Costimulatory T-cell engagement by PRS-342, a GPC3/4-1BB bispecific molecule, leads to activation of T cells and tumor growth inhibition in a HCC humanized mouse model

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Background

GPC3 is a highly expressed tumor marker on various cancers, including hepatocellular carcinoma (HCC). A recent study showed that a GPC3/B7-H3 bispecific molecule induced tumor growth inhibition in human xenografts by activating GPC3-dependent T cells [1]. However, the optimal tumor antigen-dependent T cell activity has not yet been fully explored.

4-1BB (CD137) is a key costimulatory immunoreceptor and a highly promising therapeutic target in cancer. To overcome safety and efficacy limitations of current 4-1BB-targeting antibodies, we have developed a novel 4-1BB Anticalin®-Tumor-targeting mAb bispecific that actively engages T cells in a tumor-selective fashion. We have previously reported on the generation of a GPC3/4-1BB bispecific in an HCC xenograft model [2]. Here, we describe the precise dose dependencies for the PRS-342 molecule, a 4-1BB bispecific based on the Anticalin® technology. GPC3 is an oncprotein with high tumor selectivity and high expression in not only hepatocellular carcinoma but also in a variety of other tumors with high medical need.

Anticalin® technology is a 14-16 kDa protein derived from human IgA. We utilized phage display to generate an Anticalin® protein binding to 4-1BB with high affinity and specificity. The PRS-342 bispecific construct was generated by genetic fusing of the 4-1BB-specific Anticalin® protein to a humatized high-affinity GPC3-targeting monovalent antibody with an engineered 4-1BB backbone. PRS-342 has excellent drug-like properties and can be produced with high yields. PRS-342 was designed to be highly dependent on tumor binding, which is necessary for clustering of 4-1BB, to elicit 4-1BB costimulation and T-cell activation. This was confirmed using different in vivo T cell activation assays based on mixed culture of human T cells and GPC3-expressing tumor cells. These data further demonstrate the ability of the PRS-342 to bind to both targets simultaneously. PRS-342 was also evaluated for activity in a humanized HepC2 mouse xenograft model, with results supporting its differentiation from marketed agents based on relevant benchmark controls.

Concept: tumor-specific and tumor-localized costimulatory activation of T cells

PRS-342 induces 4-1BB costimulatory signaling in a murine HepC2 tumor cell line in the presence of GPC3-positive HepC2 xenograft cells with the same GPC3 levels. GPC3 expression in HepC2 cells is highly localized. PRS-342 can activate the direct tumor-presented signal in the absence of GPC3 positive tumor cells.

An analysis of the pharmacodynamic properties of PRS-342 was performed using in vitro cell killing against GPC3-positive and GPC3-negative tumor cell lines. The results show that PRS-342 binding to GPC3-positive tumor cells significantly enhances GPC3-dependent TGF expression, which is the signal required for T-cell activation.

PRS-342 leads to tumor growth inhibition in a humanized HCC xenograft model

- Immunocompromised mice (NOD) engrafted with GPC3-positive tumor cells (HepG2) were injected with human PBMC and treated weekly with PRS-342 at two dose levels. Control mice were treated with an anti-GPC3 antibody (IgG4 variant) in equimolar doses, alone or in combination with vehicle control.
- PRS-342 showed dose-dependent tumor growth inhibition (TGI) comparable to an anti-GPC3 antibody, indicating that TGF is dominated by GPC3 inhibition in this model.

Summary

- PRS-342 was designed to elicit 4-1BB costimulatory effects in a tumor-directed manner.
- PRS-342 is a 4-1BB/GPC3 bispecific therapeutic that can substantially enhance 4-1BB-targeting effectiveness.
- PRS-342 has excellent drug-like properties and can be produced with high yields.
- PRS-342 has a pharmacodynamic profile comparable to classical antibodies.
- 4-1BB costimulation by PRS-342 leads to:
  - NAF activation in a reporter cell assay.
  - Increased production of IL-2, a proinflammatory cytokine associated with antitumor immune response in a co-culture assay.
  - Decreased-dependent cytotoxicity in impedance based real-time killing assay.
  - T cell infiltration in tumors of a HCC xenograft in humanized mice.

- The preclinical studies reported here demonstrate potent T-cell activation that is strictly dependent on the presence of GPC3-positive tumor cells.
- GPC3-dependent activation of tumor-specific T cells is expected to result in an improved safety profile.
- Collectively, our in vitro and in vivo data support the continued development of PRS-342.

References

Table 1: Tumor-Associated CD8 T cell infiltration in humanized HepG2 xenografts

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Median tumor size (mm^3)</th>
<th>TILs in tumor (CD8^+ cells/mm^3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>2000</td>
<td>50</td>
</tr>
<tr>
<td>PRS-342</td>
<td>1000</td>
<td>80</td>
</tr>
<tr>
<td>Anti-GPC3</td>
<td>500</td>
<td>20</td>
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</tbody>
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Platelet counts for the in vivo study described (a) were carried out for HE (carrageenan) and the full tumor marker GPC3 and HepC2. Percentage of TIL per tumor area excluding necrotic areas were calculated for all groups. (b) Representative pictures for CD8 staining of tumor sections demonstrating significant increased tumor infiltration for PRS-342 tumors compared to all control (vehicle, anti-GPC3 antibody and vehicle alone).