The role of liquid biopsies in the treatment of advanced colon cancer

Clara Montagut
Hospital del Mar, Barcelona

ESMO CRC Preceptorship
Valencia, May 12, 2017
ESMO consensus guidelines for the management of patients with metastatic colorectal cancer

**CLINICAL CONDITION OF THE PATIENT**

- **Fit**
  - FP+/ bevacizumab; reduced dose doublet; anti-EGFR
  - Goal: Cytoreduction (Shrinkage)
  - MOLECULAR PROFILE:
    - RAS wt
    - RAS mt
    - BRAF mt
  - Surgery alone
  - Surgery with perioperative/postoperative CT
  - Re-evaluation/assessment of response every 2 months
  - Continue; maintenance; or pause

- **Unfit* (but may be suitable)**
  - FP+/ bevacizumab; reduced dose doublet; anti-EGFR
  - Goal: Disease control (Control progression)
  - MOLECULAR PROFILE:
    - RAS wt
    - RAS mt
    - BRAF mt
  - Combination + bevacizumab
  - Triplet + bevacizumab
  - CT + biological agent
  - CT + bevacizumab
  - Unusual, see text
  - Re-evaluation/assessment of response every 2-3 months
  - Continue; maintenance; or pause

- **Unfit**
  - BSC

**GOAL**

- Cytoreduction (Shrinkage)
- Disease control (Control progression)

**MOLECULAR PROFILE**

- NED
  - Patients with clearly resectable metastases

---

*Van Cutsem et al, Ann Oncol 2016*
How do we test for Biomarkers (RAS/BRAF)?
Tissue-based testing is well established but contains challenges

- Obtain tumor tissue block
- Manual microdissection
- DNA isolation & purification

Invasive procedure
Tissue not always accessible
How do we test for Biomarkers (RAS/BRAF)?
Tissue-based RAS testing is well established but contains challenges

1. **Obtain tumor tissue block**
2. **Manual micro-dissection**
3. **DNA isolation & purification**
4. **Treatment decision**
5. **Assessment of biomarker status**
6. **Assessment of DNA quantity**

**Invasive procedure**

**Tissue not always accessible**

**Archival tissue**
Static, potentially outdated mutation profile, often degraded or unavailable
How do we test for Biomarkers (RAS/BRAF)? Tissue-based RAS testing is well established but contains challenges

- **Invasive procedure**
- **Selection bias**
  - Tumor heterogeneity
- **Tissue not always accessible**

**Archival tissue** Static, potentially outdated mutation profile, often degraded or unavailable

1. **Obtain tumor tissue block**
2. **Manual micro-dissection**
3. **DNA isolation & purification**
4. **Assessment of DNA quantity**
5. **Assessment of biomarker status**
6. **Treatment decision**
Tumor spatial heterogeneity

Adapted from Gerlinger NEJM 2012
Temporal heterogeneity - Clonal selection

Emergence of mutations of resistance

Basal RAS wt tumor → Response to anti-EGFR treatment → RAS mutant tumor at progression to treatment
New paradigm in treatment of mCRC
TREATING CLONAL EVOLUTION
Targeting spatial and temporal heterogeneity

Need to re-evaluate tumor molecular profile along the course of the disease
## Liquid Biopsy in cancer

<table>
<thead>
<tr>
<th>Targets</th>
<th>CTCs</th>
<th>cfDNA</th>
<th>cfRNA</th>
<th>Platelet</th>
<th>Exosome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Origin</td>
<td>Selected viable tumor cells leaving actively primary and/or metastasis</td>
<td>Necrotic and apoptotic tumor cells</td>
<td>Necrotic and apoptotic tumor cells</td>
<td>Active incorporation of exosomes</td>
<td>Active secretion of encapsulated particles by tumor cells</td>
</tr>
<tr>
<td>Definition</td>
<td>Tumor cells</td>
<td>Fragmented genomes released from dying tumor cells of primary and/or metastasis</td>
<td>Fragmented RNA released from dying tumor cells</td>
<td>Circulating platelets</td>
<td>Circulating encapsulated particles</td>
</tr>
<tr>
<td>Analytes</td>
<td>DNA, RNA (mRNA, miRNA), protein</td>
<td>DNA</td>
<td>RNA</td>
<td>RNA (mRNA, miRNA)</td>
<td>RNA (mRNA, miRNA), protein</td>
</tr>
</tbody>
</table>
Liquid Biopsy – genotyping in circulating tumor (ct) DNA

- Tumoral DNA (somatic mutation)
- Normal DNA (wild-Type)

Diaz & Bardelli, J Clin Oncol 2014
ctDNA: clinical applications in metastatic colorectal cancer

1. Molecular testing at diagnosis to guide treatment decision
2. Monitoring resistance to targeted therapy (antiEGFR)
3. Monitoring response to treatment (tumor burden)
RAS testing is mandatory for first line treatment decision making

"The panel strongly recommends genotyping of tumor tissue (either primary tumor or metastasis) in all patients with metastatic colorectal cancer for RAS (KRAS exon 2 and non-exon 2; NRAS) and BRAF at diagnosis of stage IV disease"

"The appropriate molecular analyses are to be carried out at the time of initial diagnosis of mCRC and should comprise a full analysis of tumour RAS mutational status (KRAS: exon 2, 3 and 4 and NRAS: exon 2, 3 and 4) with a simultaneous analysis of tumour BRAF mutational status, conducted in a validated laboratory/testing centre, to facilitate the best diagnostic and prognostic decision making possible."

"Turnaround time for RAS testing (expanded RAS analysis) should be ≤7 working days from the time of receipt of the specimen by the testing laboratory to the time of issuing of the final report, for >90% of specimens"

### Patient Characteristics N 109 (%)

<table>
<thead>
<tr>
<th>Age</th>
<th>median (range) 67 (39-86)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
</tr>
<tr>
<td>Stage at diagnoses</td>
<td></td>
</tr>
<tr>
<td>Primary site of disease</td>
<td></td>
</tr>
<tr>
<td>Primary tumor resected</td>
<td></td>
</tr>
<tr>
<td>Systemic treatment before ctDNA</td>
<td></td>
</tr>
<tr>
<td>Tumor site biopsy</td>
<td></td>
</tr>
<tr>
<td>Number of metastatic sites</td>
<td></td>
</tr>
<tr>
<td>Metastasis Location</td>
<td></td>
</tr>
</tbody>
</table>

- 109 mCRC patients
- No patient received anti-EGFR treatment before plasma collection
- The OncoBEAM™ RAS CRC assay was used to detect RAS mutations in plasma
- RAS testing in tissue was done by standard-of-care (SOC)
- For discordant cases, tissue samples were re-examined with tissue BEAMing

<table>
<thead>
<tr>
<th>Exon</th>
<th>Mutation</th>
<th>Exon</th>
<th>Mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>G12S</td>
<td>2</td>
<td>G12S</td>
</tr>
<tr>
<td></td>
<td>G12R</td>
<td></td>
<td>G12R</td>
</tr>
<tr>
<td></td>
<td>G12C</td>
<td></td>
<td>G12C</td>
</tr>
<tr>
<td></td>
<td>G12D</td>
<td></td>
<td>G12D</td>
</tr>
<tr>
<td></td>
<td>G12A</td>
<td></td>
<td>G12A</td>
</tr>
<tr>
<td></td>
<td>G12V</td>
<td></td>
<td>G12V</td>
</tr>
<tr>
<td></td>
<td>G13D</td>
<td></td>
<td>G13D</td>
</tr>
<tr>
<td>3</td>
<td>A59T</td>
<td>3</td>
<td>A59T</td>
</tr>
<tr>
<td></td>
<td>Q61L</td>
<td></td>
<td>Q61K</td>
</tr>
<tr>
<td></td>
<td>Q61R</td>
<td></td>
<td>Q61R</td>
</tr>
<tr>
<td></td>
<td>Q61H</td>
<td></td>
<td>Q61H</td>
</tr>
<tr>
<td></td>
<td>Q61H</td>
<td></td>
<td>Q61H</td>
</tr>
<tr>
<td>4</td>
<td>K117N</td>
<td>4</td>
<td>K117N</td>
</tr>
<tr>
<td></td>
<td>K117N</td>
<td></td>
<td>K117N</td>
</tr>
<tr>
<td></td>
<td>A146T</td>
<td></td>
<td>A146T</td>
</tr>
</tbody>
</table>

Vidal et al. ESMO 2016
# Plasma/Tissue Correlation with OncoBEAM RAS CRC

## Tissue RAS Result

<table>
<thead>
<tr>
<th>Plasma Ras Result</th>
<th>Positive</th>
<th>Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>49</td>
<td>5</td>
<td>54</td>
</tr>
<tr>
<td>Negative</td>
<td>2</td>
<td>53</td>
<td>55</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>51</td>
<td>58</td>
<td>109</td>
</tr>
</tbody>
</table>

- **Positive Agreement: 96,1%**
- **Negative Agreement: 91,4%**
- **Overall Agreement: 93,6%**

### Plasma/Tissue Correlation with OncoBEAM RAS CRC

**Vidal et al. Ann Oncol 2017**
## Analysis of plasma-tissue discrepant cases

<table>
<thead>
<tr>
<th>Codon</th>
<th>% plasma Mut fraction</th>
<th>Site tumor biopsy</th>
<th>Primary tumor resected</th>
<th>Days between tissue – plasma collection</th>
<th>Systemic treatment before ctDNA</th>
<th>Treatment</th>
<th>Best Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.2458%</td>
<td>primary</td>
<td>yes</td>
<td>71</td>
<td>No</td>
<td>FOLFOX</td>
<td>Panitumumab</td>
</tr>
<tr>
<td>2</td>
<td>0.128%</td>
<td>primary</td>
<td>yes</td>
<td>138</td>
<td>No</td>
<td>FOLFOX</td>
<td>Cetuximab</td>
</tr>
<tr>
<td>3</td>
<td>31.73%</td>
<td>primary</td>
<td>yes</td>
<td>122</td>
<td>No</td>
<td>XELOX</td>
<td>PD</td>
</tr>
<tr>
<td>4</td>
<td>0.896%</td>
<td>primary</td>
<td>yes</td>
<td>60</td>
<td>No</td>
<td>FOLFOX</td>
<td>Cetuximab</td>
</tr>
<tr>
<td>5</td>
<td>0.316%</td>
<td>primary</td>
<td>no</td>
<td>32</td>
<td>Yes</td>
<td>FOLFOX</td>
<td>Cetuximab</td>
</tr>
<tr>
<td>6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>No</td>
<td></td>
<td>no</td>
</tr>
<tr>
<td>7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>No</td>
<td>FOLFOX</td>
<td>Bevacizumab</td>
</tr>
</tbody>
</table>

### RAS PLASMA WT / TISSUE MUT

<table>
<thead>
<tr>
<th>Codon</th>
<th>Site tumor biopsy</th>
<th>Days between tissue – plasma collection</th>
<th>Treatment</th>
<th>Best Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td></td>
<td>1195</td>
<td></td>
<td>no</td>
</tr>
<tr>
<td>7</td>
<td></td>
<td>39</td>
<td>FOLFOX</td>
<td>PR</td>
</tr>
</tbody>
</table>

Tissue DNA sensitivity may be limited because samples fail to capture tumor heterogeneity. ctDNA sensitivity may be limited when tumor DNA is not shed into circulation.

Vidal et al. Ann Oncol 2017
Correlation between circulating RAS mutations frequency and clinical characteristics

Site of metastasis rather than number of metastasis correlates with RAS ctDNA:
- Patients with hepatic metastases have higher RAS ctDNA levels
- Patients with peritoneum or lung involvement have lower RAS ctDNA levels

RAS ctDNA levels decrease after administration of chemotherapy

Vidal et al. Ann Oncol 2017
Is plasma RAS testing a good alternative to be used in clinical practice?

PFS for patients treated with antiEGFR was the same for tissue RAS wt and plasma RAS wt patients (PFS 10.3 months)
Liquid biopsy RAS testing: Challenges and opportunities

**Technical**
- How do novel technologies compare in terms of...
  - Time to results?
  - Hands-on-time?
  - Sensitivity?
  - Cost?

**Clinical**
- What RAS ctDNA mutation percentage threshold is clinically relevant?
- Is the predictive value of RAS ctDNA the same as RAS tissue?
**Aim:** To assess the clinical relevance of monitoring RAS, BRAF and EGFR ECD mutations by liquid biopsy from RAS wt mCRC patients treated with cetuximab-based therapy.
ctDNA: clinical applications in metastatic colorectal cancer

1. Molecular testing at diagnosis to guide treatment decision

2. Monitoring resistance to targeted therapy (antiEGFR)

3. Monitoring response to treatment (tumor burden)
Emergence of mutations of resistance

Baseline RAS wt tumor → Response to anti-EGFR treatment → RAS mutant tumor at progression to treatment

Anti-EGFR treatment
Monitoring mutations of resistance in the blood of patients

Acquired resistance to anti-EGFR therapy in CRC

2. Mutations in extracellular domain of EGFR

- EGFR
- S492R, G465R/E, G464L, K467T, I491M
- KRAS
- NRAS
- PI3-K
- AKT
- BRAF
- MEK
- ERK
- HER2 ampl
- MET ampl
- LIGANDS overexpression: AREG, HRG, TGFα

1. Downstream mutations or activation of alternative receptors that converge in MEK activation

Liquid biopsy to monitor clonal dynamics in mCRC patients treated with anti-EGFR
# OncoMine ctDNA CRC—monitoring a panel of mutations in the blood of 6 CRC patients treated with panitumumab

<table>
<thead>
<tr>
<th>Gene</th>
<th>Patient 1</th>
<th>Patient 2</th>
<th>Patient 3</th>
<th>Patient 4</th>
<th>Patient 5</th>
<th>Patient 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>BRAF</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>p.V600E</td>
</tr>
<tr>
<td>EGFR</td>
<td></td>
<td></td>
<td>p.G465E</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MAP2K1</td>
<td></td>
<td></td>
<td>p.K57T</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Liquid biopsy to guide biomarker-based treatment after cetuximab / panitumumab

<table>
<thead>
<tr>
<th>mechanism of resistance</th>
<th>Therapeutic strategy</th>
<th>example</th>
<th>trial</th>
</tr>
</thead>
<tbody>
<tr>
<td>HER2 amplification</td>
<td>PanHER TKI + antiHER2</td>
<td>Trastuzumab + lapatinib</td>
<td>Heracles trial (Phase II)</td>
</tr>
<tr>
<td>MET amplification</td>
<td>anti-EGFR + MET inh</td>
<td>Cetuximab + ARQ197</td>
<td>NCT01892527 (Phase II)</td>
</tr>
<tr>
<td>RAS mutation</td>
<td>anti-EGFR + MEK inh</td>
<td>Panitumumab + MEK162</td>
<td>NCT01927341 (Phase II)</td>
</tr>
<tr>
<td>rechallenge</td>
<td></td>
<td>ERMES trial; FIRE-4</td>
<td></td>
</tr>
<tr>
<td>EGFR S492R mutation</td>
<td>panitumumab</td>
<td>panitumumab</td>
<td></td>
</tr>
<tr>
<td>EGFR ECD mutations</td>
<td>Second generation anti-EGFR</td>
<td>Sym004 PAN-HER</td>
<td>NCT02083653 (Phase II)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MM-151</td>
<td>NCT01520389 (Phase I)</td>
</tr>
<tr>
<td>BRAF mutation</td>
<td>Anti-EGFR mAbs + BRAF inhibitor</td>
<td>BRAFi + antiEGFR + PI3Ki / MEKi</td>
<td>ARRAY, BEACOn study</td>
</tr>
<tr>
<td>MEK mutation</td>
<td>Anti-EGFR + MEKi</td>
<td>Cetuximab + trametinib</td>
<td></td>
</tr>
</tbody>
</table>
Heracles trial. Treating HER2+ mCRC

Lapatinib + trastuzumab in KRAS exon 2 wild type and HER2+ mCRC patients

78% disease control

Primary endpoint met in advance with 8/23 objective responses

Cetuximab rechallenge

Mutations in RAS emerge during anti-EGFR treatment

Basal RAS wt tumor → Response to treatment → Progression to treatment
Mutations in RAS emerge during anti-EGFR treatment and **decline** when treatment is suspended.
Liquid biopsy for longitudinal monitoring of RAS mutations in blood of patients

Rechallenge with cetuximab

Siravegna et al. Nat Med 2015
Rechallenge with anti-EGFR therapy

FIRE-4 (Phase III, n=550)

- Primary endpoint: OS after randomization 2
- Results expected: January 2022

Liquid biopsy to track/identify resistance
Targeting EGFR-ECD mutations with novel anti-EGFR drugs in mCRC

Receptor binding

Detected stained cells (Normalized to EGFR WT)

<table>
<thead>
<tr>
<th></th>
<th>Cetuxima</th>
<th>Panitumumab</th>
<th>Sym004</th>
</tr>
</thead>
<tbody>
<tr>
<td>Empty</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EGFR WT</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S492R</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R451C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>K467T</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G465R</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

S492R EGFR ECD mut

Tumor volume (mm3)

Days

Control

Cetuximab

Sym004

Sánchez-Martín et al. CCR 2016
ctDNA: clinical applications in metastatic colorectal cancer

1. Molecular testing at diagnosis to guide treatment decision

2. Monitoring resistance to targeted therapy (antiEGFR)

3. Monitoring response to treatment (tumor burden)
Evaluation of response to treatment in mCRC

ctDNA as a predictor of response to chemotherapy

Liquid biopsy (ctDNA) predicts early response to chemotherapy

Prospective trial of 53 MCRC patients treated with first line chemotherapy
ctDNA as an early predictor of response to chemotherapy

Prospective trial of 53 MCRC patients treated with first line chemotherapy

Decline $>10$ fold in ctDNA at week 2 predicts response to treatment in the CT-scan (week 8)

Plasma RAS testing to evaluate response to treatment in RAS mutant patients

Significant decrease of RAS mutant alleles mirrored response to treatment

Vidal et al. Ann Oncol 2017
Take-home message - ctDNA in mCRC

- RAS and BRAF testing are mandatory for first line treatment decision in mCR.

- RAS and BRAF ctDNA testing is a less invasive, faster alternative to tissue-based RAS testing at diagnosis.

- CRC is an heterogeneous and evolutionary disease. Biomarkers change along the course of the disease, specifically under anti-EGFR treatment pressure.

- A comprehensive and dynamic monitoring of biomarkers is mandatory for a real-time precision medicine. ctDNA represents tumor heterogeneity and is useful to longitudinally monitor biomarkers in a non-invasive manner.

- Liquid biopsy-driven clinical trials to guide therapeutic strategies beyond first line are crucial to improve patient’s outcome.
Thank you
cmontagut@hospitaldelmar.cat
Guardant360 blood samples taken concurrently with tumor biopsies show high concordance but with the passage of time and treatment - the discordance grows.

Would you treat a patient based on a six month old CT scan?

- High compliance – easy for the patient
- Low risk
- Fast result
- Accessibility
- Monitoring

Talasaz et al. 2014 Abstract e22041, J Clin Oncol 32(15_suppl)
Oncomine™ Colon cfDNA Assay

Gene List

<table>
<thead>
<tr>
<th>Gene</th>
<th>Gene</th>
</tr>
</thead>
<tbody>
<tr>
<td>AKT1</td>
<td>NRAS</td>
</tr>
<tr>
<td>BRAF</td>
<td>PIK3CA</td>
</tr>
<tr>
<td>CTNNB1</td>
<td>MAD4</td>
</tr>
<tr>
<td>EGFR</td>
<td>TP53</td>
</tr>
<tr>
<td>ERBB2</td>
<td>APC</td>
</tr>
<tr>
<td>FBXW7</td>
<td></td>
</tr>
<tr>
<td>GNAS</td>
<td></td>
</tr>
<tr>
<td>KRAS</td>
<td></td>
</tr>
<tr>
<td>MAP2K1</td>
<td></td>
</tr>
</tbody>
</table>
NGS in plasma - Comprehensive analysis - Guardant 360

### Point Mutations – 73 Genes

<table>
<thead>
<tr>
<th>AKT1</th>
<th>ALK</th>
<th>APC</th>
<th>AR</th>
<th>ARAF</th>
<th>ARID1A</th>
<th>ATM</th>
<th>BRAF</th>
<th>BRCA1</th>
<th>BRCA2</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCND1</td>
<td>CCND2</td>
<td>CCNE1</td>
<td>CDH1</td>
<td>CDK4</td>
<td>CDK6</td>
<td>CDKN2A</td>
<td>CTNNB1</td>
<td>DDR2</td>
<td>EGFR</td>
</tr>
<tr>
<td>ERBB2</td>
<td>ERBB2</td>
<td>ESR1</td>
<td>EZH2</td>
<td>FBXW7</td>
<td>FGFR1</td>
<td>FGFR2</td>
<td>FGFR3</td>
<td>GATA3</td>
<td>GNA11</td>
</tr>
<tr>
<td>GNAS</td>
<td>HNF1A</td>
<td>HRAS</td>
<td>IDH1</td>
<td>IDH2</td>
<td>JAK2</td>
<td>JAK3</td>
<td>KIT</td>
<td>KRAS</td>
<td>MAP2K1</td>
</tr>
<tr>
<td>MAP2K2</td>
<td>MAPK1</td>
<td>MAPK3</td>
<td>MET</td>
<td>MLH1</td>
<td>MPL</td>
<td>MTOR</td>
<td>MYC</td>
<td>NF1</td>
<td>NFE2L2</td>
</tr>
<tr>
<td>NOTCH1</td>
<td>NPM1</td>
<td>NRAS</td>
<td>NTRK1</td>
<td>NTRK3</td>
<td>PDGFR1</td>
<td>PIK3CA</td>
<td>PTEN</td>
<td>PTPN11</td>
<td>RAF1</td>
</tr>
<tr>
<td>RB1</td>
<td>RET</td>
<td>RHEB</td>
<td>RHOA</td>
<td>RIT1</td>
<td>ROS1</td>
<td>SMAD4</td>
<td>SMO</td>
<td>STK11</td>
<td>TERT**</td>
</tr>
<tr>
<td>TP53</td>
<td>TSC1</td>
<td>VHL</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

** Includes TERT promoter region

### Indels – 23 Genes

<table>
<thead>
<tr>
<th>ATM</th>
<th>APC</th>
<th>ARID1A</th>
<th>BRCA1</th>
<th>BRCA2</th>
<th>CDH1</th>
<th>CDKN2A</th>
<th>EGFR</th>
<th>ERBB2</th>
<th>GATA3</th>
</tr>
</thead>
<tbody>
<tr>
<td>KIT</td>
<td>MET</td>
<td>ex14</td>
<td>MLH1</td>
<td>MTO1</td>
<td>NF1</td>
<td>PDGFR1</td>
<td>PTEN</td>
<td>RB1</td>
<td>SMAD4</td>
</tr>
<tr>
<td>TP53</td>
<td>TSC1</td>
<td>VHL</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>STK11</td>
</tr>
</tbody>
</table>

### Amplifications – 18 Genes

<table>
<thead>
<tr>
<th>AR</th>
<th>BRAF</th>
<th>CCND1</th>
<th>CCND2</th>
<th>CCNE1</th>
<th>CDK4</th>
<th>CDK6</th>
<th>EGFR</th>
<th>ERBB2</th>
</tr>
</thead>
<tbody>
<tr>
<td>FGFR1</td>
<td>FGFR2</td>
<td>KIT</td>
<td>KRAS</td>
<td>MET</td>
<td>MYC</td>
<td>PDGFR</td>
<td>PIK3CA</td>
<td>RAF1</td>
</tr>
</tbody>
</table>

### Fusions – 6 Genes

<table>
<thead>
<tr>
<th>ALK</th>
<th>FGFR2</th>
<th>FGFR3</th>
<th>RET</th>
<th>ROS1</th>
<th>NTRK1</th>
</tr>
</thead>
</table>

43
Take-home message - ctDNA in mCRC

- RAS and BRAF testing are mandatory for first line treatment decision in mCRC

- RAS ctDNA testing is a less invasive, highly sensitive alternative to tissue-based RAS testing at diagnosis

- Biomarkers change along the course of the disease, specifically under anti-EGFR treatment pressure

- Dynamic monitoring of biomarkers is mandatory for a real-time precision medicine

- Liquid biopsy (ctDNA) represents tumor heterogeneity and is useful to longitudinally monitor biomarkers in a non-invasive manner

- Liquid biopsy-driven clinical trials to guide treatment strategy are ongoing and are crucial to improve patient’s outcome
Gracias
cmontagut@hospitaldelmar.cat
Second generation anti-EGFR moAb

- Cetuximab
- Panitumumab
- Sym004
- MM-151
- mAb 806
- mAb 528

**Extracellular domain**

**Transmembrane domain**

**Kinase domain**

- Gefitinib
- Erlotinib
- Lapatinib
- Afatinib
- PD153035
- AG1478

PAN-HER

HER2

HER3
Sym004 is effective in colorectal cancer harbouring EGFR ECD mutations

**Receptor binding**

- Detected stained cells (Normalized to EGFR WT)

**S492R EGFR ECD mut**

- Control
- Cetuximab
- Sym004

Sánchez-Martín et al. CCR 2016
Sym004 is effective in patients progressing to cetuximab and harbouring EGFR ECD mutations.

- **EGFR G465R mutation**
- **EGFR S492R mutation**

67% disease control
High sensitivity is required to detect ctDNA

Tumoral DNA (somatic mutation)

Normal DNA (wild-Type)

Detection capacity
(mutant DNA/total DNA)

100%

Sanger Sequencing

10%

Pyrosequencing

1%

Real-Time PCR

0.1%

BEAMing

0.01%

Adapted from A. Vivancos
Acquired resistance to anti-EGFR therapy in CRC

2. Mutations in extracellular domain of EGFR

1. Downstream mutations or activation of alternative receptors that converge in MEK activation

ESMO consensus guidelines for the management of patients with metastatic colorectal cancer

**CLINICAL CONDITION OF THE PATIENT**

- Fit
- Unfit (but may be suitable)
- Unfit*

**GOAL**

- Cytoreduction (Shrinkage)
- Disease control (Control progression)

**MOLECULAR PROFILE**

- RAS wt
  - Surgery alone
  - Surgery with perioperative/postoperative CT
- RAS mt
  - Combination + bevacizumab
- BRAF mt
  - Triplet + bevacizumab

**MOLECULAR PROFILE**

- RAS wt
  - Doublet + anti-EGFR
- RAS mt
  - Combination + bevacizumab
- BRAF mt
  - Triplet + bevacizumab

- CT + biological agent

**Molecular Profile**

- Unusual, see text

**Re-evaluation/assessment of response every 2 months**

- Surgery
  - Progressive disease
  - Cytoreduction (Shrinkage)
  - Disease control
  - Continue
  - Continue; maintenance; or pause

- Second-line

- Progressive disease
  - Second-line

- BSC

Van Cutsem et al, Ann Oncol 2016
Guardant360 Dynamic Reporting
New report, new dimension in cancer management

Summary of Alterations & Associated Treatment Options
The percentage, or allele frequency, of altered circulating cell-free DNA (% cfDNA) in blood is related to the unique tumor biology of this patient. Factors that may affect the amount/percentages of detected genomic alterations in circulating cell-free DNA in blood include tumor growth, turn-over, size, heterogeneity, vascularization, disease progression, or treatment.

<table>
<thead>
<tr>
<th>Alteration</th>
<th>Mutation Trend</th>
<th>% cfDNA</th>
<th>cfDNA Amplification</th>
<th>FDA Approved in Indication</th>
<th>Available for Use in Other Indications</th>
<th>Clinical Drug Trials</th>
</tr>
</thead>
<tbody>
<tr>
<td>E746_A750 Del</td>
<td><img src="image1.png" alt="Graph" /></td>
<td>15.0</td>
<td>Afatinib, Erlotinib</td>
<td>Lapatinib, Panitumumab, Gefitinib</td>
<td>Trials Available</td>
<td></td>
</tr>
</tbody>
</table>

Guardant360® Tumor Response Map
The Guardant360 Tumor Response Map illustrates the relative changes of observed cfDNA at different sample submission time points.

Somatic Alteration Burden
- 25.2%
- 0.3%
- 15.0%

06.11.14  10.01.14  02.19.15

6 Total Alteration(s) Detected
- 4 with Associated Therapy
- 1 Associated with Lack of Response

Multiple Clinical Drug Trials Available

<table>
<thead>
<tr>
<th>MET</th>
<th>AMP</th>
<th>None</th>
<th>Orzotinib Cabozantinib</th>
<th>Trials Available</th>
</tr>
</thead>
</table>

For a more detailed Guardant360 Patient Report, log onto: https://portal.guardant360.com
To set up an account, contact CNAM Services: 800.888.3987

GUARDANT® HEALTH 15911 Old Vic Road, Suite C, C也好，CA 90803
T 800.888.3987 | clientservice@guardanthealth.com | https://portal.guardant360.com | Report version 2.8, 1217167.01.Y01, Fig. 4 of 48
## RAS testing in ctDNA in metastatic colorectal cancer

**BEAMing, Sysmex-Ionostics**

<table>
<thead>
<tr>
<th>technology</th>
<th>Quantity of plasma</th>
<th>CE marked</th>
<th>Results turn around time</th>
<th>Hand on time</th>
<th>Sensitivity in plasma</th>
<th>MAF quantification</th>
<th>coverage</th>
</tr>
</thead>
<tbody>
<tr>
<td>BEAMing</td>
<td>3-4 mL</td>
<td>April 2016 (RAS)</td>
<td>1 week</td>
<td>dedicated technician</td>
<td>0.02-0.04%</td>
<td>Quantitative</td>
<td>Restricted gene coverage; No reference truncal mutation;</td>
</tr>
<tr>
<td>Idylla</td>
<td>2 mL</td>
<td>Q2 2017 (RAS+BRAF)</td>
<td>2 hrs</td>
<td>minimum</td>
<td>0.5%</td>
<td>Semi-quantitative</td>
<td>Restricted gene coverage, but possibility to move to NGS; No reference truncal mutation;</td>
</tr>
</tbody>
</table>

**Idylla, Biocartis**
BRAF baseline diagnostic detection
Liquid biopsy versus tissue with IDYLLA (BIOCARTIS)

<table>
<thead>
<tr>
<th>Plasma ctBRAF Result</th>
<th>Tissue BRAF Result</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
</tr>
<tr>
<td>Positive</td>
<td>18</td>
</tr>
<tr>
<td>Negative</td>
<td>4</td>
</tr>
<tr>
<td>Total</td>
<td>22</td>
</tr>
</tbody>
</table>

Overall Agreement: 36/42: 85.7%
High sensitivity is necessary to detect RAS in plasma

RAS mutations (N=52)

Median MAF: 2.317%
Median MAF: 0.281%

Vidal et al. Ann Oncol 2017