Resistance to anti-EGFR therapy in colorectal cancer

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Servicio de Oncología Médica & Programa de Investigación en Cáncer
Hospital del Mar
Panitumumab and Cetuximab Bind to EGFR Extracellular Domain and Prevent Ligand Binding
Predictive biomarkers are dynamic and evolve upon time.

Primary resistance to anti-EGFR therapy

Mutations in KRAS and NRAS used in daily clinical practice

**response to treatment**

**no response to treatment**

<table>
<thead>
<tr>
<th>Exon</th>
<th>KRAS</th>
<th>NRAS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
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<tr>
<td>2</td>
<td>12</td>
<td>12</td>
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<tr>
<td>3</td>
<td>59</td>
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<tr>
<td>4</td>
<td>117</td>
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</table>

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<thead>
<tr>
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<td>61</td>
</tr>
<tr>
<td>4</td>
<td>146</td>
<td>146</td>
</tr>
</tbody>
</table>

- KRAS: 40% 4% 6%
- NRAS: 3% 4% 1%
Challenges in anti-EGFR therapy in mCRC

1. Primary resistance beyond RAS
2. Ultraselection of patients for anti-EGFR therapy (NGS)
3. Acquired resistance
4. Monitoring clonal dynamics (liquid biopsy)
5. Treating resistance to anti-EGFR therapy
Challenge 1. Primary resistance to anti-EGFR therapy

40% KRASmut (12% also PI3Kmut)

6% NRASmut

Benefit from anti-EGFR therapy

Amado, JCO 2008
Karapetis, NEJM 2008
VanCutsem, NEJM 2009
Peeters, JCO 2010
Douillard, NEJM 2013
Primary resistance to anti-EGFR therapy

- 40% KRASmut (12% also PI3Kmut)
- 6% NRASmut
- 7% BRAFmut
- 10% PI3Kmut

Quadruple negative

Benefit from anti-EGFR therapy
Effect of cetuximab treatment in 116 KRAS wildtype colorectal cancer tumors with different somatic alterations

Primary resistance to anti-EGFR therapy

- 40% KRASmut (12% also PI3Kmut)
- 6% NRASmut
- 7% BRAFmut
- 10% PI3Kmut
- 3% HER2 mut
- 1% MET ampl
- 2% FGFR1 ampl
- 2% PDGFR ampl
- 2% MEK1 mut
- 4% HER2 ampl
- 1% EGFR mut

Benefit from anti-EGFR therapy
Primary Resistance to anti-EGFR

1. Activation of **downstream signaling**: KRAS, NRAS, BRAF, MEK1
2. Activation of alternative **TK receptors**: MET, HER2, FGFR1, PDGFR
Effect of cetuximab treatment in 116 KRAS wildtype colorectal cancer tumors with different somatic alterations

EGFR dependency in colorectal cancer

Markers of response to anti-EGFR

- EGFR gene amplification
- Ligand overexpression (EGF, TGFα, amphiregulin, epiregulin, heregulin)

40% KRASmut (12% also PI3Kmut)

6% NRASmut

7% BRAFmut

10% PI3Kmut

3% HER2 mut

1% MET ampl

2% FGFR1 ampl

2% PDGFR ampl

2% MEK1 mut

4% HER2 ampl

1% EGFR mut

Benefit from anti-EGFR therapy

IRS2 mut ligands
Challenge 2
Next-generation sequencing
Ultraselection of CRC patients for anti-EGFR therapy

Multiple genes
High sensitivity
Challenge 2

Next-generation sequencing

Ultraselection of CRC patients for anti-EGFR therapy

Multiple genes
High sensitivity

Tumor heterogeneity

Standard technique

PI3K mutant
KRAS wild-type
Challenge 2
Next-generation sequencing
Ultraselection of CRC patients for anti-EGFR therapy

Multiple genes
High sensitivity

Tumor heterogeneity

Standard technique
PI3K mutant
KRAS wild-type

Next-Generation Sequencing
PI3K mutant
KRAS mutant
What is the clinical relevance of RAS mutations detected at very low frequency?

- Do these low-frequency mutations predict resistance to anti-EGFR therapy?
- Which is the threshold of detection of mutations that has a clinical impact?
Ultraselection of CRC patients for anti-EGFR therapy

PLATFORM Study

TTD participating Hospitals

ICO Hospitalet
Hospital del Mar
Hospital Vall d'Hebron

600 CRC samples
Clinical data

Central DNA extraction

Next Generation Sequencing

KRAS exon 2,3,4
NRAS exon 2,3,4
PIK3CA exon 2,9
BRAF exon 15
EGFR exon 12

Illumina MiSeq
Junior 454
Fluidigm Digital PCR
Idylla
Mutation testing using NGS with a threshold of 1% improved prediction of response to anti-EGFR therapy

<table>
<thead>
<tr>
<th></th>
<th>Conventional qPCR qualitative detection (yes/no)</th>
<th>Highly-sensitive digital PCR cut-off 1%</th>
</tr>
</thead>
<tbody>
<tr>
<td>mutated/wild-type (n/n)</td>
<td>22/80</td>
<td>37/65</td>
</tr>
<tr>
<td><strong>RAS + BRAF</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PFS, months median (mut/wt)</td>
<td>2.6 / 36.3</td>
<td>3.2 / 36.3</td>
</tr>
<tr>
<td>HR (95%CI) P-value</td>
<td>5.36 (2.57-11.18) ≤0.001</td>
<td>4.52 (2.25-9.07) ≤0.001</td>
</tr>
<tr>
<td>OS, months median (mut/wt)</td>
<td>6.6/16.3</td>
<td>10.5/18.7</td>
</tr>
<tr>
<td>HR (95%CI) P-value</td>
<td>2.63 (1.49-4.66) 0.002</td>
<td>2.5 (1.56-4.02) ≤0.001</td>
</tr>
</tbody>
</table>

Azuara et al. submitted
Mutation testing using NGS with a threshold of 1% improved prediction of response to anti-EGFR therapy
Challenge 3. Acquired resistance
Tumoral heterogeneity and acquired resistance

**Basal tumor**

**Response to treatment**

**Progression to treatment**

KRAS

**wt**

**Response to treatment**

**Progression to treatment** (expansion of resistant clones)
Tumor heterogeneity and branched evolution

Gerlinger, NEJM 2010
Tumor heterogeneity and branched evolution

Gerlinger, NEJM 2010
Tumor heterogeneity and branched evolution
Tumoral heterogeneity and acquired resistance

Basal tumor

KRAS wt

Response to treatment

KRAS wt

Progression to treatment (expansion of resistant clones)
Emergence of **mutations of resistance** during cetuximab treatment in colorectal cancer patients

<table>
<thead>
<tr>
<th>Patient #</th>
<th>Pre-Treatment</th>
<th>Post-Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td></td>
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<td>5</td>
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<td>36</td>
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<tr>
<td>37</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Montagut et al. Nat Med 2012  
Misale et al. Nature 2012  
Diaz et al. Nature 2012  
Emergence of EGFR extracellular mutations of secondary resistance to cetuximab

Montagut et al. Nat Med 2012
Acquired resistance to anti-EGFR drugs

1- mutations in ECD of EGFR

2- mutations downstream of EGFR or activation of alternative receptors that converge in MEK activation
Tumoral heterogeneity and acquired resistance

Basal tumor  →  Response to treatment  →  Progression to treatment

BRAF  wt
KRAS
BRAF  wt
KRAS  wt
EGFR
KRAS
BRAF  wt
Tumoral heterogeneity and acquired resistance is reversible?

Basal tumor → Response to treatment → Progression to treatment

Off treatment
Clonal evolution and resistance to EGFR blockade in the blood of colorectal cancer patients

Giulia Siravegna1, Benedetta Mussolin2, Michela Buscarino2, Giorgio Corti2, Andrea Cassingena4, Giovanni Crisafulli2, Agostino Ponzetti5, Chiara Cremolini6, Alessio Amatu4, Calogero Lauricella9, Simona Lamba2, Sebastjan Hobor1,10, Antonio Avallone7, Emanuele Valtorta4, Giuseppe Rospo2, Enzo Medico1,2, Valentina Motta4, Carlotta Antoniotti6, Fabiana Tatangelo7, Beatriz Bellosillo8, Silvio Veronese4, Alfredo Budillon7, Clara Montagut8, Patrizia Racca5, Silvia Marsoni2, Alfredo Falcone6, Ryan B Corcoran9, Federica Di Nicolantonio1,2, Fotios Loupakis6, Salvatore Siena4, Andrea Sartore-Bianchi4 & Alberto Bardelli1,2.
Challenge 4. Monitoring clonal dynamics
Circulating free DNA (cfDNA): “real-time” liquid biopsy

- ~3% of tumour DNA enters the blood daily
- Mutations detected in plasma show good concordance to mutations in tumoral tissue

Figure 1 | Release and extraction of cfDNA from the blood. cfDNA is released from healthy, inflamed or diseased (cancerous) tissue from cells undergoing apoptosis or necrosis. cfDNA can be extracted from a blood sample and genetic aberrations in the DNA released from cancerous tissue detected and quantified. Tumour-derived genetic alterations that can be detected in the blood include point mutations (consecutive purple, red, green and blue DNA strands), copy number fluctuations (red portion of chromosomes) and structural rearrangements (green and red DNA strands). Abbreviations: cfDNA, circulating free DNA; ctDNA, circulating tumour DNA.

Concordance of RAS mutation detection in plasma and tumor tissue samples from metastatic colorectal cancer patients for selection to anti-EGFR therapy

<table>
<thead>
<tr>
<th>Plasma Ras Result</th>
<th>Tissue RAS Result</th>
<th>Positive</th>
<th>Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>16</td>
<td>3</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>1</td>
<td>17</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>17</td>
<td>20</td>
<td>37</td>
<td></td>
</tr>
</tbody>
</table>

- **Positive Agreement**: 16/17: 94.11%
- **Negative Agreement**: 17/20: 85%
- **Overall Agreement**: 33/37: 89.19%

Vidal J, et al
Molecular monitoring of colorectal cancer patients treated with anti-EGFR therapy using serial liquid biopsies

Monitorización mediante biopsia líquida de los pacientes con cáncer colorrectal metastásico: comparación de plataformas de nueva generación para el genotipado de DNA tumoral circulante

Código (promotor): TTD-15-01
Versión y fecha: versión 1.0 de 8 Junio 2015

Promotor: Grupo de Tratamiento de los Tumores Digestivos
Plaza de Castilla 3, planta 8. D-1, 28046 Madrid
Teléfono: 92 378 82 75. Fax: 92 378 82 76
Correo electrónico: ttd@ttdgroup.org

Coordinadores:
Clinicos
Dr. Ramón Salazar Soler. Oncología Médica. Institut Català d’Oncologia.Barcelona
Dr. Josep Tabernero. Oncología Médica. Hospital Vall de Hebrón. Barcelona

Laboratorio
Liquid biopsies: Assessing the clinical relevance of tracking the molecular profile in serial plasma samples from 100 CRC patients treated with cetuximab

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Response to treatment</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Tumor sample</td>
<td>Analysis of mutations</td>
<td>Analysis of mutations</td>
</tr>
<tr>
<td>Plasma sample</td>
<td>Monthly blood extraction</td>
<td>Monthly blood extraction</td>
</tr>
</tbody>
</table>
Challenge 5. Treating resistance to anti-EGFR

Ongoing clinical trials

<table>
<thead>
<tr>
<th>KRAS wt progressing to anti-EGFR mAbs</th>
<th>Novel anti-EGFR mAbs with potent ADCC</th>
<th>Phase I/II</th>
</tr>
</thead>
<tbody>
<tr>
<td>SYM004</td>
<td>Anti-EGFR mAbs + MEK inhibitors</td>
<td>NCT01117428</td>
</tr>
<tr>
<td></td>
<td>Panitumumab + MEK162</td>
<td></td>
</tr>
<tr>
<td>KRAS wt HER2 amplified progressing to anti-EGFR mAbs</td>
<td>Dual anti-HER2 therapy</td>
<td>Phase II</td>
</tr>
<tr>
<td></td>
<td>Trastuzumab + pertuzumab or lapatinib</td>
<td>Heracles trial</td>
</tr>
<tr>
<td>KRAS wt MET high progressing to anti-EGFR mAbs</td>
<td>Anti-EGFR mAbs + MET inhibitors</td>
<td>Phase II</td>
</tr>
<tr>
<td></td>
<td>Cetuximab + ARO197</td>
<td>NCT01892527</td>
</tr>
<tr>
<td>Quadruple negative (KRAS, NRAS, BRAF, PIK3CA) progressing to anti-EGFR mAbs</td>
<td>Anti-EGFR mAbs + irreversible ERBB TKIs</td>
<td>Phase II</td>
</tr>
<tr>
<td></td>
<td>Cetuximab + neratinib</td>
<td>NCT01960023</td>
</tr>
</tbody>
</table>
New generation anti-EGFR moAb
Sym004 is a 1:1 mixture of two anti-EGFR moAbs directed against distinct non-overlapping epitopes in EGFR extracellular domain that induces efficient internalization of EGFR.
Safety and Activity of Sym004 in patients progressing to cetuximab

- Hipomagnesemia and skin toxicity G3 as limiting toxicities
- Activity:

44% tumor shrinkage
13% partial response: 1 patient with **EGFR S492R mutation**
3 patients with quadruple negative tumors

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Dientsmann. Cancer Discov 2015
Challenges in anti-EGFR therapy in mCRC

1. Primary resistance beyond RAS
2. Ultraselection of patients for anti-EGFR therapy (NGS)
3. Acquired resistance
4. Monitoring clonal dynamics (liquid biopsy)
5. Treating resistance to anti-EGFR therapy
Gracias

cmontagut@hospitaldelmar.cat
Tumors with the EGFR S492R mutation have longer duration of treatment before progression, but worse Overall Survival

<table>
<thead>
<tr>
<th></th>
<th>Panitumumab (n = 262)*</th>
<th>Cetuximab EGFR S492R (n = 46)</th>
<th>Cetuximab WT EGFR S492 (n = 238)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median OS - mos (95% CI)</td>
<td>12.5 (11.2–14.3)</td>
<td>11.9 (10.7–14.0)</td>
<td>13.8 (11.5–15.4)</td>
</tr>
<tr>
<td>HR (95% CI)</td>
<td>1.03 (0.85–1.25)</td>
<td>1.75 (1.23–2.50)</td>
<td></td>
</tr>
<tr>
<td>Median PFS - mos (95% CI)</td>
<td>4.8 (4.4–4.9)</td>
<td>5.1 (4.9–6.7)</td>
<td>4.7 (3.2–4.9)</td>
</tr>
<tr>
<td>HR (95% CI)</td>
<td>1.10 (0.92–1.30)</td>
<td>0.68 (0.10–4.92)</td>
<td></td>
</tr>
<tr>
<td>ORR - % (95% CI)</td>
<td>28.2 (22.9–34.1)</td>
<td>39.1 (25.1–54.6)</td>
<td>22.7 (17.5–28.5)</td>
</tr>
<tr>
<td>OR (95% CI)</td>
<td>1.15 (0.77–1.72)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment duration – weeks (range)</td>
<td>22 (2–70)</td>
<td>22 (6–61)</td>
<td>15 (1–94)</td>
</tr>
<tr>
<td>Treatment discontinuation due to disease progression, n (%)</td>
<td>246 (94)</td>
<td>268 (94)</td>
<td>45 (98)</td>
</tr>
</tbody>
</table>

*Patients evaluable for EGFR S492

**PFS by EGFR S492 Status in Patients Treated With Cetuximab**

**OS by EGFR S492 Status in Patients Treated With Cetuximab**
Contrary to cetuximab, Sym004 is able to bind to the EGFR ECD mutations of resistance.
A subset of mutations in EGFR of resistance to cetuximab are sensitive to panitumumab
HER2 activation as a mediator of acquired resistance to cetuximab

HER2 amplification

Heregulin overexpression

Yonesaka et al, Sci Transl Med 2011
LIGANDS: TGFα, AR, IGF2 as mediators of resistance to anti-EGFR therapy
Molecular characteristics of tumors evolve along the course of the disease

Re-biopsies should be routinely performed to monitor change in mutations

Re-biopsies will help in personalizing further treatment decisions
Liquid biopsies:
Monitoring mutational profile of cancer in the blood from patients

<table>
<thead>
<tr>
<th>Technique</th>
<th>Sensitivity</th>
<th>Optimal Application</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sanger sequencing</td>
<td>&gt; 10%</td>
<td>Tumor tissue</td>
</tr>
<tr>
<td>Pyrosequencing</td>
<td>10%</td>
<td>Tumor tissue</td>
</tr>
<tr>
<td>Next-generation sequencing</td>
<td>2%</td>
<td>Tumor tissue</td>
</tr>
<tr>
<td>Quantitative PCR</td>
<td>1%</td>
<td>Tumor tissue</td>
</tr>
<tr>
<td>ARMS</td>
<td>0.10%</td>
<td>Tumor tissue</td>
</tr>
<tr>
<td>BEAMing, PAP, Digital PCR, TAM-Seq</td>
<td>0.01% or lower</td>
<td>ctDNA, rare variants in tumor tissue</td>
</tr>
</tbody>
</table>
**PLATFORM-B Study**

**Liquid biopsies:** Assessing the clinical relevance of tracking the molecular profile in serial plasma samples from 100 CRC patients treated with Erbitux

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<td>Plasma sample</td>
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Monthly blood extraction
**Clonal dynamics in a CRC patient treated with anti-EGFR therapy**

- **Tumor load (% of baseline)**
- **EGFR p.K467T**
- **NRAS p.G12V**

<table>
<thead>
<tr>
<th>Baseline</th>
<th>AntiHER3</th>
<th>AntiHER3 +CETUX</th>
<th>IRINOTECAN+CETUX</th>
</tr>
</thead>
<tbody>
<tr>
<td>14-MAR-2012</td>
<td>28-JUN-2012</td>
<td>21-MAR-2013</td>
<td>24-APR-2013</td>
</tr>
<tr>
<td>13-MAY-2013</td>
<td>30-SEP-2013</td>
<td></td>
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</tr>
</tbody>
</table>

**Clonal dynamics timeline**

- *1st CT scan PD*
- *CT scan: PR Last administration of anti-EGFR*
Clonal dynamics in a CRC patient treated with anti-EGFR therapy

- Baseline
- 1st CT scan (1st line)
- 1st line PD
- 1st CT scan (2nd line)
- 2nd line PD
- Last administration of anti-EGFR (1st line)
- 9-DEC-2010
- 13-APR-2011
- First administration of anti-EGFR (2nd line)
- 1-NOV-2010
- Last administration of anti-EGFR (2nd line)
- 17-MAY-2010

- % mutant alleles
- Tumor load (% of baseline)

- Clonal dynamics in a CRC patient treated with anti-EGFR therapy
Rechallenging patients with anti-EGFR therapy (patient HMAR#8)

Siravegna et al. Nat Med (in press)
**KRAS activation** as a mechanism of acquired resistance to cetuximab and panitumumab in CRC

<table>
<thead>
<tr>
<th>Patient ID</th>
<th>Mutation</th>
<th>Percentage</th>
<th>Reads* / events†</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>WT*</td>
<td>0%</td>
<td>0 / 12,123</td>
</tr>
<tr>
<td>2</td>
<td>G13D*</td>
<td>10%</td>
<td>859 / 8,556</td>
</tr>
<tr>
<td>4</td>
<td>G13D*</td>
<td>5.9%</td>
<td>461 / 7,764</td>
</tr>
<tr>
<td>5</td>
<td>G13D*</td>
<td>14.3%</td>
<td>1,037 / 7,247</td>
</tr>
<tr>
<td>6</td>
<td>G13D*</td>
<td>8.6%</td>
<td>651 / 7,577</td>
</tr>
<tr>
<td>7</td>
<td>WT*</td>
<td>0%</td>
<td>0 / 17,142</td>
</tr>
<tr>
<td>8</td>
<td>Q61H†</td>
<td>17.3%</td>
<td>5,960 / 190,200</td>
</tr>
<tr>
<td>9</td>
<td>G12D†</td>
<td>0.04%</td>
<td>17 / 40,200</td>
</tr>
<tr>
<td></td>
<td>G13D†</td>
<td>0.44%</td>
<td>117 / 26,400</td>
</tr>
<tr>
<td>10</td>
<td>WT†</td>
<td>0%</td>
<td>0 / 50,300</td>
</tr>
<tr>
<td>11</td>
<td>WT† (KRAS amplified)</td>
<td>0%</td>
<td>0 / 30,400</td>
</tr>
</tbody>
</table>

Mutations detected by deep-sequencing:

*454
†BEAMing

Misale et al., Nature 2012
Acquisition of KRAS mutations in patients with resistance to panitumumab

Detection of KRAS mutations in circulating tumor DNA
Assessment every 4 weeks

Diaz et al, Nature 2012
1. EGFR ECD mut

2. HER2, c-MET amplification

3. KRAS, NRAS, BRAF, PI3K mutation

4. TGFα, AR, IGF2
Prevalence of genetic alterations associated with de novo resistance to anti-EGFR therapies in mCRC.

Sandra Misale et al. Cancer Discovery 2014;4:1269-1280
Molecular heterogeneity drives secondary resistance to anti-EGFR therapies in mCRC.

Sandra Misale et al. Cancer Discovery 2014;4:1269-1280
Molecular mechanisms of primary and secondary resistance to anti-EGFR therapies in mCRC.

Sandra Misale et al. Cancer Discovery 2014;4:1269-1280
100 metastatic CRC patients

40% mut KRAS exon 2

Benefit from anti-EGFR therapy
Valor predictiu de les mutacions de **KRAS**, **NRAS** i **BRAF** detectades per la tècniques estàndard *versus* NGS

Tècnica standard 10%

NGS 5%

P=0.002

P=0.7

NGS 1%

P=0.5

NGS 0.1%

P=0.5
% of mutated alleles

CR       PR       SD       PD

p<0.005
Acquisition of KRAS mutation in a patient with resistance to cetuximab

Misale et al., Nature 2012
Figure 3: Genetic alterations in distinct members of the EGFR signaling cascade biochemically converge to activate MEK and ERK. The indicated cell lines were analyzed for EGFR-MAPK pathway...
Figure 2: Cells lines with acquired resistance to anti EGFR antibodies are sensitive to concomitant transcriptional suppression of EGFR and MEK1/2. (A) The indicated cell lines were selected as models

Figure 4: Resistance to EGFR therapy is reverted by concomitant pharmacological inhibition of EGFR and MEK in vitro and in vivo. (A) The indicated cell lines were treated for two weeks with increasing
Cetuximab resistant cells are killed by HER2 inhibition (by Herceptin or shRNA)

Yonesaka et al, Sci Transl Med 2011
Targeting c-MET

A

Xenopatient M162

B

Xeno from Patient #3

Bardelli Can dicov 2013
Targeting HER2
Challenge 5
Can we pharmacologically target these mechanisms of resistance?

**EGFR-dependent**
1. EGFR S492R mut

**Anti-EGFR drug**
- Panitumumab
- Gefitinib/erlotinib

**EGFR-independent**
2. Other TKR

**Anti-HER2 drugs**
- Herceptin
- Lapatinib

**Downstream signaling:**
- MEK inhibitors

3. HER2 amplification or Heregulin overexpression

4. KRAS amplification or mutation

**PI3-K**

### EGFR Pathway
- EGFR
- RAS
- RAF
- MEK

### Alternative activation of downstream signaling:
- AKT
- ERK
Genetic alterations beyond KRAS associated with primary resistance to anti-EGFR Identified by xenopatients model. **HER2 amplification**
Genetic alterations beyond KRAS associated with primary resistance to anti-EGFR Identified in tissue by whole genome sequencing. C-MET amplification

Bardelli, Cancer Discov 2011
Mutation testing using NGS with a threshold of 1% improved prediction of response to anti-EGFR therapy

<table>
<thead>
<tr>
<th></th>
<th>Conventional qPCR qualitative detection (yes/no)</th>
<th>Highly-sensitive digital PCR cut-off 1%</th>
</tr>
</thead>
<tbody>
<tr>
<td>mutated/wild-type (n/n)</td>
<td>22/80</td>
<td>37/65</td>
</tr>
<tr>
<td>PFS, months</td>
<td></td>
<td></td>
</tr>
<tr>
<td>median (mut/wt)</td>
<td>2.6 / 36.3</td>
<td>3.2 / 36.3</td>
</tr>
<tr>
<td>HR (95%CI) P-value</td>
<td>5.36 (2.57-11.18) ≤0.001</td>
<td>4.52 (2.25-9.07) ≤0.001</td>
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<tr>
<td>OS, months</td>
<td></td>
<td></td>
</tr>
<tr>
<td>median (mut/wt)</td>
<td>6.6/16.3</td>
<td>10.5/18.7</td>
</tr>
<tr>
<td>HR (95%CI) P-value</td>
<td>2.63 (1.49-4.66) 0.002</td>
<td>2.5 (1.56-4.02) ≤0.001</td>
</tr>
</tbody>
</table>

Azuara et al. submitted
Challenge 5. Treating resistance to anti-EGFR

Preclinical evidence

Targeting HER2 (herceptin)

Yonesaka, STM 2011; Misale STM 2014; Bardelli Can Discov 2013; Bertotti Can Discov 2011

Targeting c-MET (crizotinib)

Targeting HER2 (lapatinib)

Targeting MEK (pimasertib)

Yonesaka, STM 2011; Misale STM 2014; Bardelli Can Discov 2013; Bertotti Can Discov 2011
Ligands promote resistance to anti-EGFR therapy in CRC
Acquired resistance to anti-EGFR drugs

1- mutations in ECD of EGFR

2- mutations downstream of EGFR or activation of alternative receptors that converge in MEK activation

3- ligands overexpression

AREG, EREG, EGF, TGFα, HB-EGF, VGF

Survival

Cell growth

KRAS

RAF

MEK

ERK

PI3-K

AKT