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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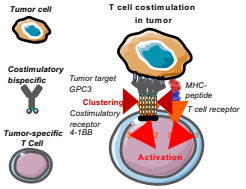
Background

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 4-1BB Anticalin®/tumor-targeting mAb bispecifics that activate T cells in a tumor localized fashion. We have previously reported on the generation and characterization of PRS-343, a clinical-stage 4-1BB/HER2 bispecific molecule, with regard to preclinical proof-of-concept and basic drug-like properties (1). Here, we describe the preclinical dataset for PRS-342, a 4-1BB/GPC3 bispecific based on the Anticalin® technology. GPC3 is an oncofetal protein with high tumor selectivity and high expression in not only hepatocellular carcinomas, but also in a variety of other tumors with high medical need.

Anticalin® therapeutics are 18 kD proteins derived from human lipocalins. 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 fusion of the 4-1BB-specific Anticalin® protein to a humanized high affinity GPC3-targeting monoclonal antibody with an engineered IgG4 backbone.

PRS-342 has excellent drug-like properties and can be produced with high yields. PRS-342 was designed to be strictly 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 vitro* T-cell costimulation assays based on mixed culture of human T cells and GPC3-expressing tumor cell lines. These data further demonstrate the ability of PRS-342 to bind both targets simultaneously. PRS-342 was also evaluated for activity in a humanized HepG2 mouse xenograft model, with results supporting its differentiated MoA compared to relevant benchmark controls.

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



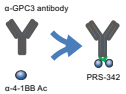
Concept of costimulatory T-cell engagement by PRS-342: Within a patient´s tumor, tumor-specific T cells are bridged with tumor cells by the costimulatory bispecific PRS-342 which simultaneously binds the tumor target GPC3 and the immune receptor 4-1BB. The resulting clustering of 4-1BB -provides a local coactivatory 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-342 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-342 design, target binding and activity in reporter and T-cell costimulation assay

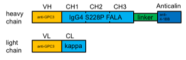
A Anti-4-1BB Anticalin Protein (Ac)

T$_M$=74°C (DSC)
K$_D$=02.3nM (SPR)

B PRS-342 Design

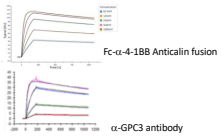


C PRS-342 Design (DNA)

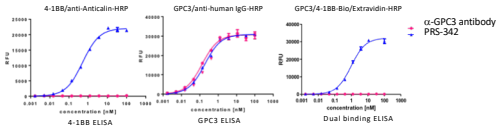


D SPR Affinity

	target name	kon [M-1*s-1]	koff [s-1]	KD [nM]
Fc-α-4-1BB Anticalin fusion	hu-4-1BB-His	3.5E+04	1.5E-04	4.1
α-GPC3 antibody	huGPC-3-His	3.6E+05	2.5E-04	0.7
PRS-342	huGPC-3-His	4.1E+05	2.7E-04	0.7



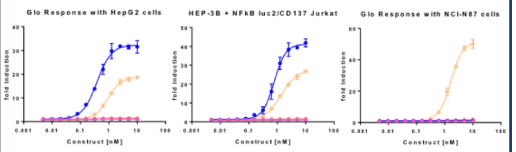
E Binding ELISA



PRS-342 Design (A, B, C) and target binding (D, E). (D) shows binding of the Fc-α-4-1BB Anticalin fusion with a KD of 4.1 nM. The GPC3 arm binds with 0.7 nM to GPC3. On and off-rate kinetic binding constant for α-GPC3 antibody and PRS-342 are similar. (E) ELISA data demonstrate PRS-342 binds GPC3 with comparable behavior to the α-GPC3 parental antibody. In a dual binding ELISA setting PRS-342 (4-1BB/GPC3 bispecific), is capable of binding both targets simultaneously.

PRS-342 reporter cell assay

- PRS-342 costimulated T cells in a Jurkat Nf-kB reporter cell assay only in the presence of GPC3-positive tumor cell lines.

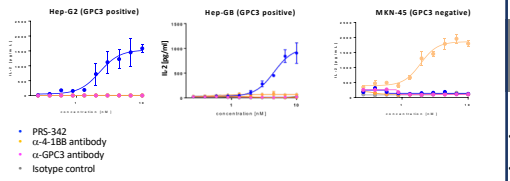


	EC50 [nM] Hep-G2	EC50 [nM] Hep-GB	EC50 [nM] NCI-N87
α-4-1BB antibody	0.94	1.39	1.77
α-GPC3 antibody	-		-
PRS-342	0.38	0.78	-

PRS-342 induces 4-1BB clustering and downstream signaling in a Jurkat Nf-kB reporter cell line in the presence of GPC3-positive HepG2 and Hep3B cells with low nM EC$_{50}$ values. GPC3-negative NCI –N87 cell are used as control. Only the 4-1BB antibody can activate the Jurkat Nf-kB reporter cell in the absence of GPC3 positive tumor cells.

PRS-342 induces 4-1BB engagement and T-cell activation in a GPC3 dependent manner

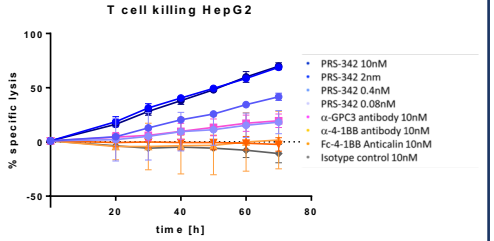
- Pan T cells were coincubated with GPC3^{high} Hep-G2, Hep-GB and MKN-45 cells and PRS-342.
- Supernatant concentrations were determined for IL-2.



IL-2 induced by human Pan T cells costimulated by PRS-342 in the presence of GPC3-positive HepG2 and Hep-GB cells in a coculture assay. No PRS-342 dependent activation was observed in presence of GPC3-negative MKN-45 cells. IL-2 levels in the culture supernatants were measured by an electrochemiluminescence (ECL) immunoassay.

PRS-342 leads to dose dependent T-cell mediated cytolysis of GPC3 expressing tumor cells

- PRS-342 induced 4-1BB costimulation results in a dose-dependent T-cell killing of GPC3 expressing tumor cells measured with an impedance based method.
- No increase of T-cell mediated killing was observed with equimolar doses of anti-GPC3 antibody, Fc-4-1BB Anticalin fusion, anti-4-1BB-antibody and isotype control.

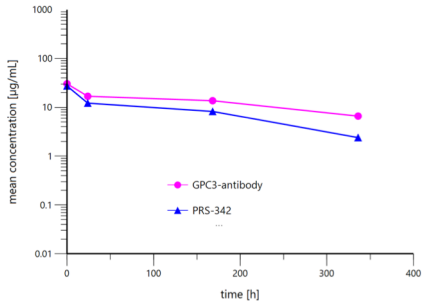


T-cell mediated cytolysis of GPC3 expressing HepG2 tumor cells was assessed using the xCELLigence RTCA HT system. Non-adherent CD8$^+$ T cells were cocultured with HepG2 cells in presence of test constructs. 4-1BB costimulation of cytotoxic T cells results in an increased cytolysis of HepG2 cells which is measured over time.

Pharmacokinetic profile of PRS-342 in mice

- Preliminary mouse PK was performed in male CD-1 mice to compare PRS-342 with an α-GPC3 antibody.
- PRS-342 has a typical antibody like PK profile in mice comparable to the α-GPC3 antibody used as building block in the bispecific PRS-342 construct.

PK in CD1 mice

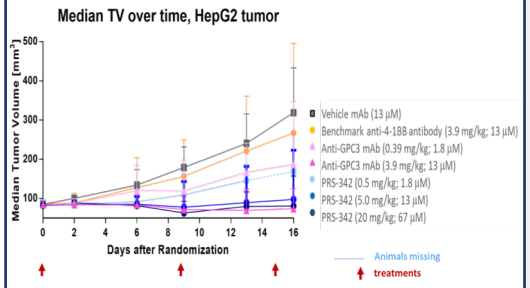


An analysis of the pharmacokinetic properties of PRS-342 as well as of an α-GPC3 antibody was performed in mice. Male CD-1 mice approximately 5 weeks of age (2 mice per timepoint) were injected into a tail vein with a dose 2 mg/kg. Plasma samples from the mice were obtained at the timepoints of 5 min, 24 h, 168 h, and 336 h. Plots of the plasma concentration over time for the anti-GPC3 antibody and PRS-342 are shown. Both the antibody and the bispecific-construct show typical antibody pharmacokinetics profiles.

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

- Immunocompromised mice (NOG) engrafted with GPC3-positive tumor cells (HepG2) were injected with human PBMC and treated weekly with PRS-342 at three dose levels.
- Control molecules were an α-GPC3 antibody (IgG4 variant) in equimolar doses, an α-4-1BB benchmark antibody in equimolar doses, and vehicle control.
- PRS-342 showed dose-dependent tumor growth inhibition (TGI) comparable to α-GPC3 antibody, indicating that TGI is dominated by GPC3 inhibition in this model.

A Median tumor growth inhibition in a humanized HepG2 xenograft model



(A) Immunocompromised female NOG mice carrying established HepG2 xenograft tumors were engrafted with 5 × 10^6 fresh human PBMC, followed by weekly i.p. treatment with PRS-342, α-4-1BB benchmark antibody, α-GPC3 antibody or isotype control at 0.5 mg up to 20 mg/kg doses (i.p.) (Charles River). Mice (n=15 per gr. remained on the study until spontaneous death or if ethical sacrifice was required, read out median tumor growth).

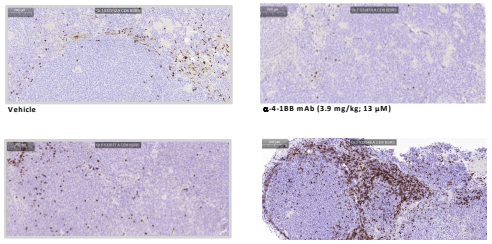
PRS-342 leads to tumor-localized increase of TILs in a humanized HCC xenograft model

- FFPE embedded xenograft tumor were analyzed histologically (HE) and immuno-histologically (IHC) for T-cell infiltration.
- Tumor IHC staining for human CD3, CD4 and CD8 shows a dose-dependent increase in the frequency of human tumor associated T cells (TILs) for PRS-342 vs controls, suggesting tumor-localized T-cell activation.

B % TIL frequency hCD3, hCD4 and hCD8 by IHC (necrotic areas are excluded)

Intratumoral T-cell infiltration	% TILs of total tumor area - necrotic area		
TILs	%CD3 T cells	%CD4 T cells	%CD8 T cells
Vehicle control	0.8	0.7	0.4
PRS-342 0.8 µM	6.2	4.7	4.5
PRS-342 13 µM	4.1	3.3	3.0
PRS-342 67 µM	10.7	4.9	7.3
α-GPC3 antibody 0.8 µM	1.6	1.3	0.7
α-GPC3 antibody 13 µM	0.8	0.6	0.5
α-4-1BB antibody 13 µM	0.8	0.7	0.2

C TIL frequency (hCD8$^+$) by IHC



(B) FFPE Xenograft tumors taken from the in vivo study described in (A) were stained for HE (not shown) and for the T-cell marker CD3, CD4 and CD8. Percentage of TILs per tumor area excluding the necrotic area were calculated for all groups (BioSiteHisto). (C) Representative pictures for CD8 staining of Hep-G2 tumors demonstrating significant increased TIL infiltration for PRS-342 tumors compared to all controls (vehicle, α-GPC3 antibody and α-4-1BB antibody).

Summary

- **PRS-342 was designed to elicit 4-1BB costimulatory effects in a tumor-localized manner.**
- **PRS-342 is a 4-1BB/GPC3 bispecific genetic fusion of a high-affinity 4-1BB-binding Anticalin® and a high affinity α-GPC3 antibody.**
- **PRS-342 has excellent drug like properties and can be produced with high yields.**
- **PRS-342 has a pharmacokinetic profile comparable to classical antibodies.**
- **T-cell costimulation by PRS-342 leads to:**
  - **Nf-kB activation in a reporter cell assay.**
  - **Increased production of IL-2, a pro-inflammatory cytokine associated with anti-tumor immune response in a co-culture assay.**
  - **Dose dependent cytolysis in impedance based real time killing assay.**
  - **TIL 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: (1) Cancer Immunol Res 2016;4(11 Suppl):Abstract nr B016.