Costimulatory T-cell engagement by PRS-343, a 4-1BB (CD137)/HER2 bispecific, leads to tumor growth inhibition and TIL expansion in humanized mouse model

Marlon J. Hinner[1], Rachida-Siham Bel Aiba[1], Corinna Schlosser[1], Thomas Jaquin[1], Andrea Allersdorfer[1], Sven Berger[1], Alexander Wiedenmann[1], Gabriele Matschiner[1], Julia Schüler[2], Ulrich Moebius[1], Christine Rothe[1], Shane A. Olwill[1]

[1]Pieris Pharmaceuticals, Inc., 255 State Street, Boston, Massachusetts; [2]Oncotest GmbH, Am Flughafen 12, Freiburg, Germany.

Abstract

Background. 4-1BB (CD137) is a potent costimulatory immunoreceptor and a highly promising target for immunostimulatory cancer therapy. Conventional 4-1BB-targeting antibodies, however, suffer from a lack of tumor-selective activity, which may lead to peripheral toxicity and reduce the available therapeutic window. To develop a therapeutic that facilitates a 4-1BB-based activation of T cells that is both tumor-target driven and tumor localized, we have generated PRS-343, a 4-1BB/HER2 bispecific. PRS-343 was made by genetic fusion of a 4-1BB-binding Anticalin® to modified trastuzumab. We have shown previously that PRS-343 targets 4-1BB and HER2 in a bispecific manner and efficiently activates T cells ex vivo in the presence of HER2-positive cells. Here, we present novel in vivo proof of concept data and results obtained during manufacturing process development.

Results. We tested PRS-343 efficacy in a humanized mouse model in immuno-compromised mice and the SK-OV-3 cell line as a HER2-positive xenograft. The data indicate that PRS-343 displays dual activity based on monospecific HER2-targeting and bispecific, tumor-localized costimulation of 4-1BB. In contrast to PRS-343, an anti-4-1BB benchmark employed in the same study had no effect on tumor growth or tumor lymphocyte frequency, but displayed an increased toxicity due to accelerated graft-versus-host-disease (GvHD). The accelerated GvHD correlated with CD8$^+$ T cell expansion in the peripheral blood. The data therefore support the concept that tumor-localized costimulatory T cell activation by a bispecific such as PRS-343 may lead to higher efficacy and reduced systemic toxicity compared to conventional anti-4-1BB mAbs.

Regarding manufacturability of PRS-343, we have successfully developed a CMC process for PRS-343 using standard processes established for antibodies. This process provides excellent results, with titers above 4g/L, overall yields above 50% and a content of high molecular weight species below 0.3%.

Conclusion. The positive functional ex vivo and in vivo data of PRS-343 as well as the excellent developability profile support investigation of its anti-cancer activity in clinical trials. A first-in-patient study is planned to commence in the first half of 2017.

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

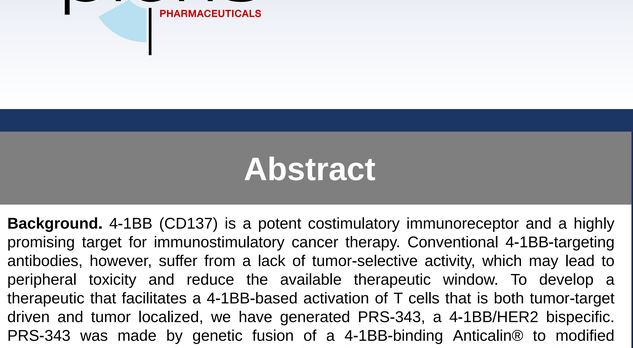
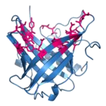


Figure 1. Concept of costimulatory T cell engagement by PRS-343: Within a patient´s tumor, tumor-specific T cells are bridged with tumor cells by the costimulatory bispecific PRS-343 which simultaneously binds the tumor target HER2 and the immune receptor 4-1BB. The resulting clustering of 4-1BB provides 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.

Building block: Anticalin targeting 4-1BB

Discovery
- Phage display of lipocalin library against 4-1BB, followed by affinity maturation

Binding to 4-1BB
- K_D = 2.3nM (SPR)
- EC50(FACS) = 5.9nM
- Non-competitive binding vs 4-1BBL



Biophysical properties
- 100% monomeric expression
- T_M =74°C (DSC)
- Fully stable after 1 week at 37°C in PBS pH7.4, human plasma or mouse plasma

Functional activity
- Ex vivo activation of T cells when coated; no activation when in solution

PRS-343 was selected from four 4-1BB/HER2 bispecifics based on functionality

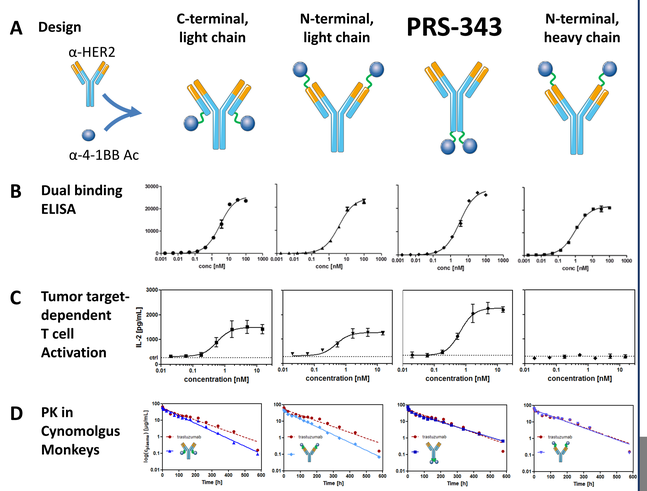
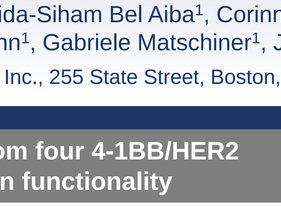
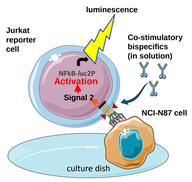


Figure 2. Design and selected functional data of four 4-1BB/HER2 bispecifics. (A) Design: genetic fusions of backbone-engineered trastuzumab to the anti-4-1BB Anticalin were generated to any of the four termini of the antibody. The IgG1 backbone of trastuzumab was exchanged for an engineered IgG4 backbone. (B) Dual binding: 4-1BB/HER2 bispecifics are capable of binding both targets at the same time according to Sandwich ELISA. (C) Ex vivo T cell activation: 4-1BB/HER2 bispecifics display different capabilities of eliciting IL-2 production by costimulatory engagement (see method description below). (D) Pharmacokinetics of PRS-343 compared to trastuzumab: male cynomolgus monkeys received test articles as an intravenous infusion at a dose of 3mg/kg. Drug levels were detected using Sandwich ELISA.

Brief methods ex vivo T cell activation.
SKBR3 tumor cells were grown overnight on 96-well plates that had been precoated with anti-CD3 antibody. The next day, T cells purified from healthy donor PBMC were added to the wells together with the titrated 4-1BB/HER2 bispecific molecules (constructs A-D). After three days in culture, IL-2 and IFN-g levels in the culture supernatants were measured by an electrochemoluminescence immunoassay.



PRS-343 activates 4-1BB pathway only in presence of HER2-expressing cells



- 4-1BB downstream signaling activation by the 4-1BB/HER2 bispecific drug candidate PRS-343 was investigated by an NF-κB-luciferase reporter assay
- PRS-343 strongly activated the 4-1BB pathway in the presence of HER2^{high} NCI-N87 target cells; no activation occurred in the absence of NCI-N87 tumor cells
- An anti-4-1BB benchmark mAb activated the 4-1BB pathway both in the absence and presence of tumor cells

Brief methods.
HER2^{high} expressing NCI-N87 tumor cells were grown overnight on 96-well plates. Jurkat NF-κB reporter cells, over-expressing 4-1BB and carrying a NF-κB-Luciferase reporter gene, were then added to the plates together with the titrated 4-1BB/HER2 bispecific construct PRS-343 (A) or anti-4-1BB benchmark mAb (B). After incubation, the T cell reporter signal was measured by luminescence. The impact of 4-1BB-targeting on 4-1BB downstream signaling was also investigated in the absence of tumor cells at a single concentration of 4-1BB agonists (10nM).

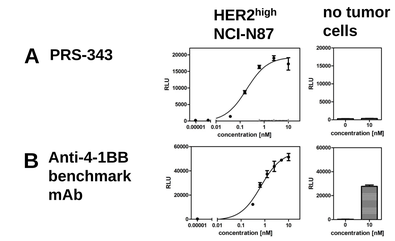


Figure 3. Activation of the 4-1BB signaling pathway in Jurkat T cells was measured by an NF-κB-Luciferase reporter assay. The luminescence signal was used as a relative measure of 4-1BB pathway activation. (A) PRS-343 drug candidate (solid line), negative control trastuzumab (light grey dashed line). (B) Anti-4-1BB benchmark mAb.

PRS-343 induces T cell activation when tumor HER2 levels are high, but not at physiological levels

- T cell activation assays were performed using the HER2^{high} SKBR3 and BT474 cell lines and cancer cell lines with HER2 expression levels that are similar to healthy tissue, HepG2 and MCF7
- IL-2 production was determined as a measure of T cell activation
- PRS-343 selectively leads to activation of T cells with SKBR3 and BT474 cells
- Anti-4-1BB benchmark mAb leads to T cell activation with all cell lines

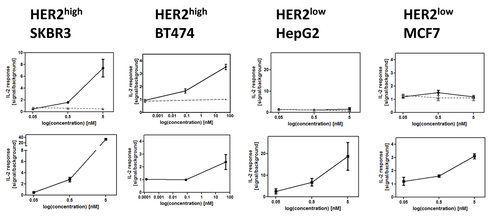


Figure 4. Relative IL-2 induction by 4-1BB-driven T-cell activation in the presence of highly HER2-positive cells (SKBR3, BT474) and cell lines expressing HER2 at a level similar to that of healthy, HER2^{low} cells (HepG2, MCF7). (A) 4-1BB/HER2 bispecific (solid lines) and negative control trastuzumab (dotted lines). (B) Anti-4-1BB benchmark mAb. The experiments were performed as described for Figure 2. The plotted relative IL-2 response corresponds to the ratio of the responses obtained in the presence and in the absence ("background") of test articles.

Activity in humanized mouse model: PRS-343 leads to TGI and increased hCD45-positive cells in tumor

- 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 different doses
- Control molecules were IgG4 isotype, an anti-4-1BB benchmark antibody and trastuzumab with an IgG4 backbone (Tras-IgG4)
- Tumor IHC staining for the human lymphocyte marker CD45 shows a dose-dependent increase in the frequency of human TIL for PRS-343, but not for controls, suggesting successful tumor-localized T cell activation by PRS-343
- PRS-343 showed dose-dependent tumor growth inhibition (TGI) comparable to Tras-IgG4, indicating that TGI is dominated by HER2 antagonism in this model

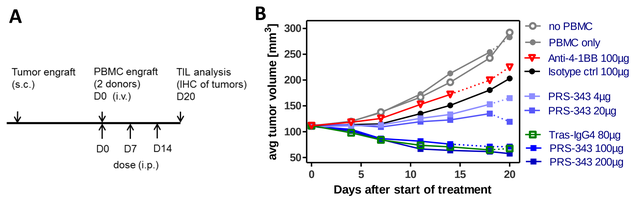


Figure 5. PRS-343 activity in NOG mice engrafted with HER-2 positive SK-OV-3 cell line and human PBMC. NOG mice were subcutaneously (s.c.) injected with SK-OV-3 cells and tumors were allowed to grow to an average of 120mm^3 prior to randomization into treatment groups (n=10). Mice were engrafted with fresh human PBMC intravenously (i.v.) into a tail vein and treatment commenced 1 hour later. Mice received 3 weekly intraperitoneal (i.p.) doses of treatment (4µg, 20µg, 100µg or 200µg) or controls. Tumor growth was recorded twice weekly. Tumors from up to six mice were harvested on day 20 post treatment (anti-4-1BB benchmark mAb: day 17) and assessed for infiltration of human T cells by immunohistochemistry. (A) Graphical overview. (B) Median of tumor growth. Data points that no longer represent the full group size of 10 mice are connected by dotted lines.

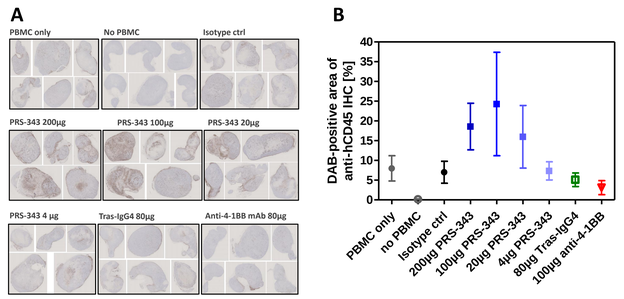


Figure 6. Immunohistochemistry of tumors after study end. (A) Sections of formalin-fixed and paraffin-embedded tumors (5 or 6 per group) were stained for human CD45. (B) The frequency of CD45$^+$ cells was quantified by dedicated software. The data shows that PRS-343 induces a dose-dependent increase in the frequency of human CD45$^+$ cells in the tumor compared to negative controls, while monospecific antibodies targeting HER2 (Tras-IgG4) or 4-1BB lack this activity.

Safety in humanized mouse model: PRS-343 avoids CD8$^+$ T_{eff} expansion in periphery unlike 4-1BB mAb

- Anti-4-1BB mAb treatment led to accelerated graft-versus-host disease with significantly increased mortality compared to control and PRS-343 groups on day 20
- PBMC phenotyping results indicate that increased mortality induced by benchmark anti-4-1BB mAb is caused by strongly increased expansion of CD8$^+$ human effector T cells in anti-4-1BB group compared to control or PRS-343 groups

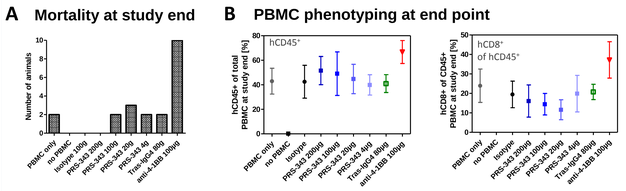


Figure 7. Mortality and PBMC phenotyping in humanized NOG mouse SK-OV-3 tumor model. (A) Mortality. Plotted values correspond to number of mice per group of ten that died spontaneously or needed to be sacrificed based on defined general condition criteria. (B) PBMC phenotyping. PBMC were isolated from mouse blood samples taken on day 20 after PBMC engraftment and analysed by multicolor FACS for human surface markers CD45, CD3 and CD8. PBMC from surviving animals of the group treated with anti-4-1BB benchmark mAb were phenotyped on day 17.

PRS-343 displays excellent developability and manufacturability with standard antibody process

- **High Stability**
  - Full activity retained after 1 week in human plasma (1 week at 37°C at 0.5mg/mL)
  - Full integrity in vivo over at least 22 days as shown during a cynomolgus monkey PK study
  - Fully stable and active after 4 weeks at 40°C in PBS (20mg/mL, aSEC and dual binding ELISA)
- **Good Manufacturability**
  - Successful manufacturing process developed for PRS-343 using standard processes established for antibodies
  - Expression titer: >4 g/L achieved in fed-batch reactor
  - Overall yield >50% over all process steps
  - Low content of high molecular weight species (<0.3%)

Summary

- **PRS-343 is a 4-1BB/HER2 bispecific based on the genetic fusion of a high-affinity 4-1BB-binding Anticalin and modified trastuzumab**
- **PRS-343 displays a differentiated profile when compared to a benchmark 4-1BB-targeting antibody**
  - **Reporter assay: PRS-343 leads to 4-1BB activation in presence of HER2-positive tumor cells, but not in their absence**
  - **Ex vivo: PRS-343 induces strong T cell activation via tumor target-dependent costimulatory T cell engagement**
  - **In vivo: PRS-343 displays dual activity based on monospecific HER2-targeting and bispecific, tumor-localized costimulation of 4-1BB, leading to increased density of hCD45$^+$ cells in the tumor**
  - **In vivo: PRS-343 avoids the systemic peripheral activation of CD8$^+$ T cells observed with benchmark 4-1BB antibody, which supports a better safety profile**
- **CMC process development for PRS-343 manufacturing has been successfully completed using standard processes established for antibodies**
- **The process provides excellent results, with titers above 4g/L, overall yields above 50% and a content of high molecular weight species below 0.3%. Optimization may further increase titer and yield.**
- **PRS-343 path to clinic: IND-enabling activities are ongoing with an anticipated first-in-patient study planned for the first half of 2017**