TRANSARTERIAL CHEMOEMBOLIZATION ENHANCES PD-1 AND PDL-1 EXPRESSION IN HEPATOCELLULAR CARCINOMA

AHMED MONTASSER¹, ², AURÉLIE BEAUFRIÈRE¹, ³, FRANÇOIS CAUCHY⁴, OLIVIER SOUBRANE⁴, NATHALIE COLNOT¹, SANDRINE COUROBLE¹, MIGUEL ALBUQUERQUE¹, ³, VALÉRIE PARADIS¹, ³

¹ PATHOLOGY DEPARTMENT, BEAUJON UNIVERSITY HOSPITAL, AP-HP, CLICHY, FRANCE.
² PATHOLOGY DEPARTMENT, THEODOR BILHARZ RESEARCH INSTITUTE, GIZA, EGYPT.
³ INSERM U1149, BEAUJON UNIVERSITY HOSPITAL, CLICHY, FRANCE.
⁴ DEPARTMENT OF HPB AND PANCREATIC SURGERY, BEAUJON UNIVERSITY HOSPITAL, AP-HP, CLICHY, FRANCE.
HCC: Where are we standing?

- The most common primary liver cancer and is the 4th most common cause of cancer-related death worldwide.

- For early stage cancer, the potentially curative treatment modalities (ablation, resection and liver transplantation) are commonly followed by recurrence or development of new cancer from the « precancerous » liver.

- Over 70% of cases present at advanced stage and are subjected to Locoregional treatment (transarterial chemoembolization; TACE and radiofrequency ablation; RFA) and systemic therapy.

HCC: Where are we standing?

- Sorafenib is the only FDA approved multi-targeted KI for HCC treatment;
  - Cost
  - Intolerable
  - Response rate 2-3%
  - Overall survival < 3 m

- Urgent need for novel effective therapies to treat advanced stages HCCs and to prevent and treat recurrences after local treatment.

- Immune checkpoint inhibitors have emerged as a frontline treatment for multiple cancers, such as metastatic melanoma, NSCLC, RCCs, and urothelial cancer.

HCC: Where are we standing?

PD-1/PDL-1 interaction:
- Lymphocyte proliferation and function.
- Tumor cell apoptosis.
- Apoptosis of tumor-specific T cells.
- Conversion of effector T cells into Treg.

**Fig. 3.** Immune checkpoint blockade: anti-PD-1, anti-PD-L1, and anti-CTLA-4 antibody. Anti-PD-1, anti-PD-L1, and anti-CTLA-4 antibodies restore cytotoxic T cell activity, resulting in tumor attack by perforin and granzyme.
Aims

- Studying the effect of TACE on PD1/PDL-1 expression.
- Studying HCC features potentially associated with higher PD-1/PDL-1 expression.
Design

- Surgically treated HCCs, inclusion criteria
  - Pure HCC
  - >1cm
  - Necrosis ≤ 80%
  - Naïve/TACE

- Morphological examination

- IHC:
  - CD3
  - CD8
  - PD-1
  - PDL-1

  - Digitalized, obtained CD8/CD3 ratio
  - Scored as %, 2 reviewers [AM & VP]

- TMA (constructed from 2 blocks):
  - MSI

- Picrosirius
  - Digitalized, obtained Tumor/Stroma ratio
Design

73 Cases

50 Surgery

23 TACE followed by surgery

11 biopsied pre-TACE

12 with no prior biopsy
Results:

With vs. Without preoperative TACE

<table>
<thead>
<tr>
<th></th>
<th>PD-1 ICs</th>
<th>PDL-1 ICs</th>
<th>PDL-1 TCs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preop. TACE</td>
<td>9,7</td>
<td>6,7</td>
<td>2</td>
</tr>
<tr>
<td>No preop. TACE</td>
<td>6,5</td>
<td>6,3</td>
<td>0,36</td>
</tr>
</tbody>
</table>

P<0.001
Results: Pre-TACE vs Post-TACE

Pre-TACE (biopsy) vs...
Results: Pre-TACE vs Post-TACE

... Post-TACE (surgical specimen)
Results: Pre-TACE vs Post-TACE

**PD-1 expression**

Surgical Specimen

Biopsy
Results: Pre-TACE vs Post-TACE
Results: Differentiation

PDL-1 expression by TCs

Well and moderately differentiated HCCs are less likely to express PDL-1 on TCs!
## Results: Macrotrabecular Pattern

<table>
<thead>
<tr>
<th></th>
<th>Sample (N=73)</th>
<th>Positive (N=10)</th>
<th>Negative (N=63)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Macrotrabecular</td>
<td>4 (40%)</td>
<td>7 (11%)</td>
<td></td>
<td>0.019</td>
</tr>
</tbody>
</table>

**PDL-1 expression by TCs**

![Image of tissue sample with bar chart showing PDL-1 expression by TCs]
## Results: Stromal Density

<table>
<thead>
<tr>
<th></th>
<th>Sample (N=73)</th>
<th>Positive (N=31)</th>
<th>Negative (N=42)</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PD-1 expression</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stroma mean (%)</td>
<td>2.8</td>
<td>3.6</td>
<td>(&lt;0.001)</td>
<td></td>
</tr>
<tr>
<td><strong>PDL-1 expression by ICs</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stroma mean (%)</td>
<td>2.8</td>
<td>3.6</td>
<td>(&lt;0.001)</td>
<td></td>
</tr>
<tr>
<td><strong>PDL-1 expression by TCs</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stroma mean (%)</td>
<td>2.8</td>
<td>3.6</td>
<td>0.002</td>
<td></td>
</tr>
</tbody>
</table>

HCCs with high Tumor/stroma ratio are more likely to express PD-1 by inflammatory cells and PDL-1 by inflammatory and tumor cells!
## Results: Vascular invasion

<table>
<thead>
<tr>
<th>Sample (N=73)</th>
<th>Positive (N=10)</th>
<th>Negative (N=63)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>No emboli</td>
<td>2 (20%)</td>
<td>35 (56%)</td>
<td>0.038</td>
</tr>
<tr>
<td>Tumor emboli</td>
<td>8 (80%)</td>
<td>28 (44%)</td>
<td></td>
</tr>
</tbody>
</table>

HCCs featuring vascular tumor emboli (whether micro- or macro-vascular) show a higher potential to express PDL-1 on TCs!
Conclusions

- **TACE alters the tumor’s immune** profile and anti-PD-1/PDL-1 therapies may be considered as lines of treatment in HCC,
  - 25% of HCCs express markers of inflammatory response (Sia D et al, Hepatogastroenterol, 2017)

- HCC is an aggressive tumor with dismal prognosis, and altering the tolerogenic tumor microenvironment may serve as an effective line of therapy.

- **Morphologic features associated with higher potential of PDL-1 expression include:**
  - Differentiation and pattern of tumor growth;
  - Stromal density
  - Vascular invasion
Recommendations:

- A consensus is needed for the validation of PDL-1 interpretation in small HCC biopsies.

- Validation by independent cohort studies is recommended.

- Ongoing clinical trials (Nivolumab + TACE):
  - NCT03143270, USA
  - NCT03572582, Germany
THANK YOU!

Beaujon Hospital Team lead by Pr. Dr. Valérie PARADIS

Pr. Dr. Elia ANIS