The *KRAS* biomarker in Colorectal Cancer

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GlaxoSmithKline
KRAS History

Timeline | A chronicle of RAS discovery

- Retroviral isolates were identified (also in 1967, 1974 and 1978)
- Ha-MSV was cloned
- Ki-MSV was cloned
- Human oncogenes were found to be related to v-ras oncogenes
- NRAS was identified
- p21 RAS was found to be a GTPase
- Ras oncogenes were found to be activated by mitogenic factors
- Ras transgenic mice were generated
- Ras proteins were shown to be farnesylated
- Ras was shown to interact with RAF
- Farnesyltransferase inhibitors were found to block Ras-induced tumours in mice

- 1964
  - Ha-MSV and K-MSV were found to contain rat cellular sequences

- 1973
  - p21 v-Ras was found to bind GTP

- 1979
  - The first human transforming gene was cloned

- 1981
  - The human H-RAS oncogene was found to be activated by point mutation

- 1982
  - FAS was found to cooperate with MYC and E1A to transform primary cells

- 1983
  - RAS-GAP was identified
  - RAS structure was resolved

- 1984
  - RAS was shown to be frequently mutated in colon tumours

- 1987
  - RAS-GFIs were identified

- 1989
  - PKC was shown to be a RAS effector

- 1990
  - Ras was shown to cooperate with MYC and E1A to transform primary cells

- 1993
  - Ras proteins were shown to be farnesylated

- 1994
  - Ras transgenic mice were generated

- 1995
  - Ras was shown to interact with RAF

Malumbres M and Barbacid M. Nature Reviews, 2003
The **KRAS Oncogene**

- The *KRAS* gene encodes the human cellular homolog of a transforming gene of the Kirsten rat sarcoma-2 virus
- *KRAS* is a self-inactivating signal transducer
  - It cycles from GDP bound ("off" state) to GTP bound ("on" state) in response to receptor activation
  - This response is transient due to the intrinsic GTPase activity
- *KRAS* oncogenes harbor activating mutations yielding proteins with reduced GTPase activity
- These activating *KRAS* mutations are among the most common oncogenic alterations in cancer\(^1\)

KRAS/BRAF mutations are early events in CRC carcinogenesis.
## Single-arm Studies Support the Hypothesis for KRAS as a Biomarker for EGFR Inhibitors

<table>
<thead>
<tr>
<th>Reference</th>
<th>Treatment (panitumumab or cetuximab)</th>
<th>N (WT:MT)</th>
<th>Objective Response N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Liévre, et al. (AACR Proceedings, 2007)</td>
<td>cmab ± CT</td>
<td>76 (49:27)</td>
<td>MT 0 (0) WT 24 (49)</td>
</tr>
<tr>
<td>S. Benvenuti, et al. (Cancer Res, 2007)</td>
<td>pmab or cmab or cmab + CT</td>
<td>48 (32:16)</td>
<td>MT 1 (6) WT 10 (31)</td>
</tr>
<tr>
<td>W. De Roock, et al. (ASCO Proceedings, 2007)</td>
<td>cmab or cmab + irinotecan</td>
<td>113 (67:46)</td>
<td>MT 0 (0) WT 27 (40)</td>
</tr>
<tr>
<td>D. Finocchiaro, et al. (ASCO Proceedings, 2007)</td>
<td>cmab ± CT</td>
<td>81 (49:32)</td>
<td>MT 2 (6) WT 13 (26)</td>
</tr>
<tr>
<td>F. Di Fiore, et al. (Br J Cancer, 2007)</td>
<td>cmab + CT</td>
<td>59 (43:16)</td>
<td>MT 0 (0) WT 12 (28)</td>
</tr>
<tr>
<td>S. Khambata-Ford, et al. (J Clin Oncol, 2007)</td>
<td>cmab</td>
<td>80 (50:30)</td>
<td>MT 0 (0) WT 5 (10)</td>
</tr>
</tbody>
</table>

WT, wild type; MT, mutant; cmab, cetuximab; CT, chemotherapy; pmab, panitumumab
**KRAS Analysis of a Phase 3, Randomized, Controlled Trial Comparing Panitumumab vs Best Supportive Care (BSC) in Colorectal Cancer**

Hypothesis: Clinical benefit of panitumumab would be confined to colorectal cancer patients with wild-type KRAS

Randomization stratification:
- ECOG score: 0-1 vs. 2
- Geographic region: Western EU vs. Central & Eastern EU vs. Rest of World

Panitumumab PD Follow-up 6.0 mg/kg Q2W + BSC

Optional Panitumumab Crossover Study

Objectives and Analysis Methodology

Primary Objective

- To assess if the effect of panitumumab on progression-free survival (PFS) was significantly greater in patients with wild-type KRAS compared to patients with mutant KRAS

Secondary Objectives

- To assess whether panitumumab improves PFS compared with BSC alone for patients with wild-type KRAS
- To assess whether panitumumab improves OS compared with BSC alone for patients with wild-type KRAS

Assay Used to Detect KRAS Mutational Status

- DNA was isolated from fixed tumor samples
- Mutant KRAS was detected using a KRAS mutation kit (DxS Ltd, Manchester, UK) that used allele-specific, real-time PCR
  - The kit can detect approximately 1% of mutant DNA in a background of wild-type genomic DNA
  - The test identifies 7 somatic mutations in codons 12 and 13
    - Gly 12 Asp
    - Gly 12 Ala
    - Gly 12 Val
    - Gly 12 Ser
    - Gly 12 Arg
    - Gly 12 Cys
    - Gly 13 Asp
DxS *KRAS* Mutation Test Kit Overview

Allele-specific ARMS* forward primer

Common Scorpions reverse primer

*Amplification Refractory Mutation System*
The ARMS Scorpions assay detects 7 common mutations in codons 12 and 13 of the K-RAS gene.

ARMS primer alignments

12cys
12arg
12ser

Normal RAS gene: 12gly

12asp
12ala
12val

13asp

With permission: DxS Ltd, Manchester, UK
## Results: Prevalence of Mutant KRAS

<table>
<thead>
<tr>
<th></th>
<th>Panitumumab + BSC</th>
<th>BSC alone</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients randomized, n</td>
<td>231</td>
<td>232</td>
<td>463</td>
</tr>
<tr>
<td>KRAS not tested, n (%)</td>
<td>11 (5)</td>
<td>7 (3)</td>
<td>18 (4)</td>
</tr>
<tr>
<td>KRAS tests failed, n (%)</td>
<td>12 (5)</td>
<td>6 (3)</td>
<td>18 (4)</td>
</tr>
<tr>
<td>Patients included in KRAS analysis, n (%)</td>
<td>208 (90)</td>
<td>219 (94)</td>
<td>427 (92)</td>
</tr>
<tr>
<td>Wild-type KRAS, n (%)</td>
<td>124 (60)</td>
<td>119 (54)</td>
<td>243 (57)</td>
</tr>
<tr>
<td>Mutant KRAS, n (%)</td>
<td>84 (40)</td>
<td>100 (46)</td>
<td>184 (43)</td>
</tr>
</tbody>
</table>

## Distribution of KRAS Mutations

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Panitumumab + BSC</th>
<th>BSC alone</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>%</td>
<td>n</td>
</tr>
<tr>
<td>12 Ala</td>
<td>8</td>
<td>9.5</td>
<td>7</td>
</tr>
<tr>
<td>12 Asp</td>
<td>34</td>
<td>40.5</td>
<td>36</td>
</tr>
<tr>
<td>12 Arg</td>
<td>0</td>
<td>0.0</td>
<td>3</td>
</tr>
<tr>
<td>12 Val</td>
<td>15</td>
<td>17.9</td>
<td>25</td>
</tr>
<tr>
<td>12 Cys</td>
<td>7</td>
<td>8.3</td>
<td>7</td>
</tr>
<tr>
<td>12 Ser</td>
<td>5</td>
<td>6.0</td>
<td>9</td>
</tr>
<tr>
<td>13 Asp</td>
<td>15</td>
<td>17.9</td>
<td>14</td>
</tr>
</tbody>
</table>
The relative effect of panitumumab versus BSC was significantly greater in patients with WT versus mutant KRAS.

The quantitative-interaction test comparing the magnitude of the relative treatment effect on PFS between WT and mutant KRAS was statistically significant (p < 0.0001).

PFS was significantly greater for panitumumab treatment compared with BSC in the WT KRAS group (stratified log-rank test p < 0.0001).

Maximum Percent Decrease in Target Lesions
Final Analysis, KRAS Evaluable Group

Mutant

Pmab + BSC

Wild-Type

BSC Alone

PR (0%)  SD (12%)  PD (70%)

PR (17%)  SD (34%)  PD (36%)

PR (0%)  SD (8%)  PD (60%)

PR (0%)  SD (12%)  PD (75%)

Overall Survival by Treatment and KRAS

Wild-type vs. Mutant (treatment arms combined)

HR = 0.67 (95% CI: 0.55–0.82) (adjusted for treatment and randomization factors; ECOG, region)

Aspects that lend robustness to the data

1. Hypothesis re: *KRAS* conferring primary resistance generated independently (from previous trials)
2. Only biomarker (in addition to EGFR) tested was *KRAS* – to avoid inflation of type-1 error
3. Analyses sufficiently powered and prespecified in statistical analysis plan before *KRAS* data known
4. Testing performed in an independent lab without patient-level knowledge of randomization or outcome
5. The magnitude of the interaction observed is substantial
6. Biological plausibility
NCI CTG CO.17: Randomized Phase III Trial in mCRC

Failed or intolerant to all recommended therapies, ECOG 0-2, No Prior EGFR directed therapy

**Primary Endpoint:** Overall Survival

**Secondary Endpoints:**
- Progression Free Survival
- Objective Response Rate (RECIST criteria)
- Safety and Quality of Life

**Cetuximab** 400 mg/m² IV week 1 then 250 mg/m² IV weekly

```
| 1:1 | REGISTER | RANDOMIZE |
```

EGFR testing by IHC

Disease Progression or Unacceptable Toxicity

BSC alone

Cetuximab* + BSC

* Cetuximab 400 mg/m² IV week 1 then 250 mg/m² IV weekly
**NCI C CTG CO.17: Overall Survival**

- **Study arm**
  - **Cetuximab + BSC**: 6.1 months (95% CI = 5.4 - 6.7)
  - **BSC alone**: 4.6 months (95% CI = 4.2 - 4.9)

- **HR**: 0.77 (95% CI = 0.64 - 0.92)

- **Stratified log rank**
  - **p-value**: 0.0046

**Subjects at Risk**

<table>
<thead>
<tr>
<th>Study arm</th>
<th>MS (months)</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cetuximab + BSC</td>
<td>6.1</td>
<td>5.4 - 6.7</td>
</tr>
<tr>
<td>BSC alone</td>
<td>4.6</td>
<td>4.2 - 4.9</td>
</tr>
</tbody>
</table>

**Proportion Alive** vs. **MONTHS**

- **CETUXIMAB + BSC**
- **BSC alone**

**NCI C CTG CO.17: Overall Survival**

**Jonker et al, NEJM 2007**
NCI C CTG CO.17 K-Ras Analysis

N=572 randomized: ITT subset

N=394: *K-ras* assessed subset (69%)

N=164 (42%) mutant

N=230 (58%) wild-type

- Genomic DNA extracted from FFPET slides or sections
- Assessed by bidirectional sequencing for codon 12/13 mutations
- No difference between *K-ras* mutated and WT patients re: demographics, previous treatment or other variables

NCI C CTG C0.17: Overall survival in K-ras Mutant patients

<table>
<thead>
<tr>
<th>Study arm</th>
<th>MS (months)</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cetuximab + BSC</td>
<td>4.5</td>
<td>3.8 - 5.6</td>
</tr>
<tr>
<td>BSC alone</td>
<td>4.6</td>
<td>3.6 - 5.5</td>
</tr>
</tbody>
</table>

HR 0.98  95% CI (0.70,1.37)

Log rank p-value: 0.89

NCI C CTG C0.17: Overall survival in K-ras Wild-Type patients

<table>
<thead>
<tr>
<th>Study arm</th>
<th>MS (months)</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cetuximab + BSC</td>
<td>9.5</td>
<td>7.7 - 10.3</td>
</tr>
<tr>
<td>BSC alone</td>
<td>4.8</td>
<td>4.2 - 5.5</td>
</tr>
</tbody>
</table>

HR 0.55 95% CI (0.41,0.74)

Log rank p-value: <0.0001

NCI C CTG C0.17: Overall Survival by KRAS Status in BSC ARM

**KRAS status** | **MS (months)** | **95% CI**
---|---|---
Mutated | 4.6 | 3.6 - 5.5
Wild-Type | 4.8 | 4.2 - 5.5

HR 1.01  95% CI (0.74,1.37)

Log rank p-value: 0.97

**NO PROGNOSTIC IMPACT OF K-ras STATUS**

### Chemotherapy combination studies

<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></td>
<td></td>
<td></td>
<td>Antibody</td>
<td>Control</td>
</tr>
<tr>
<td>van Cutsem et al, 2008(^10); CRYSTAL trial of first line therapy</td>
<td>FOLFIRI ± cetuximab</td>
<td>N</td>
<td>172</td>
<td>176</td>
</tr>
<tr>
<td></td>
<td></td>
<td>RR (%)</td>
<td>59.3</td>
<td>43.2</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>
</tr>
<tr>
<td></td>
<td></td>
<td>P</td>
<td>.0025</td>
<td>.46</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PFS (m)</td>
<td>9.9</td>
<td>8.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HR</td>
<td>0.68</td>
<td>1.07</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P</td>
<td>.017</td>
<td>.47</td>
</tr>
<tr>
<td>Bokemeyer et al, 2008(^3); OPUS trial of first line therapy</td>
<td>FOLFOX ± cetuximab</td>
<td>N</td>
<td>61</td>
<td>73</td>
</tr>
<tr>
<td></td>
<td></td>
<td>RR (%)</td>
<td>60.7</td>
<td>37.0</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>
</tr>
<tr>
<td></td>
<td></td>
<td>P</td>
<td>.011</td>
<td>.106</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PFS (m)</td>
<td>7.7</td>
<td>7.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HR</td>
<td>0.57</td>
<td>1.83</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P</td>
<td>.016</td>
<td>.0192</td>
</tr>
<tr>
<td>Punt et al, 2008(^9); CAIRO2 trial of first line therapy</td>
<td>(Capecitabine + oxaliplatin + bevacizumab) ± cetuximab</td>
<td>N</td>
<td>153</td>
<td>152</td>
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<tr>
<td></td>
<td></td>
<td>PFS (m)</td>
<td>10.5</td>
<td>10.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P</td>
<td>.10</td>
<td>.043</td>
</tr>
<tr>
<td></td>
<td></td>
<td>OS (m)</td>
<td>22.2</td>
<td>23.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P</td>
<td>.49</td>
<td>.35</td>
</tr>
</tbody>
</table>
## Panitumumab: Modification to ongoing studies

<table>
<thead>
<tr>
<th>Study</th>
<th>Regimens</th>
<th>N</th>
<th>Study Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemotherapy + Panitumumab</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20050181</td>
<td>FOLFIRI ± panitumumab 2nd line</td>
<td>1150 (1187 enrolled)</td>
<td>Fully enrolled</td>
</tr>
<tr>
<td>20050203 (PRIME)</td>
<td>FOLFOX ± panitumumab 1st line</td>
<td>1150 (1183 enrolled)</td>
<td>Fully enrolled</td>
</tr>
<tr>
<td>20060141 (SPIRITT)</td>
<td>FOLFIRI + panitumumab vs. FOLFIRI + bevacizumab 2nd line</td>
<td>210</td>
<td>Ongoing</td>
</tr>
<tr>
<td>Chemotherapy + Bevacizumab ± Panitumumab</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20040249 (PACCE)</td>
<td>Oxaliplatin or irinotecan regimen + bevacizumab ± panitumumab 1st line</td>
<td>823 oxaliplatin 230 irinotecan</td>
<td>Complete</td>
</tr>
</tbody>
</table>
# Cetuximab: Modification to ongoing studies

## Revisions and Current Status of Trials

<table>
<thead>
<tr>
<th>Clinical Setting</th>
<th>N (initial/revised)</th>
<th>Required Protocol Revisions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td><strong>K-ras Testing</strong></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Prior to Randomization</td>
</tr>
<tr>
<td>N0147* (NCCTG)</td>
<td>Adjuvant</td>
<td>2300 / 3768</td>
</tr>
<tr>
<td>PETACC 8</td>
<td>Adjuvant</td>
<td>2000 / 2549</td>
</tr>
<tr>
<td>C80405* (CALGB)</td>
<td>1st line</td>
<td>2300 / 3610</td>
</tr>
<tr>
<td>COIN † (MRC)</td>
<td>1st line</td>
<td>2421</td>
</tr>
<tr>
<td>S0600* (SWOG)</td>
<td>2nd line</td>
<td>1260 / TBD</td>
</tr>
</tbody>
</table>

* Accrual suspended as per 06-Jun-08 Action Letter issued by NCI / CTEP

† Accrual completed prior to amendment
**KRAS Conclusions**

- The efficacy of panitumhumab and cetuximab monotherapy is confined to patients with wild-type KRAS.

- **KRAS** mutation status does not have a treatment-independent prognostic effect.

- **KRAS** genotyping of tumors should be done in patients with mCRC being treated with anti-EGFR antibodies.

- Ongoing studies in mCRC in various lines of therapy will further elucidate the role of **KRAS** mutational status in patient selection in combination with chemotherapy.
### Beyond KRAS: PIK3CA Mutations do not appear to predict outcome

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Best Response</th>
<th></th>
<th></th>
<th></th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>CR</td>
<td>PR</td>
<td>SD</td>
<td>PD</td>
</tr>
<tr>
<td><strong>n (row %)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>KRAS WT/PI3K WT</td>
<td>1 (0.9)</td>
<td>31 (29)</td>
<td>55 (51)</td>
<td>21 (19)</td>
<td>108 (100)</td>
</tr>
<tr>
<td>KRAS WT/PI3K mutant</td>
<td>0</td>
<td>5 (36)</td>
<td>8 (57)</td>
<td>1 (7)</td>
<td>14 (100)</td>
</tr>
<tr>
<td>KRAS mutant/PI3K WT</td>
<td>0</td>
<td>1 (1)</td>
<td>42 (62)</td>
<td>25 (37)</td>
<td>68 (100)</td>
</tr>
<tr>
<td>KRAS mutant/PI3K mutant</td>
<td>0</td>
<td>0</td>
<td>7 (78)</td>
<td>2 (22)</td>
<td>9 (100)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>1 (0.5)</td>
<td>37 (19)</td>
<td>112 (56)</td>
<td>49 (25)</td>
<td>199* (100)</td>
</tr>
</tbody>
</table>

**NOTE:** %, distribution of responses in each mutation group.

**Abbreviations:** CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease.

* KRAS data were not available for one of the patients.

Lambrechtets et al, JCO 2009
Effect of B-Raf mutations

WT KRAS

Di Nicolantonio et al. JCO 26:5705, 2008

N=113; WT KRAS: 30%; mutant B-Raf: 10%; WT KRAS & B-Raf: 60%
Effect of Ligand expression

**High EREG by pre-specified threshold**

- Cetuximab + BSC
- BSC alone
- HR 0.43 [0.29-0.64], p<0.0001

**High EREG by minimum-p threshold**

- Cetuximab + BSC
- BSC alone
- HR 0.46 [0.32-0.65], p<0.0001

- OS was better for cetuximab than BSC among patients with high EREG based on both approaches
  - HR 0.43 and 0.46 respectively; p<0.0001

From NCI CTG Co.17, Jonker et al, ASCO 2009
EGFR antibodies in CRC: Evolving Predictive Signature

- **KRAS**: Accepted (EMEA approvals, NCCN guidelines, ASCO PCO)

- **B-Raf**: Evolving as a powerful negative predictor

- **AREG/EREG**: Seem predictive in KRAS WT, need validation and assay definition (binary vs. continuous)

- **PTEN**: Suggested effect in small series

- **PIK3CA**: No apparent effect

- **Polymorphisms**:  
  - FcγRIIa-H131 and IIIa-V158F, role of ADCC?  
  - EGFR intron 1 CA repeats