Preclinical toxicology and pharmacology for the 4-1BB/HER2 bispecific PRS-343: A first-in-class costimulatory T cell engager

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Background

4-1BB (CD137) is a key costimulatory immunoreceptor and a highly promising therapeutic target in cancer. To overcome toxicity and efficacy limitations of current 4-1BB-targeting antibodies, we have developed PRS-343, a 4-1BB/HER2 bispecific based on Anticalin® technology. We have previously reported on the generation and characterization of PRS-343 with regard to preclinical proof-of-concept and basic drug-like properties (1). Here, we describe the preclinical dataset supporting initiation of a first-in-patient trial.

The pharmacology of PRS-343 is investigated by ex vivo assays based on mixed culture of human PBMC and tumor cell lines. The assays are used to determine the cytokine profile of T cells costimulated by PRS-343-induced 4-1BB clustering. Using a set of immortal cancer cell lines and primary cells spanning a range of HER2 surface copy numbers, we identify the threshold required to elicit a costimulatory response, and a lower threshold below which costimulation can be reliably excluded. The risk of PRS-343-mediated, systemic 4-1BB activation and concomitant toxicity is investigated in a cytokine release assay and in a mouse toxicology model of human PBMC-induced xenograft-vs-host disease (xGvHD). HER2-mediated toxicity is studied in a GLP-compliant, repeat-dose toxicology study in cynomolgus monkeys.

The combined dataset provides an overview on the pharmacology, mode of action and safety profile of PRS-343.

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

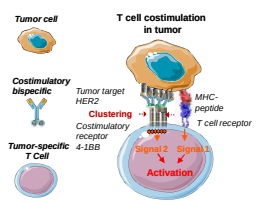
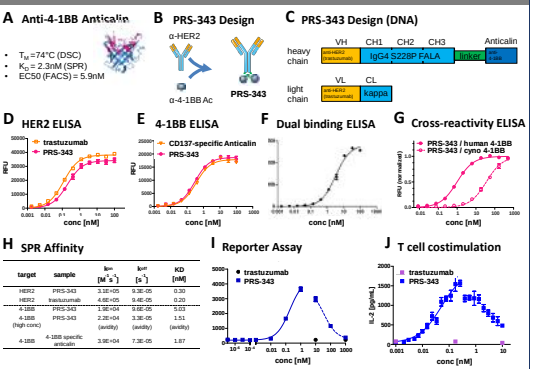


Figure 1. Concept of costimulatory T cell engagement by PRS-343: Within a patient´s tumor, tumor-specific T cells are bridged with tumor cells by the costimulatory bispecific PRS-343 which simultaneously binds the tumor target HER2 and the immune receptor 4-1BB. The resulting clustering of 4-1BB provides a local co-activatory signal to the T cell, further enhancing its T cell receptor (TCR)-mediated activity and leading to tumor destruction. Toxic side effects are expected to be manageable, as PRS-343 does not induce clustering and activation of 4-1BB in the absence of target-positive cells, and healthy tissue is spared by tumor-costimulated T cells due to the absence of a primary, TCR-mediated signal.

PRS-343 design, target binding and activity in reporter and T cell costimulation assay



H SPR Affinity

target	sample	k_{on} ($M^{-1}s^{-1}$)	k_{off} (s^{-1})	KD [nM]
HER2	PRS-343	3.1E+05	9.3E-05	0.30
HER2	trastuzumab	4.6E+05	9.4E-05	0.20
4-1BB	PRS-343	3.19E+04	9.6E-05	5.03
4-1BB (high conc)	PRS-343	2.2E+04 (avidity)	3.3E-05 (avidity)	1.51 (avidity)
4-1BB	4-1BB specific anticalin	3.9E+04	7.3E-05	1.87

Figure 2. PRS-343 Design, target binding and cell-based activity. (A) 4-1BB binding Anticalin. (B,C) Design. (D) HER2 ELISA shows similar potency of PRS-343 compared to trastuzumab. (E) 4-1BB ELISA shows similar potency of PRS-343 compared to 4-1BB-specific Anticalin. (F) Dual binding: 4-1BB/HER2 bispecifics are capable of binding both targets at the same time according to Sandwich ELISA. (G) Cross-reactivity: PRS-343 displays reduced cross-reactivity to 4-1BB from cynomolgus monkey. (H) On-rate, off-rate and KD of binding to targets HER2 and 4-1BB for PRS-343 and reference molecules trastuzumab and 4-1BB specific Anticalin. 4-1BB binding affinity was determined using different target coating concentrations minimizing or favoring avidity effects, respectively. (I) PRS-343 induces 4-1BB clustering and downstream signaling in a Jurkat Nf-kB reporter cell line in the presence of HER2-positive NCI-N87 cells with a potency of 50pM. (J) PRS-343 induces IL-2 production in a costimulatory T cell activation assay in the presence of HER2-positive SKBR-3 cells, with a potency of 35pM. In both types of cell-based assays, the response is bell-shaped as expected for ternary complex formation between PRS-343 and target cells (2).

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

- T cells were co-incubated with HER2^{high} NCI-N87 cells and PRS-343.
- Supernatant concentrations were determined for a panel of cytokines
- Cytokines prominently induced by PRS-343-mediated costimulation were GM-CSF, IL-2, IFNγ and TNFα
- These cytokines may serve as pharmacodynamic biomarkers in clinical studies

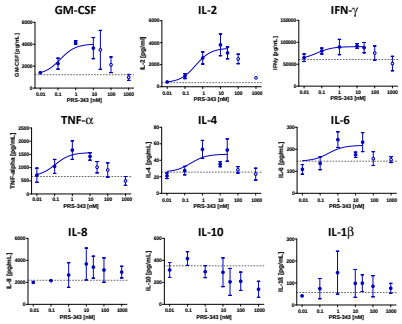


Figure 3. Cytokines induced by human T cells co-stimulated by PRS-343 in the presence of HER2-positive NCI-N87 cells in a T cell co-stimulation assay. Cytokine levels in the culture supernatants were measured by an electrochemiluminescence (ECL) immunoassay.

PRS-343 induced cytokine release in the absence of T cell receptor stimulation is negligible

- A cytokine release assay (3) was performed in the absence of T cell receptor (TCR) stimulation and presenting PRS-343 to PBMC in solution, wet-coated and air-dried
- PRS-343 shows negligible cytokine induction activity compared to the positive anti-CD3 control OKT3 independent of presentation strategy
- The data confirms that 4-1BB is a costimulatory receptor that requires a primary TCR signal; the risk of systemic cytokine release syndrome in clinical studies appears low

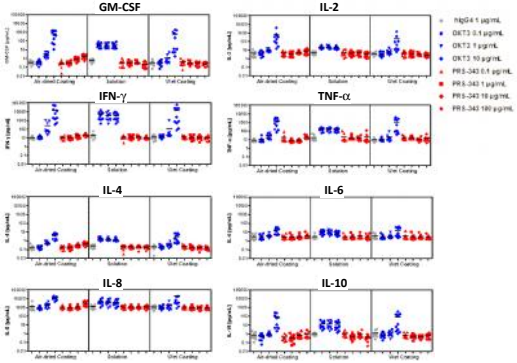


Figure 4. Cytokine release assay with PRS-343. PBMC were isolated from the blood of twelve healthy donors and incubated for 72 hours with PRS-343 either air dried, in soluble form, or wet coated. Four concentrations of PRS-343 in a volume of 50µl were tested in each setting as indicated in the figure. The anti-CD3 monoclonal antibody OKT3 at three different concentrations served as the positive control, and an IgG4 isotype antibody was the negative control. Supernatant levels of ten cytokines (IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12p70, GM-CSF, IFN-γ and TNF-α) were analyzed. The figure shows the average response for the ten donors that displayed a significant response to OKT3, and for a selection of the most relevant cytokines.

PRS-343-mediated T cell costimulation requires supraphysiological HER2 levels

- 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
- An anti-4-1BB benchmark mAb was used as a positive control
- Response specificity was controlled by competition with an excess of trastuzumab
- The series of experiments shows
  - (i) reliable costimulation above HER2 levels corresponding to 14% of SKBR-3 (HER2 2+)
  - (ii) no costimulation in the physiological HER2 expression range (<2% of SKBR-3)
  - (iii) variable donor-dependent results in the intermediate range (2%-11%)
- Costimulatory activity was observed in SUM225 and JIMT-1 cell lines described as resistant to conventional HER2-targeted therapy (4-6)

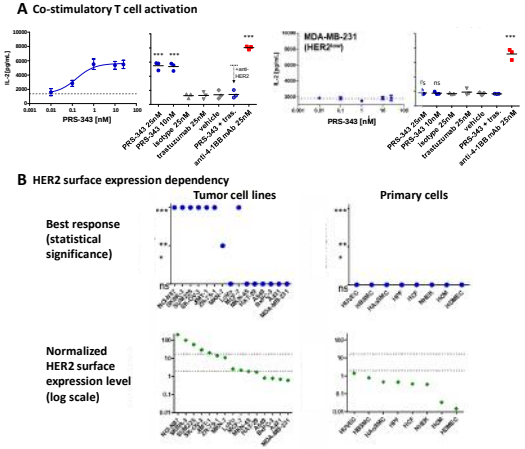


Figure 5. PRS-343 costimulation dependence on target cell HER2 level. Tumor or primary cells of different HER2 positivity were subjected to a T cell costimulatory activation assay using IL-2 supernatant levels as the readout. Experiments for each cell line were performed with at least two different donors. (A) Exemplar results. PRS-343 at various concentrations, target cells and healthy donor T cells were co-incubated in the presence of coated anti-CD3 antibody. Negative controls used were IgG4 isotype, trastuzumab or vehicle. Anti-4-1BB benchmark mAb was the positive control. The experiment was performed also in the presence of an excess of trastuzumab to inhibit the binding of PRS-343 to the SKBR3 cells. (B) Top: The best statistical significance obtained for any donor vs. control levels is reported for tumor cell lines and primary cells (p<0.001 (***), p<0.01 (**) or p<0.05 (*). Values of p>0.05 were considered not statistically significant (ns)). Bottom: relative cell surface HER2 levels (normalized against SKBR3 expression levels) are plotted for each tumor cell line and primary cell type on a logarithmic scale.

PRS-343 leads to TGI and tumor-localized increase in hCD45(+) cells in tumor in humanized 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 IgG4 isotype, an anti-4-1BB benchmark antibody and trastuzumab with an IgG4 backbone (Tras-IgG4)
- Tumor IHC staining for human CD45 shows a dose-dependent increase in the frequency of human TIL for PRS-343 vs controls, suggesting tumor-localized T cell activation
- PRS-343 showed dose-dependent tumor growth inhibition (TGI) comparable to Tras-IgG4, indicating that TGI is dominated by HER2 antagonism in this model

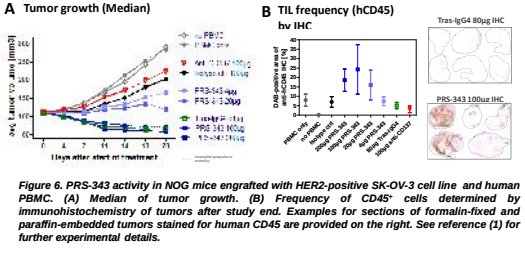


Figure 6. PRS-343 activity in NOG mice engrafted with HER2-positive SK-OV-3 cell line and human PBMC. (A) Median of tumor growth. (B) Frequency of CD45$^+$ cells determined by immunohistochemistry of tumors after study end. Examples for sections of formalin-fixed and paraffin-embedded tumors stained for human CD45 are provided on the right. See reference (1) for further experimental details.

Humanized Mouse Toxicology: PRS-343 avoids systemic 4-1BB activation in contrast to benchmark

- Immuno-compromised, tumor-free mice were injected with human PBMC and treated over 3 weeks with PRS-343 or controls (IgG4 isotype or anti-4-1BB benchmark mAb)
- PRS-343 showed unchanged dynamics of xenograft-versus-host disease compared to isotype control, while anti-4-1BB benchmark significantly accelerated mortality
- The results support a potentially improved safety profile of PRS-343 over benchmark by lack of systemic activation and concomitant toxicity

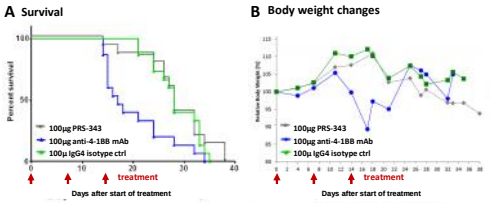


Figure 7. Immuno-compromised female NOG mice were engrafted with 7 × 10^6 fresh human PBMC, followed by weekly i.p. treatment with PRS-343, a 4-1BB benchmark agonist or isotype control at 100 µg/dose (i.p.) for 3 weeks. Mice (n=15 per group) remained on the study until spontaneous death or if ethical sacrifice was required. (A) Survival plot. (B) Relative median body weight of surviving animals.

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, 10 and 120mg/kg over 4 weeks as an intravenous infusion of 120 min duration (see Table 1 for study design)
- Delayed onset or reversibility of toxicity was studied in recovery groups (0 and 120 mg/kg)
- PRS-343 was well tolerated at both doses tested, with no significant findings
- TK analysis demonstrated full, dose-proportional exposure at both dose levels, with a terminal half-life of 5-6 days

Table 1. Study Design.

Group	Group Description	Dose Level (mg/kg/week)	Number of Animals: Toxicity Male	Toxicity Female	Recovery Male	Recovery Female
1	Control	0	3	3	2	2
2	Low	10	3	3	-	-
3	High	120	3	3	2	2

Conclusion

- **PRS-343 is a 4-1BB/HER2 bispecific based on the genetic fusion of a high-affinity 4-1BB-binding Anticalin and modified trastuzumab**
- **The presented preclinical pharmacology and toxicology studies confirm previous results (1) and support that PRS-343 elicits its costimulatory effects strictly on T cells also receiving a primary TCR signal and strictly localized to HER2-positive tumors:**
  - **PRS-343-mediated 4-1BB activation requires supraphysiological HER2 levels**
  - **PRS-343 costimulation leads to increased production of multiple pro-inflammatory cytokines associated with anti-tumor immune response**
  - **The risk of systemic 4-1BB activation is low based on negligible cytokine release in the absence of primary T cell receptor stimulation**
  - **This is supported by a humanized mouse toxicology study, where PRS-343 avoids the systemic peripheral activation of CD8$^+$ T cells observed with a benchmark 4-1BB antibody**
  - **A GLP-compliant cynomolgus monkey toxicology study demonstrates that the benign toxicity profile of trastuzumab is retained in PRS-343 with regard to HER2 targeting**
- **The reported data support evaluation of PRS-343 in a Phase 1 study in patients with HER2-positive advanced or metastatic solid tumors.**

References: (1) Cancer Immunol Res 2016;4(11 Suppl):Abstract nr B016. (2) J Am Chem Soc 2013; 135, 6092-6099. (3) J Immunol 2007; 179, 3325-3331. (4) Mol Cancer Ther 2010; 9, 1489-1502. (5) Oncogene 2007; 26, 7163-7169. (6) Mol Canc Ther 2004; 3, 1585-1592.