Estado mutacional y respuesta a terapia en CCR

Ana Vivancos, VHIO
Critical aspects in implementation of liquid biopsy in the clinics

- **ctDNA shedding**
  - mCRC
  - Frequency of cases with detectable ctDNA (%)

- **Intratumor heterogeneity**
  - Tumor load
  - Met locations
  - Necrosis, other biological processes

- **Targeted therapies**
  - Clonality
    - mCRC: APC, KRAS vs BRAF, PIK3CA
    - Lung: EGFR indel 19 vs T790M vs C797S
  - Selection
    - mCRC: anti-EGFR, RAS clones
    - Lung: EGFR T790M
Some technical considerations (in mCRC)
Technical characteristics of cfDNA

Stanislav Volik et al., MCR, 2016

cfDNA vary in terms of yield from patient to patient

1st line mCRC: up to 100-fold

Vivancos Lab, unpublished data
No major Shedding issues in mCRC

(n = 177)
Mutant allele fractions are frequently low in liquid biopsy

<table>
<thead>
<tr>
<th>Median MAF</th>
<th>Tumor</th>
<th>Literature</th>
</tr>
</thead>
<tbody>
<tr>
<td>4%</td>
<td>mBC</td>
<td>Dawson et al., 2013</td>
</tr>
<tr>
<td>1.5%</td>
<td>mCRPC</td>
<td>Azad et al., 2015</td>
</tr>
<tr>
<td>4%</td>
<td>mCRC</td>
<td>Grasselli et al., 2017</td>
</tr>
<tr>
<td>6.6%</td>
<td>NSCLC</td>
<td>Fairclough et al., J Clin Oncol 34, 2016 (suppl; abstr e23021)</td>
</tr>
</tbody>
</table>

**Concordance Study** (VHIO, ICO, H.Mar)

**Detection Capability** (mutant DNA/ total DNA)
- Sanger Sequencing
- Pyrosequencing
- Real-Time PCR
- Digital PCR

**MAFs**
- 0.01-0.1%: 39%
- 0.1-1%: 29%
- >1%: 21%
- >5%: 11%
Technical considerations: Available technologies

- **Digital PCR** (~0.01% sensitivity)
- **Real-time PCR** (~0.1% sensitivity)
- **NGS** (~5% sensitivity)
Different technologies show different analytical sensitivities

- mCRC patients tested with OncoBEAM RAS CRC: n = 559
  - OncoBEAM KRAS-MUT+ results: n = 265 (47.4%)
  - OncoBEAM KRAS-MUT+ results ≤ 5% MAF: n = 147 (147/265) = 55.5%
  - OncoBEAM KRAS-MUT+ results ≤ 1% MAF: n = 99 (99/265) = 37.4%
  - OncoBEAM KRAS-MUT+ results ≤ 0.1% MAF: n = 26 (26/265) = 9.8%

- Primary Objective: Comparison of SOC FFPE and OncoBEAM (≤ 1% MAF) and Idylla: n = 43

- Secondary Objective: qPCR (Idylla) = Detection of 1 mutant count in 10,000 total counts

Ana Vivancos et al. - Poster 592
ASCO GI Congress
Detectability drops <1% MAF for qPCR-based methods

<table>
<thead>
<tr>
<th>MAF % Range</th>
<th>PPA Idylla vs OncoBEAM</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤ 5%, but &gt; 1%</td>
<td>(32/35) = 91.4%</td>
</tr>
<tr>
<td>≤ 1%, but &gt; 0.1%</td>
<td>(42/63) = 66.7%</td>
</tr>
<tr>
<td>≤ 0.1%</td>
<td>(7/17) = 41.2%</td>
</tr>
</tbody>
</table>

Table 1: Comparison of Idylla and OncoBEAM KRAS mutation detection sensitivity in plasma at different MAF%

- Idylla detected KRAS mutations in **81 out of 115** OncoBEAM KRAS-MUT⁺ samples with < 5% MAF  
  **Idylla ctKRAS PPA vs OncoBEAM = 70.4%**

- Idylla detected KRAS mutations in **49 out of 80** OncoBEAM KRAS-MUT⁺ samples with ≤ 1% MAF  
  **Idylla ctKRAS PPA vs OncoBEAM = 61.2%**

Concordance for ≤1% MAF:

- dPCR (OncoBEAM) with SOC: **72.1% (31/43)**
- qPCR (Idylla) with SOC: **46.5% (20/43)**
A comprehensive panel is required for many applications: NGS based

- Minimal Residual Disease
  - Monitoring Tumor Burden
- Biomarkers of sensitivity to treatments
- Biomarkers of resistance to treatments

- Gene-panel capture approaches (up to 24000 genes)
- Exome-seq (24000 genes)
- Whole genome sequencing
A comprehensive panel required: a Pilot study with Amplicon-seq in mCRC

n=90 (RAS mutant enriched)

NGS VHIO-card (Amplicon-seq), 60 genes

65 samples with available BEAMing test:

25 WT by BEAMing, 21 by NGS (3 likely FP)

40 mutant by BEAMing, 33 by NGS (7 missed by NGS MAF <0.5%)

RAS MAF% cfDNA

\[ r^2 = 0.7 \]
A comprehensive panel required: a Pilot study with Amplicon-seq in mCRC

n=90 (RAS mutant enriched)

NGS VHIO-card (Amplicon-seq), 60 genes

Using a routine NGS panel, 87% of cases showed at least 1 mutation

The remaining 13% are negative, most likely due to analytical sensitivity issues
In order to improve sensitivity issues with NGS, high coverage and UMI-based chemistries are required.

Guardant360

SafeSeq (Sysmex)

Avenio (Roche)
PCR Amplicons

Shear/ligation libraries with random start/stop

Shear / Single UMI ligation

Shear / Duplex UMI ligation

Low MAF: UNIQUE MOLECULAR IDENTIFIERS + DUPLEX SEQUENCING
**Best option for a possible routine?**

**VHIO-plasma panel**

<table>
<thead>
<tr>
<th>Genes</th>
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<tbody>
<tr>
<td>SF3B1</td>
</tr>
<tr>
<td>KIT1</td>
</tr>
<tr>
<td>ROS</td>
</tr>
<tr>
<td>FGFR2</td>
</tr>
<tr>
<td>BRCA2</td>
</tr>
<tr>
<td>NF1</td>
</tr>
<tr>
<td>RNF43</td>
</tr>
<tr>
<td>CTNNB1</td>
</tr>
<tr>
<td>IDH1</td>
</tr>
<tr>
<td>NRAS</td>
</tr>
<tr>
<td>ALK1</td>
</tr>
<tr>
<td>PDGFRA</td>
</tr>
<tr>
<td>MET</td>
</tr>
<tr>
<td>RET</td>
</tr>
<tr>
<td>BRCA1</td>
</tr>
<tr>
<td>TP53</td>
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<tr>
<td>AKT1</td>
</tr>
<tr>
<td>ESR1</td>
</tr>
<tr>
<td>IDH2</td>
</tr>
<tr>
<td>PIK3Ca</td>
</tr>
<tr>
<td>FGFR3</td>
</tr>
<tr>
<td>APC</td>
</tr>
<tr>
<td>EGFR</td>
</tr>
<tr>
<td>ERBB3</td>
</tr>
<tr>
<td>MAP2K1</td>
</tr>
<tr>
<td>ERBB2</td>
</tr>
<tr>
<td>BRAF</td>
</tr>
<tr>
<td>FBXW7</td>
</tr>
<tr>
<td>KRAS</td>
</tr>
<tr>
<td>TertPromoter</td>
</tr>
</tbody>
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Includes several unstable homopolymers: MSI

![Graphs showing reads for L18-3029, L18-3030, and L18-3031](image)

We are around 2.8 – 3.5 SSCS for 1 DCS, maybe due to panel design intended to capture both strands of DNA. This would mean an improvement compared to the other two published methods.

Duplex sequencing Kennedy et al., 2014
CAPP-seq Newman et al., 2016
Beyond mutation status in plasma...
Impact in prognosis of circulating tumor DNA Mutant Allele Fraction (MAF) in RAS mutant metastatic Colorectal Cancer (mCRC)

E. Elez1,2, C. Chianese3(*), E. Sanz-García1,2(*), E. Martinelli4, A. Noguerido1,2, F.M. Mancuso3, J. Grasselli1,5, G. Caratù3, J. Matito3, C. Santos5, T. Macarulla1,2, G. Argilés1,2, J. Capdevila1,2, A. García1,2, N. Mulet 1,2,5, M. Martínez, G. Soler, F. Jones6, J. Taberner01,2, F. Ciardello4, R., Salazar5(**), A. Vivancos3(**)

1-Department of Medical Oncology, Vall d’Hebron Institute of Oncology, Barcelona, Spain; 2-Department of Medical Oncology, Vall d’ Hebron University Hospital, Universitat Autònoma de Barcelona, Barcelona, Spain; 3-Cancer Genomics Group, Vall d’Hebron Institute of Oncology, Barcelona, Spain; 4-Medical Oncology, Department of Clinical and Experimental Medicine “F. Magrassi,” Università della Campania “L. Vanvitelli,” Napoli 5-Department of Medical Oncology, Catalan Institute of Oncology, Universitat de Barcelona, L’Hospitalet, Spain; 6- Sysmex Inostics, Illinois, United States.
Mutant Allele Fraction (MAF) could be determined with Beaming (quantitative) technology. However, its prognosis impact in CRC has not been explored.

ctDNA has emerged as an alternative to tumor biopsy to detect mutations in mCRC.

Concordance between RAS mt in plasma and tissue is high (90%)

- Exploratory analysis of this study showed a shorter OS in mCRC with RAS MAF higher than 10%.
- This exploratory analysis was in the whole population which was very heterogenous in terms of treatment and moment of the plasma analysis.
110 pts from Concordance Cohort and VHIO Plasma Library

47 pts RAS wt

63 pts RAS mt (BEAMing in plasma)

21 pts with liver-limited disease (resected)

42 pts with non-resectable metastases (47 samples)
Plasma MAF distribution

<table>
<thead>
<tr>
<th>MAF</th>
<th>1st LINE</th>
<th>2nd LINE</th>
<th>3rd LINE</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 1%</td>
<td>6 (21%)</td>
<td>6 (50%)</td>
<td>1 (17%)</td>
</tr>
<tr>
<td>1-10%</td>
<td>9 (31%)</td>
<td>2 (17%)</td>
<td>1 (17%)</td>
</tr>
<tr>
<td>&gt;10%</td>
<td>14 (48%)</td>
<td>4 (33%)</td>
<td>4 (66%)</td>
</tr>
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</table>
Plasma MAF correlation with treatment line and tumor location

No correlation of MAF with line or tumor location
Plasma MAF correlation with number and type of metastatic sites

Higher plasma MAFs when hepatic or lymph node metastatic affection is present
Plasma MAF does not correlate with serum biomarkers

- Ca19.9 (ng/mL)
  - Correlation: 0.232
  - P-value: 0.311

- CEA (U/mL)
  - Correlation: 0.056
  - P-value: 0.792

serum biomarkers
Plasma MAF does not correlate with tumor burden

- Hepatic volume (mm):
  - Correlation: 0.174
  - P-value: 0.405

- Num. of hepatic lesions:
  - Correlation: 0.181
  - P-value: 0.347
A non-statistically significant trend for better PFS was observed when MAF < 5.8% (optimized cut-off)
A statistically significant better OS was observed when MAF < 5.8%, resulted as an independent variable in Multivariant analysis*(p=0.0043)

* Tumor Side, N of M1 sites and CEA were added in the model
PFS and OS in 2\textsuperscript{nd} line
Best response and MAF in 1st and 2nd line.
Independent dataset CAPRI-GOIM trial (2\textsuperscript{nd} line)

340 pts in CAPRI-GOIM trial

92 pts with baseline plasma sample

33 pts RAS mut in plasma

1 pt with lack follow-up data

32 pts RAS mut in plasma

340 pts in CAPRI-GOIM trial

92 pts with baseline plasma sample

33 pts RAS mut in plasma

1 pt with lack follow-up data
mCRC mutations can be easily detected in plasma by different techniques, NGS is close.

*RAS* MAF is a prognostic factor in 1st and 2nd line of treatment for mCRC.

The underlying biological or molecular mechanisms that contribute to or modulate plasma MAFs are still to be determined.