CLINICAL IMPLICATIONS OF THE PRESENCE OF CTCs POSITIVE FOR VEGFR EXPRESSION IN PATIENTS WITH ADVANCED COLORECTAL CÁNCER UNDERGOING TREATMENT FOR FOLFOX+BEVACIZUMAB.

Mª José Serrano, PhD

Division of Oncological Research.
“Biodynamic of Circulating Tumour Cells, Microenvironment and Metastases Group”
Colorectal cancer (CRC) is one of the most common forms of cancer, in regard to both incidence and mortality. In the Western world, CRC is the second most common malignancy diagnosed in women, after breast cancer, and the third most common in men, after prostate and lung cancer, accounting for 13.1% and 12.8% of all forms of cancer, respectively.

For the time being, surgical resection of the tumor remains the prominent choice for treatment followed by adjuvant chemotherapy.
Metastasis process

RATIONALE

VEGF-VEGFR

• Proliferation
• Metastases

Pattern of expression of VEGFR in CTCs
Material and Methods

● A Prospective study.

● 50 patients enrolled with metastatic colon cancer.

● 10 ml of whole blood were drawn from colon cancer patients into the CellSave Preservative Tube containing a cellular preservative and processed within 72 hours.

● MACs Miltenyi system was then utilized to separate the CTCs by treatment with iron particles coated with antibodies against cytokeratine for capturing CTCs.

● Multi-Cytokeratin antibody (AE1/AE3) was used to detect CTCs by immunohistochemistry method.
<table>
<thead>
<tr>
<th>Age</th>
<th>N(%) Basal</th>
<th>N(%) week 6</th>
<th>N(%) week 12</th>
<th>N(%) week 24</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CTC-</td>
<td>CTC+</td>
<td>CTC-</td>
<td>CTC+</td>
</tr>
<tr>
<td>&lt;50</td>
<td>6 (46.2)</td>
<td>7 (53.8)</td>
<td>4 (40)</td>
<td>6 (60)</td>
</tr>
<tr>
<td>&gt;50</td>
<td>20 (54.1)</td>
<td>17 (45.9)</td>
<td>14 (50)</td>
<td>14 (50)</td>
</tr>
<tr>
<td>Sex</td>
<td>Man</td>
<td>17 (53.1)</td>
<td>15 (46.9)</td>
<td>12 (48)</td>
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<tr>
<td></td>
<td>Woman</td>
<td>8 (44.4)</td>
<td>10 (55.6)</td>
<td>7 (53.8)</td>
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<tr>
<td>Menopausal state</td>
<td>Pre</td>
<td>1 (25)</td>
<td>3 (75)</td>
<td>1 (33.3)</td>
</tr>
<tr>
<td></td>
<td>Post</td>
<td>7 (50)</td>
<td>7 (50)</td>
<td>6 (60)</td>
</tr>
<tr>
<td>Tumor location</td>
<td>Rectum</td>
<td>22 (54.5)</td>
<td>10 (45.5)</td>
<td>9 (52.9)</td>
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<tr>
<td></td>
<td>Left colon</td>
<td>5 (31.25)</td>
<td>11 (68.75)</td>
<td>5 (38.5)</td>
</tr>
<tr>
<td></td>
<td>Transverse colon</td>
<td>3 (75)</td>
<td>1 (25)</td>
<td>3 (75)</td>
</tr>
<tr>
<td></td>
<td>Right colon</td>
<td>6 (75)</td>
<td>2 (25)</td>
<td>2 (50)</td>
</tr>
<tr>
<td>KRAS</td>
<td>Wild type</td>
<td>10 (50)</td>
<td>10 (50)</td>
<td>9 (52.9)</td>
</tr>
<tr>
<td></td>
<td>Mutated</td>
<td>8 (47.1)</td>
<td>9 (52.9)</td>
<td>6 (40)</td>
</tr>
<tr>
<td></td>
<td>unknown</td>
<td>8 (61.5)</td>
<td>5 (38.5)</td>
<td>4 (66.7)</td>
</tr>
<tr>
<td>Metástasis location</td>
<td>1 organ</td>
<td>18 (60)</td>
<td>12 (40)</td>
<td>13 (54.2)</td>
</tr>
<tr>
<td></td>
<td>&gt; 1 organ</td>
<td>8 (40)</td>
<td>12 (60)</td>
<td>6 (42.9)</td>
</tr>
</tbody>
</table>

1. CTC detection and their correlation with clinical-pathological characteristics

2. Expression of VEGF Receptor in CTCs and their relation with response to Bevacizumab.
STUDY METHOD

IF POSITIVE RESPONSE
BLOOD DRAWN 24 WEEKS AFTER
AFTER INITIAL TREATMENT
:FOLFOX+BEVAZUCIMAB

BLOOD DRAWN AT BASELINE

BLOOD DRAWN 6 WEEKS

BLOOD DRAWN 12 WEEKS

ASSESSMENT OF TREATMENT RESPONSE
USING RECIST CRITERIA

IFNEGATIVE RESPONSE
Patient is excluded from this study
**ISOLATION AND DETECTION METHOD**

**STEP 1:** Preparation of samples for isolation

**STEP 2:** Isolation of CTCs

**STEP 3:** Detection and Characterization of CTCs

- CK Negative cells
- CK positive cells

- Phenotype characterization
- Genetic characterization

- Immunohistochemistry

- Cytospin
RESULTS

HUVE cell line. Positive Control for expression of VEGFR.

CTCs positive sample for VEGFR and CK expression from a MCC patients

VEGFR

LEUCO

HT29

HCT116

CK
Immunocytochemistry for CK expression was performed on 50 samples and 27 (54%) of cases were positive for CK.

RESULTS

<table>
<thead>
<tr>
<th>% of patients positive for CTCs at baseline</th>
</tr>
</thead>
<tbody>
<tr>
<td>negative samples 46%</td>
</tr>
<tr>
<td>positive samples 54%</td>
</tr>
</tbody>
</table>

AVERAGE NUMBER OF CTCs DETECTED DURING FOLLOW-UP

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>6 weeks</th>
<th>12 weeks</th>
<th>24 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>CTC ratio</td>
<td>1-100</td>
<td>1-40</td>
<td>1-40</td>
<td>1-15</td>
</tr>
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</table>
No positive correlation was found between CTCs and clinical-pathological characteristics.
RESULTS

- PREVALENCE OF CTCs:

EVALUATION OF THE PRESENCE OR ABSENCE OF CTCs AT 6 AND 12 WEEKS DURING FOLLOW-UP

![Graph showing the prevalence of CTCs at different follow-up points (BASAL, 6 WEEKS, 12 WEEKS) with bars for patients positive and negative for CTCs.]

Patients Positive for CTCs

Patients Negative for CTCs
RESULTS

- After treatment the prevalence of CTCs was shown in the 60% of patients:

![Pie chart showing 51% negative, 40% increased, and 9% decreased CTCs.]

![Bar chart showing CTCs before and 6 weeks of treatment for patients P23, P20, P22, P31, P17, P6.]

- Patients CTCs number
- CTCs to 6 weeks of treatment
- CTCs before treatment
RESULTS

- Characterization of CTCs based on the VEGFR expression:
  - 30% of CTC were negative for VEGFR
  - 70% of CTC were positive for VEGFR

88% of patients with CTC\textsuperscript{VEGFR+} IMPROVED PFS with positive response

83.3% of patients with CTC\textsuperscript{VEGFR-} Developed a negative response and short PFS
Our findings indicate that VEGFR-2 can be expressed in CTCs.

We found that VEGFR-2 was expressed in most metastatic colon cancer CTCs.

Our study indicates the presence of heterogeneity in VEGFR distribution among the population of CTCs analyzed in the same sample, identifying two subpopulations: $\text{CTC}^{CK/VEGFR+}$ and $\text{CTC}^{CK/VEGFR-}$.

In this study, we observed a negative response to FOLFOX+Bevacizumab treatment in patients $\text{CTC}^{CK/VEGFR-}$ while the patients with $\text{CTC}^{CK/VEGFR+}$ responded favorably to Treatment with FOLFOX+Bevacizumab.
Biomarker Characterization of Circulating Tumour Cells in Colon Cancer Patients

- Biomarker characterization in CTCs may become a useful tool for selecting patients for tailored therapies and targeted drug development.
- The mere detection of CTCs is not sufficient to understand the biological properties of the cells. Specific biologic characterization of all types of CTCs will be necessary for eventual clinical application.
- Our study also suggests that more importance must be placed on CTC phenotypes when determining an appropriate treatment regimen instead of basing all treatment solely on primary tumor type.
GROUP OF BIODYNAMIC OF CIRCULATING TUMOR CELLS,
MICROENVIRONMENT AND METASTASIS
Division of
CIRCULATING TUMOR CELLS

Group Director & Principal Investigator:
Prof. JOSE A. LORENTE, M.D., Ph.D.

Division Scientific Director:
Dra Mª JOSE SERRANO, PhD

Division Clinical Director:
Prof. Dr. JOSE LUIS GARCIA-PUCHE, MD, PhD.

Scientific staff:
Gabriel Ortega-Sánchez (M.S.), María Jesús Álvarez-Cubero (Ph.D.), María Sáiz-Guinaldo (M.S.), Juan Carlos Alvarez (Ph.D.), Lucas González Herrera (M.D.)

Technical staff:
Carmen Feixas

Clinical Associates:
José Manuel Cozar/ Armando Zuluaga/ Juan Torres Melero/ Pedro Sánchez Rovira/ Juan de la Haba Rodriguez/ José Exposito Hernandez/ Enrique Aranda/ Javier Valdivia
¡MUCHAS GRACIAS!

THANK YOU SO MUCH!