Background

The pharmacology of PRS-343 is investigated by in vivo assays based on mixed culture of human PBMC and tumor cell lines. The assays are used to determine the cytokine profile of cells co-cultured with PRS-343-induced 4-1BB clustering. Using an in vitro model of 4-1BB-mediated apoptosis and activation. The result of PRS-343-mediated, systemic 4-1BB activation and concomitant toxicity is investigated in a cytokine release assay and in a mouse tumor model of human PBMC-induced xenograft-cells disease (xGvHD). PRS-343-mediated toxicity is studied in a GLP-compliant, repeated-toxicology study (xGvHD). The combined dataset provides an overview on the pharmacology, mode of action and safety profile of PRS-343.

PRS-343-costimulated T cells express IL-2, GM-CSF, IFNγ and TNFα.

- T cells were co-incubated with HER2-positive SKBR-3 and PRS-343.
- Supernatant concentrations were determined for a panel of cytokines.
- Cytokines prominently induced by PRS-343-costimulation were GM-CSF, IL-2, IFNγ and TNFα.
- These cytokines may serve as pharmacodynamic biomarkers in clinical studies.

PRS-343-mediated T cell costimulation requires supraphysiologic levels of HER2.

- The costimulatory T cell activation assay was performed for a series of tumor cell lines and primary cells covering a wide range of HER2 positivity.
- IL-6 production was positive and significant.
- Response specificity was confirmed by competition with an excess of trastuzumab.
- The cytokine release assay shows (relative) cytokine expression above HER2 levels corresponding to 14% of SKBR-3 (HER2+ 2) (k) to variable donor-dependent results in the intermediate range (1%–2%).
- Cytokine activity was observed in SKBR-3 and JTV-1 cell lines described as resistant to conventional HER2-targeted therapy (4).

PRS-343 is Well Tolerated in Repeat-Dose Cynomolgus Monkey Toxicology Study

- The safety of PRS-343 was investigated in a GLP-compliant cynomolgus monkey study. PRS-343 was given in weekly doses of 0.01, 0.1, 1, 10, 100 and 1000 mg/kg over 4 weeks as an intravenous injection (0.1 ml/kg) for 14 days.
- Delayed onset or reversibility of toxicity was studied in recovery groups (0 and 10 mg/kg) and in the study group for both doses, with no significant findings.
- TK analysis demonstrated full, dose-proportional exposure at both doses, with a terminal half-life of 5.6 days.

Table 1: Study Design

<table>
<thead>
<tr>
<th>Group</th>
<th>Description</th>
<th>Dose Level (mg/kg/week)</th>
<th>Number of Animals</th>
<th>Toxicity Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>Control</td>
<td>0</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Female</td>
<td>Control</td>
<td>0</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Male</td>
<td>Low</td>
<td>10</td>
<td>3</td>
<td>-</td>
</tr>
<tr>
<td>Female</td>
<td>Low</td>
<td>10</td>
<td>3</td>
<td>-</td>
</tr>
<tr>
<td>Male</td>
<td>High</td>
<td>120</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Female</td>
<td>High</td>
<td>120</td>
<td>3</td>
<td>2</td>
</tr>
</tbody>
</table>

Conclusion

PRS-343 leads to TGI and tumor-localized increase in hCD45(+) cells in tumor-humianized mice.

- Immuno-compromised mice engrafted with HER2-positive tumor cells (SK-OV-3) were injected with human PBMC and treated over 3 weeks with PRS-343 at four dose levels.
- Control molecules were anti-CD40, an anti-4-1BB benchmark antibody and trastuzumab with an IgG4 backbone (IgG4-anti-HER2).
- Tumor (HER2 positive) showed a dose-dependent increase in the frequency of circulating CD45(+) cells, with no significant effects in both control groups, with no significant findings.

PRS-343- mediated 4-1BB activation requires supraphysiologic HER2 levels.

- PRS-343 costimulation leads to increased production of multiple pro-inflammatory cytokines associated with activation of the innate immune system.
- The results were consistent with increased IL-6 activity in both control groups.
- A GLP-compliant cynomolgus monkey toxicology study demonstrates that the binding affinity profile of trastuzumab is retained in PRS-343 with regard to HER2 targeting.

The data reported support evaluation of PRS-343 in a Phase 1 study in patients with HER2-positive advanced or metastatic solid tumors.