Biomarkers of primary resistance to targeted therapies

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Link of interest

• AstraZeneca
• Boehringer-Ingelheim
• InteGragen
• Merck-Serono
• Sanofi
Landscape of colorectal cancer treatment

- T1T2N0 (I)
  - No treatment
  - 5FU IV/PO
  - FOLFOX

- T3T4N0 (II)
  - Surgery
  - M+ (IV)
  - FOLFOX
  - ± BE
  - ± CET

- N+ (III)
  - FOLFOX

- ADJ
- L1
- L2
- L3

- Twelves NEJM 2005
- André NEJM 2004
- Van Cutsem NEJM 2009
- Saltz J Clin Oncol 2008
- Tournigand JCO 2004
- Hurwitz NEJM 2004
- Adam Ann Surg 2004

BEV: Bevacizumab (Avastin Roche)
CET: Cetuximab (Erbitux. Merck KGaA/Imclone/BMS)
IRI: Irinotecan
PAN: Panitumumab
REGO Regorafenib
**KRAS Mutation and Anti-EGFR therapy in advanced colorectal cancer**

<table>
<thead>
<tr>
<th>KRAS Status</th>
<th>Responders*</th>
<th>Non responders*</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>KRAS mutation (%)</td>
<td>0 (0)</td>
<td>13 (100)</td>
<td>13</td>
</tr>
<tr>
<td>Wildtype (%)</td>
<td>11 (65)</td>
<td>6 (35)</td>
<td>17</td>
</tr>
</tbody>
</table>

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Overall survival according to KRAS mutation

- Wildtype: 16.3 months
- Mutated KRAS: 6.9 months

\[ p=0.016 \]


* Response according to RECIST criteria
Target therapies

EGFR

Cetuximab
Panitumumab

PTEN
PI3K
AKT
mTOR

RAS
RAF
MEK
ERK

Survival
Proliferation

KRAS mutation (40%)

Lievre et al, JCO 2008
Douillard et al, NEJM 2013
Mekenkamp et al, BMC cancer 2012
• Candidate gene
  – BEYOND KRAS
    • RAS rare mutation
    • Amplification of KRAS
  – Role of minor KRAS mutant allele

• Without assumption
  – RNA
  – MirRNA
  – DNA
Impact of RAS mutation in PFS and OS in PRIME study

**KRAS**

- **Advanced CRC 1st line**
  - ECOG:0-2
  - N=1183

- **KRAS MT**
  - 40%

- **KRAS WT**
  - 60%

- **Exon 2 Codons 12, 13**
  - 40%

**NRAS**

- **Advanced CRC 1st line**
  - ECOG:0-2
  - N=1183

- **NRAS**
  - 3%

- **Exon 2 Codons 12, 13**
  - 3%

**Main objective**

- **PFS**

**FOLFOX4 + panitumumab 6 mg/kg N=593**

**FOLFOX4 (J1-J14)**

- **N=590**

## Impact of RAS mutation in PFS and OS in PRIME study

<table>
<thead>
<tr>
<th>RAS mutation</th>
<th>Panitumumab+FOLFOX</th>
<th>FOLFOX</th>
</tr>
</thead>
<tbody>
<tr>
<td>No KRAS mutation in exon 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PFS</td>
<td>9.6 CI$_{95%}$[9.2 – 11.1]</td>
<td>8 CI$_{95%}$[7.5 – 9.3]</td>
</tr>
<tr>
<td>OS</td>
<td>23.8 CI$_{95%}$[20.0-27.7]</td>
<td>19.4 CI$_{95%}$[17.4 – 22.6]</td>
</tr>
<tr>
<td>KRAS mutated in exon 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PFS</td>
<td>7.3 CI$_{95%}$[6.3 – 8]</td>
<td>8.8 CI$_{95%}$[7.7 – 9.4]</td>
</tr>
<tr>
<td>OS</td>
<td>15.5 CI$_{95%}$[13.1 – 17.6]</td>
<td>19.2 CI$_{95%}$[16.2 – 21.5]</td>
</tr>
<tr>
<td>No RAS mutation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PFS</td>
<td>10.1 CI$_{95%}$[9.3 – 12.0]</td>
<td>7.9 CI$_{95%}$[7.2 – 9.3]</td>
</tr>
<tr>
<td>OS</td>
<td>25.8 CI$_{95%}$[21.7-29.7]</td>
<td>20.2 CI$_{95%}$[17.6 – 23.6]</td>
</tr>
<tr>
<td>No KRAS mutation in exon 2, other RAS mutation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PFS</td>
<td>7.3 CI$_{95%}$[5.3 – 9.2]</td>
<td>8.0 CI$_{95%}$[6.4 – 11.3]</td>
</tr>
<tr>
<td>OS</td>
<td>17.1 CI$_{95%}$[10.8 – 19.4]</td>
<td>17.8 CI$_{95%}$[13.0 – 23.2]</td>
</tr>
</tbody>
</table>

Impact of RAS mutation in PFS and OS in PRIME study

Figure 1. Hazard Ratio for Disease Progression or Death and Hazard Ratio for Death from Any Cause, According to KRAS and RAS Mutation Status.
• Candidate gene
  – BEYOND KRAS
    • RAS rare mutation
      • Amplification of KRAS
    – Role of minor KRAS mutant allele

• Without assumption
  – RNA
  – MirRNA
  – DNA
Other KRAS alterations
KRAS gene amplification in colorectal cancer and impact on response to EGFR-targeted therapy

Emanuele Valitorta1, Sandra Misiale2,3, Andrea Sartore-Bianchi2, Iris D. Nasteghaei1, François Peret1, Calogero Lauricella1, Valentina Dimandolo1, Sebastiano Hobor4, Bart Jacobs5, Cristina Ennoling1, Simona Lamb45, Elisia Scalz1, Silvio Veronese1, Pierre Laurent-Puig6, Salvatore Siena7, Sabine Tejpar8, Marcello Mottola8, Ermelindo J.A. Punt9,10, Marcello Gambarota1, Alberto Bardelli11,12,13 and Federica Di Nicolantonio1,4,5

KRAS Amplification

![Image of KRAS gene amplification in colorectal cancer](image_url)

**a** KRAS IHC

**b** KRAS FISH

**c** KRAS AMPL n=7 (0.7%)

**d**

- KRAS AMPL
  - n=7 (0.7%)
- KRAS MUT
  - n=317 (35%)
- KRAS WT
  - n=582 (64%)

**e**

- KRAS WT and not amplified
- KRAS WT amplified

**Number of mCRC patients**

- Responder (10): 60
- Non Responder (10): 50

Response to EGFR targeted monoclonal Ab
• Candidate gene
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Can we go further in the prediction?

Recent papers showed
- secondary resistance to anti EGFR is associated with
  - KRAS selected clones or
  - acquired KRAS mutant clones

We decided to explore the intra tumor KRAS heterogeneity
- By exploring the existence of KRAS minority sub clones
  - We used a digital droplet PCR (Raindance technologies)
  - By studying the clinical impact in response and in PFS and OS in advanced colorectal cancer patients treated by antiEGFR therapy.
Multiple assays and DNA sample are mixed and compartmentalized into droplets.
The emulsion is thermocycled to amplify targets.
Endpoint fluorescence is measured from each droplet.
Data analyzed by counting the number of droplets corresponding to each assay.

2 Multiplex Panels developed covering KRAS Codon 12 &13

**Patients’ characteristics**

<table>
<thead>
<tr>
<th>Patients’ characteristics (n = 177)</th>
<th>Cases (n)</th>
<th>Percentages</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age, y</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤65</td>
<td>104</td>
<td>58.7</td>
</tr>
<tr>
<td>&gt;65</td>
<td>73</td>
<td>41.3</td>
</tr>
<tr>
<td><strong>Gender</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>101</td>
<td>57.1</td>
</tr>
<tr>
<td>Female</td>
<td>76</td>
<td>42.9</td>
</tr>
<tr>
<td><strong>Number of previous chemotherapy treatments</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>1</td>
<td>0.6</td>
</tr>
<tr>
<td>1</td>
<td>22</td>
<td>12.4</td>
</tr>
<tr>
<td>2</td>
<td>86</td>
<td>48.6</td>
</tr>
<tr>
<td>3</td>
<td>44</td>
<td>24.9</td>
</tr>
<tr>
<td>4</td>
<td>16</td>
<td>9.0</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>2.8</td>
</tr>
<tr>
<td>6</td>
<td>3</td>
<td>1.7</td>
</tr>
<tr>
<td><strong>EGFR-targeted therapies</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cetuximab</td>
<td>2</td>
<td>1.1</td>
</tr>
<tr>
<td>Cetuximab + chemotherapy</td>
<td>144</td>
<td>81.4</td>
</tr>
<tr>
<td>Panitumumab</td>
<td>10</td>
<td>5.6</td>
</tr>
<tr>
<td>Panitumumab + chemotherapy</td>
<td>21</td>
<td>11.9</td>
</tr>
<tr>
<td><strong>Response to EGFR-targeted therapies</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Complete response</td>
<td>7</td>
<td>4.0</td>
</tr>
<tr>
<td>Partial response</td>
<td>54</td>
<td>30.5</td>
</tr>
<tr>
<td>Stable disease</td>
<td>62</td>
<td>35.0</td>
</tr>
<tr>
<td>Progressive disease</td>
<td>53</td>
<td>29.9</td>
</tr>
<tr>
<td>Not evaluable</td>
<td>1</td>
<td>0.6</td>
</tr>
</tbody>
</table>
Responses to anti-EGFR–based therapies and KRAS mutational status as determined by conventional procedures (qPCR) or droplet-based dPCR.

Fraction of mutated KRAS alleles (including multiple subclones) in the patients detected by both conventional and droplet dPCR procedures.

Patients response to cetuximab according to the fraction of KRAS-mutated alleles.

Association between fraction of KRAS mutant allele and survival

- In a Cox model:
  - the fraction of allelic KRAS mutant is associated with a shorter PFS and a shorter OS
- Considering an incremental of 1% mutant allele:
  - The hazard ratio of PFS was 1.03 (CI$_{95\%}$[1.02–1.04], P<0.001)
  - The hazard ratio of OS was 1.02 (CI$_{95\%}$[1.01–1.03], P<0.001)
Correlation between the fraction of mutated KRAS alleles in the tumor and patient survival.

Target therapies

Cetuximab
Panitumumab

EGFR

PI3K
AKT
mTOR

Survival

KRAS mutation (40%)
NRAS mutation (5%)
KRAS amplification

BRAF mutation (10%)

PTEN

PIK3CA mutation (30%)
PTEN inactivation

RAS
RAF
MEK
ERK

Proliferation

Lievre et al, JCO 2008
Douillard et al, NEJM 2013
Mekenkamp et al, BMC cancer 2012
• Candidate gene
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Modeling RAS phenotype in colorectal cancer

A

B

C

Guinney, Ferté....... Laurent-Puig Clin Cancer Res 2014
Modeling RAS phenotype in colorectal cancer

HR = 2.3, CI_{95\%} [1.2-4.9], p = .016

HR = 2.0, CI_{95\%} [1.2-3.3], p = 6.4e-03

Guinney, Ferté...... Laurent-Puig Clin Cancer Res 2014
• Candidate gene
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microRNAs

- microRNAs are non-coding RNA of 22 nucleotides
- Around 1,000 microRNAs have been identified in the human genome
- These microRNAs are linked to transcribed RNA in their 3’ end non-translated
- The role of these microRNAs is to regulate gene expression mostly by inhibiting the RNA translation and/or by promoting the RNA degradation
- 60% of human genes are regulated by microRNAs.

Flow chart.

Identification of microRNA associated with survival

• We analyzed 1154 microRNA on a Illumina microarray, 11 were associated with PFS according to a Cox model and principal component analysis allowing to calculate a progression score.

• Among these 11 microRNA tested by qPCR, only one (hsa-mir31-3p) has a significantly different expression between patients with high and low risk (p<0.0004)

![Graph showing expression levels of miR-31-3p in high risk and low risk groups.](image-url)
Kaplan–Meier curves for risk groups obtained from the second training set (A) and the validation set 1a (B).

Kaplan–Meier PFS curves and the waterfall plot for RECIST criteria for the whole series of validation (PIMABI phase II; n = 45).

All patients

HR=2.4 CI_{95\%} [1.6-3.5]; P<0.001
Median PFS high risk versus low risk: 12.8-35.7 weeks
miR-31-3p is a predictive biomarker of cetuximab effects in a post-hoc analysis of New EPOC phase III trial

Laurent-Puig P et al, ESMO 2014
miR-31-3p is a predictive biomarker of cetuximab effects in a *post-hoc* analysis of New EPOC phase III trial

P = 0.12
HR = 1.7 CI=[0.88- 3.08]

Laurent-Puig P et al ESMO 2014
hsa-mir31-3p maturation regulated by AGO2

MicroRNAs (miRNAs) are generated by two-step processing to yield small RNAs that negatively regulate target gene expression at the post-transcriptional level.

Among those some have large loop for which the maturation is negatively controlled by AGO2 which is phosphorylated by EGFR in hypoxia condition.

hsa-mir31-3p is one of these microRNA.

High level of these microRNA suggested the absence of EGFR response to hypoxia in tumors.

It should be linked to the absence of effect to anti-EGFR therapy.

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  – DNA
Exome Sequencing
37 tumours and normal DNA

All treated by cetuximab
All  *KRAS, NRAS, BRAF* WT

• 18 responders
• 19 non responders
Correlations #SNV

Responders
Non-responders

Moyenne
70 SNV/sample
Correlations #SNV

- **OS (months)**: #SNV vs. OS, \( p = 0.42 \)
- **PFS (weeks)**: #SNV vs. PFS, \( p = 0.97 \)
- **Age (years)**: #SNV vs. Age, \( p = 0.10 \)

**Legend**:
- Green: Responders
- Red: Non-responders

**Mean #SNV/sample**: 70
The mapkinase pathway

- EGFR
- ERBB2
- ERBB3
- MET

- PIK3CA
- PTEN
- AKT
- mTOR
- MAP3K13
- MAP2K7
- MAP2K4
- JNK1/2/3
- MAP3K1
- MAP2K1
- MEK1
- MAPK3
- ERK1
- MAPK4
- ERK4

- KRAS
- NRAS
- BRAF
- RAF1
The mapkinase pathway mutations in responders and non-responders patients
Conclusions

• Need of INTEGRATION

• The unique marker for a clinical problem is probably an illusion

• Different faces of the same god

Sanjusangendo temple Kyoto
1001 God of Mercy