Evaluation of the Prognostic and Predictive Significance of Hepatocellular Carcinoma Circulating Tumor Cells Expressing Programmed Death-Ligand 1 (PD-L1)

Pin Jun Chen, Paul Winograd, Shuang Hou, Colin Court, Saeed Sadeghi, Richard Finn, Yazhen Zhu, Fady Kaldas, Ronald Busuttil, James Tomlinson, Hsian-Rong Tseng, Vatche G. Agopian
Nothing to Disclose
Hepatocellular Carcinoma: Epidemiology

- HCC is the 6th most common cancer and 3rd leading cause of cancer death worldwide.

- HCC incidence and mortality continues to rise in the United States.

- Majority of patients present with surgically unresectable, incurable disease.

Hepatocellular Carcinoma: Systemic Treatment

**Sorafenib**
- Median OS 10.7
- ΔOS 2.8 months

**Regorafenib**
- Median OS 10.6
- ΔOS 2.8 months

**Lenvatinib**
- Median OS 13.6
- ΔOS 1.3 months

Kudo M et al., Lancet 2018
Immunotherapy: PD-1/PD-L1 Inhibitors

PD-L1/PD-1 binding inhibits T cell killing of tumor cell

Blocking PD-L1 or PD-1 allows T cell killing of tumor cell

Immunotherapy in HCC

**Nivolumab: CheckMate 040**

FDA approval as 2nd line treatment for HCC patients progressing on sorafenib

- Objective response rate: ~20%
- Durable response observed (median 9.9 mo)

**Pembrolizumab: KEYNOTE-224**

<table>
<thead>
<tr>
<th>Objective response*</th>
<th>All treated participants (n=104)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td><strong>18 (17%; 11-26)</strong></td>
</tr>
</tbody>
</table>

- Best overall response†
  - Complete response: 1 (1%)
  - Partial response: 17 (16%)
  - Stable disease: 46 (44%)
  - Progressive disease: 34 (33%)
  - Not assessable‡: 6 (5%)

- Disease control§: 64 (62%; 52-71)
- Median time to response, months (IQR)¶: 2.1 (2.1-4.1)

- Median duration of response, months (range)¶¶: Not reached (3.1-14.6+)**
- Duration of response ≥ 9 months¶¶: 12 (77%)

†Zhu A et al., Lancet Oncology 2018
Biomarkers for Immunotherapy Treatment Response

Nivolumab: CheckMate 040

- Tumor PD-L1 status by IHC
- CheckMate 040 trial: no association between treatment response and PD-L1 status

Pembrolizumab: KEYNOTE-224

- Tumor Proportion Score (43% vs 22% ORR; not significant)
- Tumor/Immune Combined Score (32% vs 20% ORR, p=0.021)

Zhu A et al., Lancet Oncology 2018
“Liquid Biopsy” as a Biomarker

- Limited Data on HCC CTCs as a Biomarker
- No study to date has reported on PD-L1+ HCC CTCs
Specific Aims

1. Identify and enumerate HCC CTCs, and evaluate feasibility of phenotyping CTCs expressing PD-L1

2. To evaluate the potential of PD-L1+ CTCs to serve as a prognostic biomarker in discriminating early/advanced stage diseases and survival

3. Assess ability of PD-L1+ CTCs as a predictive biomarker in a subset of patients undergoing anti-PD-1 therapy
Our HCC CTC Approach: NanoVelcro

Blood Draw & Shipment (<72 h)
Depletion of RBCs And PBMC Banking (1.5 hr)
CTCs Capture in NanoVelcro Chips (1 hr)
Immunostaining 1st Ab (Overnight) 2nd Ab (45 min)
Microscopy Imaging + Image Analysis / Archiving (10 min)
NanoVelcro: HCC CTC Definitions

Epithelial CK+ CTC: DAPI+/CK+/PD-L1-/CD45-

PD-L1+ CTC: DAPI+/CK+/PD-L1+/CD45-
Study Design

Total Patients (n = 109)
- Excluded Patients (n = 2)
- Enrolled Patients (n = 107)

Healthy Controls (n = 8)
- Cirrhosis (n = 7)
- Benign Liver Lesions (n = 5)
- HCC (n = 87)
- Non-Malignant Liver Disease (n = 12)

Within Transplant Criteria
- NMLD and Healthy Controls (n = 20)
- Early stage * (n = 49)

Outside Transplant Criteria
- Locally Advanced * (n = 22)
- Metastatic (n = 16)

CTC enumeration

*UCSF criteria: 1 lesion ≤ 6.5 cm or 2-3 lesions ≤ 4.5 cm and a total tumor diameter ≤ 8 cm, no vascular involvement

Median follow up: 19 months
HCC CK+ CTCs are an Accurate Diagnostic Marker

Unpublished data

Sensitivity 92%
Specificity 85%
PD-L1+ CTCs are a Prognostic Biomarker

A ROC curve showing AUROC of 0.81 with p < 0.0001. Sensitivity 71% and Specificity 92%.
Presence of PD-L1+ CTCs and Overall Survival

Unpublished data

P < 0.0001

Median 14.0 mo
## PD-L1+ CTCs: Multivariate Cox Survival Analysis

<table>
<thead>
<tr>
<th>Variable</th>
<th>Multivariate</th>
</tr>
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<tbody>
<tr>
<td>Laboratory MELD (per unit)</td>
<td>1.1 (1.1-1.2)</td>
</tr>
<tr>
<td></td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>AFP (per log unit)</td>
<td>1.6 (1.2-2.0)</td>
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<td></td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Radiologic Tumor Burden &gt; UCSF</td>
<td>7.2 (3.0-17)</td>
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<td>CTC Characteristics</td>
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# PD-L1+ CTCs: Multivariate Cox Survival Analysis

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<tr>
<th>Variable</th>
<th>HR (95% CI)</th>
<th>P-value</th>
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<tr>
<td>PD-L1+ CTC ≤ 1/2mL</td>
<td>1.0</td>
<td>ref</td>
</tr>
<tr>
<td>PD-L1+ CTC &gt; 1/2mL</td>
<td>3.2 (1.3-7.8)</td>
<td>&lt;0.01</td>
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PD-L1+ CTCs and Response to Immunotherapy

13th ILCA Annual Conference
20–22 September 2019 | Chicago, USA
Summary

• First study to characterize PD-L1+ CTC phenotyping in HCC

• PD-L1+ CTCs are **prognostic:**
  – Discriminate early stage/curable and advanced stage/incurable HCC
  – Independently portend poor survival after controlling for MELD, AFP, and tumor stage

• PD-L1+ CTCs are **potentially predictive:**
  – Associated with treatment response to anti-PD1 treatment
Thank You

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