to 18.7  
-  
12  
7.0 to 17.0  
-  
27.3  
9.5 to 45  
-  
25.3  
0/0 to 70.0  
- |
## Table 3 | Single arm studies of treatment of metastatic CRC with anti-EGFR monoclonal antibodies and KRAS mutational status, page 2 of 2

<table>
<thead>
<tr>
<th>Study and population</th>
<th>Treatments by Arm</th>
<th>Variable</th>
<th>KRAS WT</th>
<th>KRAS Mutated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Khambata-Ford <em>et al.</em> 2007&lt;sup&gt;7&lt;/sup&gt;</td>
<td>Cetuximab ; second or third line treatment</td>
<td>No. of patients Response rate, %</td>
<td>50</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>Di Fiore <em>et al.</em> 2007&lt;sup&gt;5&lt;/sup&gt;</td>
<td>Cetuximab plus chemotherapy</td>
<td>No. of patients Response rate, %</td>
<td>43</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>28</td>
<td>0</td>
</tr>
<tr>
<td>Benvenuti <em>et al.</em> 2007&lt;sup&gt;2&lt;/sup&gt;</td>
<td>Panitumumab or cetuximab , or cetuximab plus chemotherapy</td>
<td>No. of patients Response rate, %</td>
<td>32</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>31</td>
<td>6</td>
</tr>
</tbody>
</table>

Abbreviations: EGFR, epidermal growth factor receptor; CRC, colorectal cancer; WT, wild type; PFS, progression-free survival; OS, overall survival.

Adapted with permission<sup>11</sup>: Blue Cross – Blue Schield Association. Technology Evaluation Center. KRAS Mutations and Epidermal Growth Factor Receptor Inhibitor Therapy in Metastatic Colorectal Cancer TEC Assessments 2008; volume 23, tab 6. Copyright

Adapted from Blue Cross – Blue Schield Association. Technology Evaluation Center KRAS and Epidermal Growth Factor Receptor Inhibitors

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Allegra CJ *et al.* J Clin Oncol. 2009 Apr 20;27(12):2091-6
Figure 4. Panitumumab + BSC vs. BSC alone in a wild-type KRAS subgroup

<table>
<thead>
<tr>
<th>Patients at Risk</th>
<th>Weeks</th>
<th>Proportion with PFS</th>
</tr>
</thead>
<tbody>
<tr>
<td>P’mab + BSC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BSC alone</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>PFS by treatment</th>
<th>Events/n (%)</th>
<th>Median, weeks</th>
<th>Mean, weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>P’mab + BSC</td>
<td>115/124 (93)</td>
<td>12.3</td>
<td>19.0</td>
</tr>
<tr>
<td>BSC alone</td>
<td>114/119 (96)</td>
<td>7.3</td>
<td>9.3</td>
</tr>
</tbody>
</table>

HR = 0.45 (95% CI: 0.34–0.59)
Stratified log-rank test, \( P < 0.0001 \)
Figure 4. Panitumumab + BSC vs. BSC alone in a mutant KRAS subgroup

**PFS by treatment**

<table>
<thead>
<tr>
<th></th>
<th>Events/n (%)</th>
<th>Median, weeks</th>
<th>Mean, weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>P'mab + BSC</td>
<td>76/84 (90)</td>
<td>7.4</td>
<td>9.9</td>
</tr>
<tr>
<td>BSC alone</td>
<td>95/100 (95)</td>
<td>7.3</td>
<td>10.2</td>
</tr>
</tbody>
</table>

HR = 0.99 (95% CI: 0.73–1.36)
**Figure 5. CRYSTAL: Cetuximab + FOLFIRI vs. FOLFIRI**

**FOLFIRI first line - primary endpoint (PFS)**

**ITT population; independent review**

- **HR:** 0.851
- **P = 0.0479**

**RR**
- FOLFIRI + cetuximab: 46.9%
- FOLFIRI: 38.7%

**1-year PFS rate**
- 23% vs 34%

Van Cutsem et al. N Engl J Med. 2009 Apr 2;360(14):1408-17
Crystal: Relating KRAS status to efficacy: data quality

577 subjects analyzed for K-RAS mutation

540 subjects: K-RAS evaluable population (ITT)

348 (64.4%) K-RAS wt
- Group A: 172 (49.4%)
- Group B: 176 (51.6%)

192 (35.6%) K-RAS mt
- Group A: 105 (54.74%)
- Group B: 87 (45.3%)

171 subjects with events (49.1%)

101 subjects with events (52.6%)

Van Cutsem et al. N Engl J Med. 2009 Apr 2;360(14):1408-17
Figure 6. Crystal: progression free survival (PFS) – KRAS wild-type

<table>
<thead>
<tr>
<th></th>
<th>Patients, n (progressed/ censored)</th>
<th>median PFS, mo [95% CI]</th>
<th>HR [95% CI]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cetuximab + FOLFIRI</td>
<td>172 (76/96)</td>
<td>9.9 [8.7, 14.6]</td>
<td>0.68 [0.501, 0.934]</td>
</tr>
<tr>
<td>FOLFIRI</td>
<td>176 (95/81)</td>
<td>8.7 [7.4, 9.9]</td>
<td>-</td>
</tr>
</tbody>
</table>

Van Cutsem et al. N Engl J Med. 2009 Apr 2;360(14):1408-17
Figure 7. Crystal: PFS – KRAS mutant

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Patients, n</th>
<th>median PFS, mo [95% CI]</th>
<th>HR [95% CI]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cetuximab + FOLFIRI</td>
<td>105 (58/47)</td>
<td>7.6 [6.7,9.4]</td>
<td>1.07 [0.710, 1.610]</td>
</tr>
<tr>
<td>FOLFIRI</td>
<td>87 (43/44)</td>
<td>8.1 [7.5,9.4]</td>
<td>-</td>
</tr>
</tbody>
</table>

Van Cutsem et al. N Engl J Med. 2009 Apr 2;360(14):1408-17
Figure 1. Overall survival in the KRAS wild-type population from the CRYSTAL study

- **Cetuximab+FOLFIRI** (n=316)
  - No. of Events: 242
  - Median OS: 23.5 months [21.2–26.3]
  - HR (95% CI): 0.796 [0.670–0.946]
  - p-value: 0.0094 (log-rank)

- **FOLFIRI** (n=350)
  - No. of Events: 288
  - Median OS: 20.0 months [17.4–21.7]
  - HR (95% CI): 1.0

Figure 8. Overall Survival CRYSTAL
Figure 9. Opus: Cetuximab + FOLFOX vs. FOLFOX in first line treatment Primary endpoint: overall response rate

Overall response rate (%)

- **K-RAS wt**
  - FOLFOX: 37
  - Cetuximab + FOLFOX: 61
  - Odds ratio: 2.54; \( P = 0.011 \)

- **K-RAS mt**
  - FOLFOX: 49
  - Cetuximab + FOLFOX: 33
  - Odds ratio: 0.51; \( P = 0.106 \)

No benefit for Cetuximab

\( P = 0.106 \)
Figure 10. Opus: Cetuximab + FOLFOX vs. FOLFOX in first line; PFS KRAS wild-type

<table>
<thead>
<tr>
<th></th>
<th>Patients, n (progressed/censored)</th>
<th>median PFS, mo [95% CI]</th>
<th>HR [95% CI]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cetuximab</td>
<td>61 (30/31)</td>
<td>7.7 [7.1,12.0]</td>
<td>0.57 [0.351, 0.894]</td>
</tr>
<tr>
<td>FOLFOX</td>
<td>73 (48/25)</td>
<td>7.2 [5.6,7.4]</td>
<td>-</td>
</tr>
</tbody>
</table>

K-RAS wt: HR=0.57

43% risk reduction for progression

Log Rank P-value: 0.016
Figure 11. Opus: Cetuximab + FOLFOX vs. FOLFOX in first line; PFS KRAS mutant

<table>
<thead>
<tr>
<th></th>
<th>Patients, n (progressed/censored)</th>
<th>median PFS, mo [95% CI]</th>
<th>HR [95% CI]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cetuximab + FOLFOX</td>
<td>52 (39/13)</td>
<td>5.5 [4.0, 7.4]</td>
<td>1.83 [1.095, 3.056]</td>
</tr>
<tr>
<td>FOLFOX</td>
<td>47 (26/21)</td>
<td>8.6 [6.5, 9.5]</td>
<td>-</td>
</tr>
</tbody>
</table>

Log Rank P-value: 0.0192

K-RAS mt: HR=1.83
Randomized phase 3 study of panitumumab with FOLFOX4 vs FOLFOX4 alone as first-line treatment in patients with metastatic colorectal cancer: the PRIME trial

## Results: KRAS Ascertainment

<table>
<thead>
<tr>
<th></th>
<th>Panitumumab + FOLFOX</th>
<th>FOLFOX</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients randomized, n</td>
<td>593</td>
<td>590</td>
<td>1183</td>
</tr>
<tr>
<td>Patients included in <em>KRAS</em> analysis – %</td>
<td>92</td>
<td>93</td>
<td>93</td>
</tr>
<tr>
<td><em>WT</em> <em>KRAS</em> – %</td>
<td>60</td>
<td>60</td>
<td>60</td>
</tr>
<tr>
<td><em>MT</em> <em>KRAS</em> – %</td>
<td>40</td>
<td>40</td>
<td>40</td>
</tr>
<tr>
<td>Patients with <em>KRAS</em> unevaluable, %</td>
<td>8</td>
<td>7</td>
<td>7</td>
</tr>
</tbody>
</table>

KRAS tumor status was determined using the DxS kit (Manchester, UK) that tests the 7 most common KRAS mutations in codons 12 and 13.

Figure 12. WT KRAS: Progression-Free Survival

Figure 13. PFS by KRAS Mutation Status

<table>
<thead>
<tr>
<th></th>
<th>Events</th>
<th>Median (95% CI)</th>
<th>Events</th>
<th>Median (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n (%)</td>
<td>months</td>
<td>n (%)</td>
<td>months</td>
</tr>
<tr>
<td>Panitumumab + FOLFOX</td>
<td>199 (61)</td>
<td>9.6 (9.2–11.1)</td>
<td>167 (76)</td>
<td>7.3 (6.3 – 8.0)</td>
</tr>
<tr>
<td>FOLFOX</td>
<td>215 (65)</td>
<td>8.0 (7.5–9.3)</td>
<td>157 (72)</td>
<td>8.8 (7.7 – 9.4)</td>
</tr>
</tbody>
</table>

HR = 0.80 (95% CI: 0.66–0.97)
P-value = 0.02

HR = 1.29 (95% CI: 1.04 – 1.62)
P-value = 0.02

Two main findings emerged from the assessment of these studies:

1. A consistent correlation exists between the presence of a KRAS mutation in codon 12 or 13 and lack of response to anti-EGFR MoAb therapy in patients with metastatic colorectal cancer.

2. Evidence of improved tumor response, progression-free and/or overall survival, in response to anti-EGFR MoAb therapy is seen only in those patients with no mutation in codon 12 or 13 (wild type) versus abnormal (mutated) KRAS tumors in analyses from 6 of 8 randomized controlled trials (RCTs).
No strong prognostic effect of KRAS was observed in the control arm (best supportive care) of the NCIC CO 17 study.

When an EGFR inhibitor was combined with FOLFOX in patients with KRAS mutations, a reduced efficacy was observed compared to patients with mutations receiving chemo alone (see Opus study and Prime study).

This emphasizes the need for KRAS selection prior to therapy!
To date, the data cover only mutations in codons 12 and 13 of KRAS.

Mutations that activate KRAS also occur at codons 61, 15 and 146. However, these mutations are very uncommon (<2%) and are currently not taken into account for decision making.

It is presently unclear how differences in sensitivity and specificity among the various assays that are available for KRAS mutation testing may affect the predictive value of the test.

In addition, other mutations that affect the response to anti-EGFR MoAbs have been reported (eg, mutation in BRAF and PI3K). These subjects are either the focus of current research, or there are insufficient data to justify an opinion at present.
Current guidelines for EGFR monoclonal antibody usage

- **December, 2007:** EMEA granted a conditional marketing authorization for panitumumab as monotherapy for treatment of mCRC patients with EGFR-expressing wild-type KRAS genes after failure of standard chemotherapy regimens.

- **July, 2008:** EMEA approved cetuximab for use in mCRC patients with KRAS wild-type tumors.

- **November, 2008:** Testing for KRAS gene mutations was added to the updated National Comprehensive Cancer Network (NCCN) clinical practice guidelines for colon cancer. These guidelines stipulate that only patients whose tumors have the wild-type (normal) KRAS genes should receive treatment with the epidermal growth-factor receptor inhibitors cetuximab and panitumumab.

- **February, 2009:** ASCO PROVISIONAL CLINICAL OPINION - based upon systematic reviews of the relevant literature, all mCRC patients who are candidates for anti-EGFR antibody therapy should have their tumor tested for KRAS mutations in a CLIA-accredited laboratory. If KRAS mutation in codon 12 or 13 is detected, these patients should not receive anti-EGFR therapy as part of their treatment for mCRC.

- **July, 2009:** Change in the US FDA labelling of cetuximab and panitumumab - retrospective subset analyses of mCRC trials have not shown a treatment benefit in patients whose tumor had a KRAS mutation in codon 12 or 13. Use of these drugs is not recommended for the treatment of colorectal cancer in patients with these mutations.
Patients diagnosed with metastatic CRC should be tested for KRAS status to allow the optimal treatment strategy to be implemented.
3. How to test for KRAS?

- KRAS is now a validated predictive biomarker for the use of EGFR targeting antibodies in metastatic colorectal cancer.

- In the last years routine KRAS testing has been set up worldwide, a process involving the oncologist, the pathologist and the molecular diagnostics laboratory.

- KRAS testing may be the first test in colorectal cancer, but certainly not the last. It is important that access to molecular testing on CRC samples be well organized and controlled, for current and future use.
Workflow - theranostic

*Cycle: 5 days minimum for test*
Role of the oncologist

• When to conduct KRAS testing?
  1) On diagnosis of CRC (any stage)?
  2) On diagnosis of liver metastases?

• As soon as possible. Currently knowledge of KRAS status is necessary in metastatic setting only, but the Workflow is optimized and delays avoided when testing is done at diagnosis.

• Where to conduct KRAS testing?

• Together with pathologist, oncologists identify a certified laboratory that will perform KRAS testing. Issues to discuss include test procedures, turn around time, cost and reimbursement procedures.

Using existing sample of tumor tissue makes further invasive procedures unnecessary
Role of the pathologist

The area of interest for DNA extraction should be selected specifically by the pathologist.

- Requirements of minimum tumor content (%) or tumor area (surface) will depend on the test utilized and should be discussed with the molecular diagnostics laboratory.

The pathologist's selection of the appropriate tissue block for molecular diagnostic assessment is crucial.

What material can be used for DNA extraction and KRAS testing?

- Archival formalin fixed tumor specimens that are obtained at surgery.
- Primary tumor and metastasis can be used (over 95% concordance, studies in progress).
- Endoscopic and needle biopsies may be used if verified to contain sufficient invasive tumor cells, studies in progress.
KRAS testing procedures

- DNA will be extracted from the tissue on the slides.
- KRAS mutant cells have one mutant gene copy and one wild type gene copy (50/50% mut/wt ratio).
- Admixture of normal tissue on the slide should be kept to a minimum as it decreases the ratio of mutant versus wt signal in the DNA extracted and may hamper detection of mutations (false negative).
- This is why sensitivity of the assays is important; several approaches are possible:
- Extraction of DNA from FFPE tissue can be performed by using different approaches, including commercially available kits.
  - Select only slides that contain a high % of tumor cells vs. normal cells - 70% is suggested.
  - Perform macrodissection of tumor areas and use these for DNA extraction, as depicted.
Molecular analysis

- KRAS testing should be performed in a certified laboratory.

- Laboratory certification and method validation is obtained via national and international certification bodies.

- National and European quality assessment programs and training for KRAS testing have been set up. (See European Society of Pathology at www.esp-pathology.org).

Molecular analysis

Choice of test:

• The DNA is analyzed for KRAS mutations by various, generally PCR based, methods (*Table 4*). Each of these methods has advantages and disadvantages related to their sensitivity and specificity. More detailed descriptions of the different procedures can be found in van Krieken *et al.* Virchows Arch. 2008 Nov;453(5):417-31.

  o Test sensitivity (*Table 4*) is an issue because of the admixture of tumor and normal cells in the patient material, which can potentially affect the ability to detect low copy tumor specific alterations.

  o Specificity: the clinical impact of KRAS is shown when considering the seven most frequent mutations, representing nearly 98% of all KRAS alterations (*Table 1*). A test that specifically and only detects these seven mutations is therefore acceptable. Techniques like sequencing may detect other genomic alterations in KRAS, of which the clinical significance is unknown.
Table 4 | Laboratory analysis of KRAS mutations

<table>
<thead>
<tr>
<th>Method for assessing gene status</th>
<th>Sensitivity (%)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Direct dideoxy sequencing</td>
<td>20 - 30</td>
</tr>
<tr>
<td>Direct pyrosequencing</td>
<td>5</td>
</tr>
<tr>
<td>Allele specific probes</td>
<td>10</td>
</tr>
<tr>
<td>High-resolution melting analysis</td>
<td>5</td>
</tr>
<tr>
<td>ARMS/scorpion probes</td>
<td>1</td>
</tr>
</tbody>
</table>

*The lowest level of mutant DNA that can be detected, expressed as a percentage of total DNA in the tumor sample analysed. Abbreviation: ARMS, amplification refractory mutation system.
Molecular analysis

Choice of KRAS test:

- Currently there is not enough information to assess whether one method is superior to others.

- In view of the need for standardization and quality control it is advisable to use the commercial assays available, which have extensive method validation performed by the producer and are continuously monitored for quality assurance purposes (CE marking).

- In Europe there is no regulation on which test to use.

- In the US, the FDA controls the tests to be used.

- The UK based vendor DxS, offers a kit (TheraScreen, based on qPCR and ARMS Scorpion probes) for KRAS mutation detection that has been widely used in the clinical trials reported so far. DxS is expected to seek US Food and Drug Administration approval for their assay.
A qPCR-based KRAS test workflow (therascreen)

DNA is extracted from formalin fixed paraffin embedded tissue using standard methodology.

Each DNA sample is added to 8 separate reaction tubes and placed onto a real-time PCR instrument.

The PCR reaction takes around 90 minutes.

The analysis is completed by comparing the mutant reactions to the normal.

Source: Therascreen, DxS Ltd 48 Grafton Street, Manchester, M13 9XX, UK
Reporting Criteria for KRAS testing

Reporting of the result should be clear for the oncologist:

- **KRAS normal** = no mutation was identified.
  - Report will specify assay type and controls used.

- **KRAS abnormal** = mutation was found.
  - Report will specify what mutation was found.
  - Report will specify assay type and controls used.

- Laboratory reports should specify:
  - assay sensitivity and specificity,
  - limitations due to insufficient sample quality,
  - and other technical issues useful to test evaluation.

No EGFR targeting drugs should be used when a KRAS mutation is present
Conclusion: KRAS fulfils Criteria for a good biomarker

- Based on sound scientific evidence
  - Reproduced in different tumour types
  - Understood mechanistically
- Can be measured reproducibly with high sensitivity and specificity using the patient material
- Has a clinically relevant impact on treatment
THANK YOU!