Liquid biopsies to track clonal evolution and resistance to EGFR inhibition in mCRC

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University of Torino - Medical School
## Disclosures

<table>
<thead>
<tr>
<th></th>
<th>Relationship</th>
</tr>
</thead>
<tbody>
<tr>
<td>Horizon discovery</td>
<td>SAB</td>
</tr>
<tr>
<td>Biocartis</td>
<td>SAB</td>
</tr>
<tr>
<td>Trovagene</td>
<td>SAB</td>
</tr>
</tbody>
</table>
Tumor evolution in the blood of patients
Colorectal cancer as a model system for liquid biopsies

- Hyperproliferation
- Early Adenoma
- Adenomatous Polyps
- Severe Dysplasia
- Adenocarcinoma
- Hepatic Metastasis
Actionable targets in CRC
Liquid biopsies of actionable targets in CRC

- EGFR
- HER2
- MET
- TRKA
- ALK
- crizotinib
- Lapatinib
- trastuzumab
- panitumumab
- cetuximab
- entrectinib
- ALK
- crizotinib
- LGR4-6
- RSPO
- Anti RSPO Ab
- Wnt 1
- Porcupine inhibitor
- LRP5/6
- Frizzled
- KRAS
- GRB2
- SOS
- Dabrafenib/EGFRi
- PI3K
- PIP2
- PIP3
- AKT
- mTORC1
- RAF
- MEK
- ERK
- Trametinib
Validation in animal models

First patient treated (Niguarda Hospital)

Publication

First clinical response (PR)

Clinical trial HERACLES
HER2+ mCRC xenopatients are sensitive to dual HER2 blockade with lapatinib and trastuzumab.

Bertotti A. et al, Cancer Discovery 2012
HER2 amplification in CRC

HER2 IHC

IHC 0

IHC 1+

IHC 2+

IHC 3+

FISH

10X

10X

20X

10X

100X

100X

100X

100X

Positive tumor cells \( \geq 50\% \)

Eligible for HERACLES Trial
Representative CE-CT scans of 2 responders

Patient # 121016
baseline
Week 8 - PR
Week 54 - PR +

Patient # 121023
baseline
Week 8 - PR
Week 24 - PR +
Response and resistance to HER2 blockade

Sartore-Bianchi et al., Lancet Oncology 2016
HER2 blockade secondary resistance

Patient 121006

Siravegna et al., Unpublished
HER2 blockade secondary resistance

Patient 122025

Siravegna et al., Unpublished
Actionable targets in CRC

- crizotinib
- trastuzumab
- Lapatinib
- panitumumab
- cetuximab
- entrectinib
- ALK
- MET
- EGFR
- HER2
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- LRP5/6
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- PIP3
- AKT
- mTORC1
- RAF
- MEK
- ERK
- dabrafenib/EGFRi
- trametinib

Porcupine inhibitor
CRC patient: ALK translocated

CRC patient: NTRK1 translocated

Medico et al., Nature Commun 2015
Targeting translocated NTRK1 in CRC
Monitoring NTRK1 blockade in cfDNA

Russo et al., Cancer Discovery 2016
TRKA resistance mutations

<table>
<thead>
<tr>
<th>Gene</th>
<th>Codon</th>
<th>Mutation at resistance</th>
<th>Tissue of detection</th>
<th>Associated with resistance to</th>
</tr>
</thead>
<tbody>
<tr>
<td>NTRK1</td>
<td>595</td>
<td>p.G595R</td>
<td>Colon</td>
<td>Entrectinib</td>
</tr>
<tr>
<td>ALK</td>
<td>1202</td>
<td>p.G1202R</td>
<td>Lung</td>
<td>Crizotinib/ceritinib</td>
</tr>
<tr>
<td>ROS1</td>
<td>2032</td>
<td>p.G2032R</td>
<td>Lung</td>
<td>Crizotinib</td>
</tr>
<tr>
<td>EGFR</td>
<td>796</td>
<td>p.G796A/R</td>
<td>Lung</td>
<td>Erlotinib/gefitinib</td>
</tr>
</tbody>
</table>
Anti EGFR therapy in colorectal cancer

Cetuximab
Panitumumab

Ligand EGFR/Erbb2/Erbb3/Erbb4

EGFR

Ras

Raf

Raf

MEK

ERK

PI3K

AKT

Cytoplasm

Cell Proliferation
Survival
Migration

Nucleus
Monitoring EGFR blockade in cfDNA

% mutated alleles

Misale et al., Nature 2012
Siravegna et al., Nat Med 2015
Approx 70% of patients
Mechanism (2)
Mutations of the target: EGFR ECD

- EGFR S492R
- EGFR R451C
- EGFR S464L
- EGFR G465R
- EGFR K467T
- EGFR I491M

Approx 30% of patients
Resistance to EGFR blockade drives lesion-specific responses in colorectal cancer
MAP2K1 K57T emerges during EGFR blockade

<table>
<thead>
<tr>
<th>TISSUE SAMPLES</th>
<th>TP53</th>
<th>RAS/RAF</th>
<th>MAP2K1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary Tumor, 2011</td>
<td>p.E171*</td>
<td>WT</td>
<td>WT</td>
</tr>
<tr>
<td>Liver metastasis (pre-cetux), 2012</td>
<td>p.E171*</td>
<td>WT</td>
<td>WT</td>
</tr>
</tbody>
</table>
Clonal evolution and lesion-specific responses

Panitumumab + trametinib (5 months)

MEK1 p.K57T

Russo et al., Cancer Discovery 2016
Clonal evolution and lesion specific response in cfDNA

- % MEK p.K57T
- % KRAS p.Q61H
- % TP53 p.E171*

Panitumumab + trametinib
Tracing cancer’s evolutionary trees with liquid biopsies
What are we up against?

Evolution
First cell: 4 billion years

Mass extinction events

THE EMERGENCE OF LIFE
Land-Based Life

Water-Based Life

Bottlenecks
Tumor evolution and targeted therapies

Response to therapy

Resistance to therapy
Liquid biopsies to monitor cancer evolution

1. Exploiting clonal evolution

2. Therapies that adapt to tumor evolution
Exploiting clonal evolution
What happens to resistant clones upon progression?
KRAS clones decline upon withdrawal of EGFR antibodies

Siravegna et al., Nature Medicine 2015
When KRAS clone decline in blood, re-challenging with anti-EGFR antibodies can be clinically effective.
Tumor sensitivity to anti-EGFR

sensitive  resistant

Andrea Sartore Bianchi, Salvatore Siena, Silvia Marsoni
Therapies that adapt to tumor evolution
EGFR ECD resistant mutations

EGFR S492R
EGFR R451C
EGFR S464L
EGFR G465R
EGFR K467T
EGFR I491M

Approx 30% of patients

Sabrina Arena and Clara Montagut
# Emergence of RAS or EGFR extracellular domain mutations and duration of response to EGFR blockade

<table>
<thead>
<tr>
<th>Male Sex (%)</th>
<th>All (N=27)</th>
<th>EGFR only (N=7)</th>
<th>RAS only (N=13)</th>
<th>EGFR + RAS (N=7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male Sex (%)</td>
<td>13 (48%)</td>
<td>5 (71%)</td>
<td>6 (46%)</td>
<td>2 (29%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Age (median, range)</th>
<th>All (N=27)</th>
<th>EGFR only (N=7)</th>
<th>RAS only (N=13)</th>
<th>EGFR + RAS (N=7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (median, range)</td>
<td>60 (31-81)</td>
<td>64 (44-78)</td>
<td>59 (42-81)</td>
<td>55 (31-78)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Anti-EGFR drug</th>
<th>Cetuximab</th>
<th>Panitumumab</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cetuximab</td>
<td>23 (85%)</td>
<td>6 (86%)</td>
</tr>
<tr>
<td>Panitumumab</td>
<td>4 (15%)</td>
<td>1 (14%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Chemotherapy</th>
<th>Irinotecan -based</th>
<th>Oxaliplatin -based</th>
<th>None</th>
</tr>
</thead>
<tbody>
<tr>
<td>Irinotecan -based</td>
<td>20 (74%)</td>
<td>4 (57%)</td>
<td>11 (77%)</td>
</tr>
<tr>
<td>Oxaliplatin -based</td>
<td>4 (15%)</td>
<td>2 (29%)</td>
<td>1 (8%)</td>
</tr>
<tr>
<td>None</td>
<td>3 (11%)</td>
<td>1 (14%)</td>
<td>2 (15%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Line of treatment</th>
<th>1st</th>
<th>2nd</th>
<th>≥ 3rd</th>
</tr>
</thead>
<tbody>
<tr>
<td>Line of treatment</td>
<td>5 (19%)</td>
<td>2 (29%)</td>
<td>1 (8%)</td>
</tr>
<tr>
<td>2nd</td>
<td>9 (33%)</td>
<td>2 (29%)</td>
<td>6 (46%)</td>
</tr>
<tr>
<td>≥ 3rd</td>
<td>13 (48%)</td>
<td>3 (42%)</td>
<td>6 (46%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Best Response</th>
<th>Stable Disease &gt;16w</th>
<th>Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stable Disease &gt;16w</td>
<td>12 (44%)</td>
<td>1 (14%)</td>
</tr>
<tr>
<td>Response</td>
<td>15 (56%)</td>
<td>6 (86%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Progression Free Survival</th>
<th>Median (weeks; 95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All (N=27)</td>
<td>39.1 (33-46)</td>
</tr>
<tr>
<td>EGFR only (N=7)</td>
<td>45 (42-48)</td>
</tr>
<tr>
<td>RAS only (N=13)</td>
<td>25.6 (24-27)</td>
</tr>
<tr>
<td>EGFR + RAS (N=7)</td>
<td>38.7 (21-56)</td>
</tr>
</tbody>
</table>
Emergence of RAS or EGFR extracellular domain mutations and duration of response to EGFR blockade

Probability of Progression-free Survival

Number of patients

Stable Disease

Partial Response

RAS group

EGFR group

Van Emburgh et al submitted
EGFR ECD mutations appear after K-RAS clones during EGFR blockade.
Dynamics of RAS and EGFR-ECD clonal evolution in cancer cells populations

- **NRAS G12C**
- **NRAS G12C+EGFR S492R**

![Graph showing clonal evolution with NRAS G12C and EGFR S492R mutations over time](image)

- Baseline
- 1 Month
- 2 Months
- 3 Months
- 4 Months
- 6 Months

**Legend:**
- **NRAS G12C**
- **NRAS G12C+EGFR S492R**
- **0.5-5%**
- **<0.5%**

Cetuximab
MM-151 and EGFR ECD mutations

S464L
G465R,E
K467T
I491M
S492R

R451C

cetuximab
panitumumab
MM-151 (x3)

Arena et al., Science Transl Med 2016
MM-151 on patient’s avatar

mCRC patient at CTX progression

EGFR G465E: 53.5% (ddPCR)

EGFR G465E: 50.9% (ddPCR)

2D cell culture

Pharmacological and biochemical testing

CRC G465E-XL

Cell viability (% of control)

Anti-EGFR moABs [M]
CRC patient

% mutated alleles

GE/ml plasma

EGFR S464L

FOLFIRI+cetuximab

baseline

CT scan: PR

CT scan: PD

0 1 2 3 4 5 6 7 8 9

0 1 2 3 4

0 100000 200000 300000 400000 500000

FOLFIRI+cetuximab
MM-151 and EGFR ECD mutations

Baseline
August 29, 2014

First Assessment
November 26, 2014

EGFR ECD TRIAL
DiAGNOSIS: genotyping cfDNA in the blood to determine the tumor profile

SURGERY: tumor cfDNA is not present, the patient is disease free

RESISTANCE: emergence of genetic alterations associated with drug resistance

MINIMAL RESIDUAL DISEASE: tumor cfDNA is still present in the circulation

FOLLOW UP: patient monitoring throughout the treatment course to assess response and resistance

TREATMENT: analysis tumor cfDNA for real time monitoring of response to treatment

In blood *veritas*
Monitoring tumor evolution

Tissue Biopsy

Liquid Biopsy

Russo and Bardelli