Discussion abstracts #O-025, O-026, O-027

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Catalan Cancer Network
DISCLOSURE

**Scientific Advisory Boards & IDMC (last 5 years):**

**Bio-Techs:** VCN-BCN, Agendia, Guardant Health, Roche Diagnostics, Ferrer

**Pharma:** Pfizer, Novartis, Ipsen, Amgen, Merck, Roche Farma, Tayhoo, Lylli, MSD

**Speaker (last 5 years):**

**Pharma:** Pfizer, Novartis, Ipsen, Amgen, Merck, Roche Farma, Lylli, MSD, AZD, Celgene.
Association between tumor mutation burden (TMB) and MLH1, PMS2, MSH2, and MSH6 alterations in 395 microsatellite instability-high (MSI-High) gastrointestinal (GI) tumors

Descriptive study

• Rationale: the loss of mismatch repair system causes mutations in Microsatellites (MSI)
  – Higher tumor mutational burden (TMB)
  – higher chances to respond to Inmune checkpoint inhibitors
• Methods: MSI+ G-I series of 395 patients
  – Association between TMB (NGS panel) and
    • tumor primaries and sides
    • MMR protein analysis (IHC)
• Ultimate aim: optimize patient selection for Inmune Check Point Inhibitors (ICI)
Tumor Mutational Burden

- Total number of nonsynonymous somatic mutations per MB of the genome coding area
- Validated in different NGS approaches:
  - Exome (about 30 MB)
  - Gene panels (about 1.7 MB)
    - Salem et al: 592 genes and 1.4 megabases [MB] sequenced per tumor
- The larger the TMB the higher chances of neoantigens and subsequent T cell activation & response to ICI
  - CRC poor response outlier (immunosuppressive microenvironment)

1 Yarchoan et al., N Engl J Med 377;25
Among MSI-High gastrointestinal cancers, CRC exhibited the highest TMB level (concordant with BioPortal analysis).
Sidedness in CRC

Left-sided tumors exhibited higher TMB than right-sided tumors. (conflicting with TCGA analysis)

TMB and MMR alterations

MSH2 and/or MSH6 alterations were associated with a significantly higher TMB than MLH1/PMS2 alterations across all gastrointestinal cancer types. (concordant with BioPortal analysis)

Coexistent MSH2 & MSH6 alteration is very common and yields the highest TMB, but close to MSH2 or MSH6 isolated alterations.
Rationale for MSH2 & MSH6 higher TMB yield?

Therapeutic Targeting of the DNA Mismatch Repair Pathway
Sarah A. Martin, Christopher J. Lord and Alan Ashworth, CCR 2010

Vilar et al. Nat Clin Oncol 2010

Human mismatch repair proteins

<table>
<thead>
<tr>
<th>Protein</th>
<th>Heterodimer</th>
<th>Repair function</th>
</tr>
</thead>
<tbody>
<tr>
<td>MutSα</td>
<td>MSH2 ♦ MSH6</td>
<td>binds base-base mismatches and insertion-deletion mismatches</td>
</tr>
<tr>
<td>MutSβ</td>
<td>MSH2 ♦ MSH3</td>
<td>binds insertion-deletion mismatches</td>
</tr>
<tr>
<td>MutLα</td>
<td>MLH1 ♦ PMS2</td>
<td>early step before excision</td>
</tr>
</tbody>
</table>

Repairs both base-base and Indels
Context

• MSI or high TMB in G-I cancer are infrequent
• MSH2/MSH6 dMMR specific subgroup
  – Improve PPV for response?

• Can we enlarge the target population?
  – Treat more patients effectively with ICI
Tumor Mutational Burden/Load

- Some **MSS** G-I tumors have high TMB
  - 8.3 % anal ca
  - 1.3% pNETs
  - 3.5 % sq esophageal (tobacco induced)
PD-L1 in G-I tumors

- Predicts response to ICI in some non G-I tumors
- No role of PD-L1 in MSI+
- Role of PD-L1 in MSS tumors?
  - Pembro approved in PD-L1+ gastric or GE adenos
  - 48% sq esophageal PD-L1 +
    - Only 3.5% high TMB

Expressed by tumor cells
Binds to PD1-R on T cells, inactivates them

Salem et al. Mol Cancer Res; 16 (5)
Other potential predictors of response to ICI

(check session XVII: Guillem Argiles and Opening Lecture by Herbert Tilg, Innsbruck)

- CMS-1 & 4 in CRC
  - PD1 + tgf-beta inhibitors in tgf-beta driven cancer
- EBV and MSI-like in gastric
- HPV+ involved in >80% anal ca,
  - Yet only 8.3% TML high

- HLA genotype?

- Microbiome subtypes?
Total circulating cell-free DNA as prognostic biomarker in metastatic colorectal cancer prior to first-line oxaliplatin-based chemotherapy

**Presenting author:** Julian Hamfjord*  |  Co-authors: Tormod Kyrre Guren, Olav Dajani, Bengt Glimelius, Halfdan Sorbye, Per Pfeiffer, Thoralf Christoffersen, Ole Christian Lingjærde, Kjell Magne Tveit, Elin H. Kure, Niels Pallisgaard and Karen-Lise Garm Spindler

*Oslo University Hospital and University of Oslo

Oral Presentation at the ESMO 20th World Congress on Gastrointestinal Cancer, Session XX, Saturday 23 June 2018
Materials and Methods


• Droplet dPCR

• Data for this substudy were analyzed across the three treatment arms, endpoint OS, <10% drop outs (499/566)
**cfDNA Levels Associated with OS**

<table>
<thead>
<tr>
<th>Performance status (PS)</th>
<th>HR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>WHO 0</td>
<td>1.00</td>
<td>1.30 – 2.00</td>
</tr>
<tr>
<td>WHO 1</td>
<td>1.62</td>
<td>1.35 – 3.38</td>
</tr>
<tr>
<td>WHO 2</td>
<td>2.13</td>
<td>1.35 – 3.38</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Biochemical investigations</th>
<th>HR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALP level (below/above UNL)</td>
<td>1.35</td>
<td>1.08 – 1.69</td>
</tr>
<tr>
<td>CEA level (below/above UNL)</td>
<td>1.37</td>
<td>1.03 – 1.81</td>
</tr>
<tr>
<td>cfDNA level (+SD)</td>
<td>1.22</td>
<td>1.09 – 1.37</td>
</tr>
</tbody>
</table>
cfDNA vs ctDNA

- cfDNA is simpler, easier, cheaper... **BUT**
- Weaker prognosticator (HR 1.2) than ALP and CEA.
  - No external validation (cut-off)
- Meta-analysis (N ~ 1000):
  - cfDNA levels are higher in metastatic disease and confer worse prognosis,
  - prospective studies to validate a cut-off are warranted¹
- Levels of cfDNA are not necessarily a surrogate of ctDNA or tumor activity²,³.
  - cfDNA levels depend on several other physiological conditions: stress, sport, hormonal status, etc.
  - Release of ctDNA varies according to a number of parameters, such as tumor burden, stage, vascularity, cellular turnover, and response to therapy

¹. Spindler et al. The Oncologist 2017; 22:1–7
Independent dataset CAPRI-GOIM trial

340 pts in CAPRI-GOIM trial

92 pts with baseline plasma sample

33 pts RAS mut in plasma

32 pts RAS mut in plasma


ctDNA RAS Mutant Allele Frequency and Prognosis
BEACON CRC Phase 3 Study Design¹

Safety Lead-in Completed

ENCO 300 mg QD + BINI 45 mg BID + CETUX 400 mg/m² (initial) then 250 mg/m² QW

N=10

Phase 3 Currently Enrolling

Triplet therapy ENCO + BINI + CETUX n=205

Doublet Therapy ENCO + CETUX n=205

Control Arm FOLFIRI + CETUX, or IR + CETUX n=205

Disease progression

Continued follow-up for evaluation of OS

Disease progression

Disease progression

Continued follow-up for evaluation of OS

R 1:1:1


FOLFIRI=folinic acid, fluorouracil, and irinotecan hydrochloride; OS=overall survival.
CRC BRAF+ BACKGROUND

- Small population:
  - 8-10% early stage
  - 4-5% late stage
  - Very poor prognosis in late stage (mCRC)
- Non specific approved treatments in 1st and 2nd Lines:
  - TRIBE\(^1\) (28 BRAF Mt pts): mOS 19 m; PFS 7'5 m
  - VOLFI\(^2\) (16 BRAF MT) RR 74% OR 8.750 (0.9-84.80) P=0.1262
  - VELOUR\(^3\) (36 BRAF MT pts): mOS 10’3 vs 5’5m; mPFS 5’5 vs 2’2m

Differential response of BRAF inhibition in *BRAF* mutant melanoma versus colon cancer

Vemurafenib (PLX4032) - A selective *BRAF* V600E kinase inhibitor

PLX4032 response rate in B-RAF V600E-positive tumours

- Melanoma: 81%
- Colon cancer: 5.20%

Kopetz et al., ASCO 2010
Targeting the RAF pathway in mCRC

Signaling in BRAF mt CRC

Reactivation of EGFR signaling upon BRAF inhibition

Corcoran et al., Cancer Discovery 2012
Prahallad et al., Nature 2012
BEACON
Confirmed Best Overall Response

<table>
<thead>
<tr>
<th>CONFIRMED BEST OVERALL RESPONSE*</th>
<th>PATIENTS (N=29)†</th>
</tr>
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<tbody>
<tr>
<td>ORR (CR + PR)</td>
<td>14 (48%)</td>
</tr>
<tr>
<td>CR</td>
<td>3 (10%)</td>
</tr>
<tr>
<td>PR</td>
<td>11 (38%)</td>
</tr>
<tr>
<td>SD</td>
<td>13 (45%)</td>
</tr>
<tr>
<td>PD</td>
<td>0</td>
</tr>
<tr>
<td>Not evaluable for response‡</td>
<td>2 (7%)</td>
</tr>
</tbody>
</table>

- ORR for patients with 1 and 2 prior regimens were 62% and 31% respectively
- Median DOR: 5.5 mo (95% CI, 4.1–NR)

*Local assessed confirmed responses per RECIST 1.1
†Patients with $BRAF^{V600E}$ mutations.
‡Non-responders per intent-to-treat analysis.

CR=complete response; DOR=duration of response; NR=not reached; ORR=objective response rate; PD=progressive disease; PR=partial response; SD=stable disease.
BRAF INHIBITION

<table>
<thead>
<tr>
<th>Regimen</th>
<th>Response rate</th>
<th>PFS</th>
<th>Citation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single/Doublet BRAF/MEK</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vemurafenib</td>
<td>5%</td>
<td>2.1 months</td>
<td>Kopetz, ASCO '10</td>
</tr>
<tr>
<td>Dabrafenib</td>
<td>11%</td>
<td>NR</td>
<td>Falchook, Lancet '08</td>
</tr>
<tr>
<td>Encorafenib</td>
<td>16%</td>
<td>NR</td>
<td>Gomez-Roca, ESMO '14</td>
</tr>
<tr>
<td>Dabr + Tramet</td>
<td>12%</td>
<td>3.5 months</td>
<td>Corcoran, ASCO '14</td>
</tr>
<tr>
<td>Doublet with EGFR</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vem + Panit</td>
<td>13%</td>
<td>3.2 months</td>
<td>Yeager et al CCR '14</td>
</tr>
<tr>
<td>Vem + Cetux</td>
<td>20%</td>
<td>3.2 months</td>
<td>Tabernero et al ASCO '14</td>
</tr>
<tr>
<td>Encorf + Cetux</td>
<td>22%</td>
<td>4.2 months</td>
<td>Tabernero et al ESMO GI 2016</td>
</tr>
<tr>
<td>Dabr + Panit</td>
<td>10%</td>
<td>3.5 months</td>
<td>Corcoran, ESMO 2016</td>
</tr>
<tr>
<td>Triplet with EGFR</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vem + Cetux + Irinotecan</td>
<td>16%</td>
<td>4.4 months</td>
<td>Kopetz et al, ASCO GI '17</td>
</tr>
<tr>
<td>Dabr + Tramet + Panit</td>
<td>21%</td>
<td>4.2 months</td>
<td>Corcoran, ESMO '16</td>
</tr>
<tr>
<td>Encoraf + Cetux + Alpelisib</td>
<td>27%</td>
<td>5.4 months</td>
<td>Tabernero et al ESMO GI '16</td>
</tr>
</tbody>
</table>
Efficacy of BRAFi/EGFRi combos

Are we closing the Gap?

• Good news, we have a potential new standard
  – Yet, lower clinical activity of combos in CRC than single agent activity in MM\(^1\)

• Intense signal reverberation in CRC MAPK pathways\(^2\)
  – KRAS mutations, KRAS amplifications, BRAF amplifications, MEK mutations

• Robust gene programs\(^3,4\)
  – Different Molecular gene expression programmes in CRC BRAF+\(^5\)
  – Overlapping phenotypes (\(~30\%\) BRAF+ are MSI+)

\(^1\)Chapman PB et al. NEJM 2011, \(^2\)Ahronian et al. Cancer Discovery 2015
What if BRAF+/ MSI+?

Checkmate 142
Nivolumab 3 mg/kg Q2W

Checkmate 142
Ipilimumab + nivolumab

<table>
<thead>
<tr>
<th>Nivolumab + ipilimumab (N = 119)$^a$</th>
<th>n</th>
<th>ORR</th>
<th>DCR$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>BRAF/KRAS mutation status, n (%)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wild type</td>
<td>31</td>
<td>17 (55)</td>
<td>24 (77)</td>
</tr>
<tr>
<td><strong>BRAF mutant</strong></td>
<td>29</td>
<td>16 (55)</td>
<td>23 (79)</td>
</tr>
<tr>
<td>KRAS mutant</td>
<td>44</td>
<td>25 (57)</td>
<td>37 (84)</td>
</tr>
</tbody>
</table>

Responses were observed irrespective of tumor PD-L1 expression, BRAF or KRAS mutational status or clinical history of Lynch.

Take Home Messages

• Inmune-Oncology is not contradictory with Precision Medicine
  – MSI and TMBare universal biomarkers
    • Differential TMB amongst specific MMR genes
    • PD-L1 valid in Gastric and G-E adenocarcinoma
  – more biomarkers urgently needed

• cfDNA levels are prognostic in mCRC, yet weak
  – Prospective validation studies
  – Predefined cut-off value based on normal level in healthy people.

• We have a potential second line standard for BRAF+ mCRC
  – Unfortunately we do not have a 1st Line specific standard
  – BRAF+ MSI+ have now 2 valid alternatives in second line
Acknowledgements

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Cristina Santos
Elena Élez

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