Synergistic effect of c-Met inhibitor Savolitinib in combination with a VEGFR inhibitor Frquitinib in clear cell renal cell carcinoma xenograft models

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Abstract # 189

Introduction

- Renal cell carcinoma (RCC) is the most common type of kidney tumor in human, of which approximatively 85-95% is clear cell renal cell carcinoma (ccRCC).1
- c-Met/VEGFR targeted therapies brought significant advancement in the treatment of RCC, however, resistance still occurs in most cases following a transient period of clinical benefit.2,3 The hepatocyte growth factor (HGF) receptor c-Met activation appeared to be a novel mechanism of resistance to anti-VEGF therapies in ccRCC.4-6 Therefore, targeting both c-Met and VEGF pathways simultaneously may offer additional clinical benefit.

In this report, a survival study of c-Met+expressed in treatment-naive Chinese patients will be described. In addition, animal tumor-effect of the combination of Savolitinib (K草T11342) and Frquitinib (K草T113724) was evaluated in multiple ccRCC xenograft models.

Materials and Methods

- Human tumor samples: ccRCC archived tumor samples from 62 treatment-naive patients were obtained from a local hospital tumor bank fixed in formalin and embedded in paraffin.
- PDG-F signaling: development and anti-tumor efficacy study: Fresh tumor specimens from treatment-naive patients were collected during surgery. The tumor was subsequently imbedded into 10mm-thick frozen sections, and subsequently mouse-to-mouse passage were made in additional NOD-SCID in nude mice once the tumor size reached 500-600 mm³. After several passages in vivo, the PDG-F (PDGF) over-expression was evaluated to evaluate the anti-tumor efficacy. For the in vitro derived xenografts, tumors were implanted subcutaneously into female BALB/c nude mice. Tumor cells (4x10⁶) and 4T1 cells were subcutaneously implanted into nude mice for tumor anti-tumor evaluation.
- Synergistic activity of c-Met inhibitor in ccRCC xenografts: The anti-tumor efficacy of c-Met inhibitor was investigated in ccRCC xenografts models. The combinatorial c-Met inhibitor and tumor were treated with 10% recombinant buffer solution. Formalin, embedded in paraffin and sectioned at 4 μm. Sections were manually treated with rabbit monoclonal antibody and the DAB substrate.
- IHC staining: ccRCC patient samples: The IHC staining on ccRCC patient samples was performed using the Kitabkin anti-c-Met (Clone: 14C12) mouse monoclonal antibody (Abcam). The IHC staining was conducted at Wuxi Clinical Laboratory (Shanghai).
- Met-IHC scoring: wild type: The whole breast section were carefully examined. The staining intensity was categorized scored on a scale of 0, 1+, 2+, or 3+. The categorical score was determined as the intensity score with the highest percentage of tumor cells. Scores of 0, 1+, 2+, or 3+ had a percentage of wild type, 15%, 30%, 50%, or 75% respectively.

Results

- The expression of c-Met in ccRCC pulmonary samples of Chinese patients with ccRCC

<table>
<thead>
<tr>
<th>PDL-F</th>
<th>Met-IHC</th>
<th>Total IHC</th>
<th>Met-IHC Dual IHC</th>
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</thead>
<tbody>
<tr>
<td>11</td>
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<td>24</td>
<td>11</td>
<td>12</td>
<td>5</td>
</tr>
<tr>
<td>Overall</td>
<td>62</td>
<td>60</td>
<td>56</td>
</tr>
</tbody>
</table>

The expression of c-Met in ccRCC pulmonary samples of Chinese patients with ccRCC

Conclusions

The c-Met expression was frequently found in ccRCC patient (56%). Among them, 25% tumor showed IHC score 1+. The mean of IHC score in Grade 1+ expression was higher than that in ccRCC xenograft model high dose of IHC expression. Significantly increased anti-tumor effect was observed in all models when the two agents were used in combination. The combination treatment produced stronger inhibition than single treatment of tumor proliferation marker MET and angiogenesis marker CD31, compared to other combination of VEGFR1 and VEGFR2 alone. These results indicated that the observed synergistic effect might be attributed to the dual inhibition on tumor signaling and tumor microenvironment.

Summary

- c-Met expression was frequently detected in Chinese patients with ccRCC.
- Treatment with Savolitinib or Frquitinib alone did not result in significant tumor growth inhibition as single agent.
- The combination of Savolitinib and Frquitinib significantly improved anti-tumor effect.
- The combined treatment produced stronger inhibition than single treatment of tumor proliferation marker MET and angiogenesis marker CD31, compared to other combination of VEGFR1 and VEGFR2 alone.
- These results indicated that the observed synergistic effect might be attributed to the dual inhibition on tumor signaling and tumor microenvironment.

References