Efficacy of anti-PD1/PDL1 antibody monotherapy in patients with advanced non-small cell lung cancer with increased hepcidin expression

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Background
• Hepcidin (Hep), a key regulator of iron metabolism, is overexpressed in kinds of cancer cells. While malignant cells require high amounts of iron for active proliferation, iron also facilitates the production of oxygen radicals, which may result in ferroptosis.
• T cell-promoted tumor ferroptosis, a form of iron-induced regulated cell death, is involved in the anti-tumor activities of immune checkpoint inhibitors (ICIs).
• From an analysis using the The Cancer Genome Atlas lung adenocarcinoma (TCGA-LUAD) and squamous cell carcinoma (TCGA-LUSC) database, Hep expression showed a positive correlation with the infiltration levels of immune cells and the expression of diverse immune cell marker sets.
• The Efficacy of ICIs on advanced non-small cell lung cancer (aNSCLC) with high iron metabolism is unknown.
• Aim of this study is to examine the efficacy of anti-PD1/PD-L1 monoclonal antibody (Ab) therapy on survival in patients with aNSCLC with increased hepcidin expression.

Methods
• A retrospective observational study in 108 histologically confirmed aNSCLC patients treated with nivolumab, pembrolizumab, or atezolizumab monotherapy as any lines from January 1, 2016, to August 24, 2018. Eligible subjects with residual formalin-fixed tumor samples for immunohistochemical stain (IHC) were selected.
• IHC for Hep was conducted with anti-Hepcidin-25 antibody ab07670 (Abcam). Tissue proportion scores (TPS; High: >= 50%, None and Moderate: 1-49%) of Hep were assessed by a pathologist with uninformmed clinical outcomes.
• The Kaplan-Meier method was used to estimate the survival time. The data cutoff for OS was May 5, 2023. Statistical analysis was conducted using GraphPad Prism 9.
• The Cancer Genome Atlas Lung Cancer data was analyzed to explore the expression profile of HAMP (gene encodes Hep) and its prognostic value with UCSC Xena browser (http://xena.ucsc.edu/).
• Human adenocarcinoma cell line (A549) was obtained from the Japanese Collection of Research Bioresources Cell Bank.

Result 1
• Characteristics of 45 eligible subjects are shown in Table 1; men: 39 (86.7%), the median age: 72 years (range, 36-83), with driver gene mutations: 2 (4.4%). Representative Hep IHC results were shown in Figure 1. No significant difference in Hep TPS between histological types or PD-L1 TPS (data not shown).

Table 1
<table>
<thead>
<tr>
<th>Table 1</th>
<th>Total</th>
<th>Hep High</th>
<th>Hep Moderate</th>
<th>Hep None/Mixed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of cases</td>
<td>45</td>
<td>36</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>Number of cases</td>
<td>WC (MET.2014)</td>
<td>36 (97.8%)</td>
<td>3 (8.3%)</td>
<td>6 (95.8%)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>69.5 (60.0-92.2)</td>
<td>68.4 (60.0-92.2)</td>
<td>78.1 (60.0-90.9)</td>
<td>76.3 (60.0-92.2)</td>
</tr>
<tr>
<td>Number of Anti-PD-L1 Ab lines</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>PD-L1 Ab Doses</td>
<td>Number of cases</td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Number of cases</td>
<td>0.5</td>
<td>0.25</td>
<td>0.125</td>
<td>0.032</td>
</tr>
<tr>
<td>Probability of Survival Hep (Hep)</td>
<td>0.5</td>
<td>0.3</td>
<td>0.4</td>
<td>0.6</td>
</tr>
<tr>
<td>Days</td>
<td>1000</td>
<td>2000</td>
<td>3000</td>
<td>2000</td>
</tr>
<tr>
<td>Median</td>
<td>29.7</td>
<td>24.5</td>
<td>24.5</td>
<td>24.5</td>
</tr>
<tr>
<td>OS (Events/Cases)</td>
<td>26/8</td>
<td>26/8</td>
<td>26/8</td>
<td>26/8</td>
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<tr>
<td>CI</td>
<td>26/8</td>
<td>26/8</td>
<td>26/8</td>
<td>26/8</td>
</tr>
</tbody>
</table>

Result 2
A) The median patient follow-up was 15.3 months (range, 26 days to 87.8 months). The median time to death was 69.6 (95% CI, not reached) and 15.3 months (95% CI, 0.94-29.7) for Hep High (TPS ≥ 50%) and None or Moderate (TPS < 50%), respectively (A). The hazard ratio and p-value for OS with Hep High versus None or Moderate were 0.55 (95% CI, 0.23 to 1.34) and p = 0.19.
B) High HAMP expression in primary and recurrent tumor is likely to have a favorable effect on survival in all stages, but not in advanced stages (stages IIB and IV).

Result 3
A) IFNγ-induced ROS production, a feature of ferroptosis, in A549 was enhanced by the addition of recombinant Hep. IFNγ-induced cytotoxicity was enhanced by Hep.
B) IFNγ-induced cytotoxicity was enhanced by Hep.

Discussion
• In our cohort, aNSCLC patients with increased Hep expression and anti-PD1/PD-L1 Ab monotherapy showed a longer survival time, but statistically insignificant probably due to a small sample size. Further study with a larger sample size is required to validate the effect of hepcidin on anti-PD1/PD-L1 monotherapy.
• Because iron is important for DNA synthesis, various types of cancer cells alter the expression of iron metabolism-related factors. Hep overexpression in cancer cells of different origins has already been reported. Hep induces the degradation of the main iron export protein, ferroportin, increasing iron supply in cancer cells. Accumulation of iron in cancer cells may contribute to ferroptosis caused by CD8+ T cells activated by cancer immunotherapy.
• As for limitations, this is a single-center study with a small number of subjects. Second, increased iron levels or other alterations in cancer cells caused by Hep overexpression were not evaluated with tissue samples and in vitro examinations. Third, the correlation of Hep expression with the infiltration levels of immune cells or the expression levels of diverse immune cell markers was not investigated.

References

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