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Simultaneous costimulatory T-cell engagement and checkpoint inhibition by PRS-344/S095012, a 4-1BB/PD-L1 bispecific compound for tumor-localized activation of the immune system

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INTRODUCTION

- **4-1BB** (CD137) is a **key co-stimulatory immunoreceptor** and a promising oncology target.
- Peripheral immune activation by 4-1BB agonistic antibodies has been associated with on-target toxicity and a limited therapeutic window.
- To overcome 1st generation 4-1BB agonist safety and efficacy drawbacks, we have generated **PRS-344/S095012, a 4-1BB/PD-L1 bispecific Anticalin® protein/mAb fusion protein** (Figure 1) designed to have a 4-1BB localized activity, while also offering the benefit of checkpoint inhibition (Figure 2).
- Here we describe the **preclinical *in vitro* and *in vivo* activity of PRS-344/S095012.**

This program is part of the strategic alliance between Pieris and Servier.

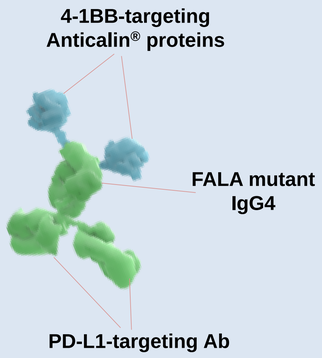


Figure 1. Structure of PRS-344/S095012

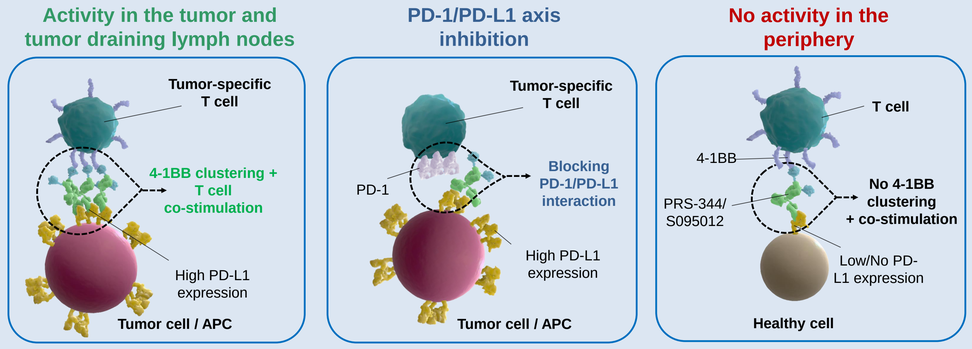


Figure 2. PRS-344/S095012 dual MoA: selective activation of 4-1BB$^+$ T cells in PD-L1$^+$ tumor and/or antigen-presenting cells in the tumor microenvironment or tumor-draining lymph node (dLN) and blocking of the PD-1 / PD-L1 interaction. No clustering of 4-1BB is expected in the periphery.

PRS-344/S095012 is capable of dual target engagement

- **PRS-344/S095012 binds to 4-1BB and PD-L1 in a comparable way to the respective single building blocks and can bind both targets simultaneously.**
- **PRS-344/S095012 effectively blocks the PD-1/PD-L1 binding and shares an overlapping 4-1BB-binding epitope with a clinically active anti-4-1BB benchmark mAb.**

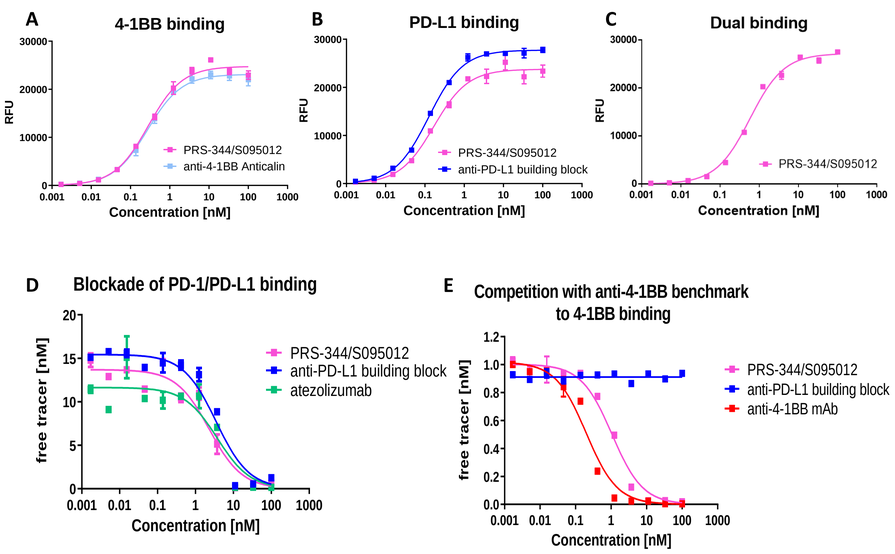


Figure 3. Binding A,B) Direct binding to human recombinant 4-1BB and PD-L1 C) Simultaneous binding of PRS-344/S095012 to 4-1BB and PD-L1. D) Blockade of PD-1/PD-L1 interaction. E) Competition with an anti-4-1BB benchmark mAb. All experiments were conducted with an ELISA-based approach

PRS-344/S095012 stimulates activated T cells in a PD-L1-dependent fashion and enhances their proinflammatory and cytotoxic potential

- **PRS-344/S095012-mediated co-stimulation is strictly PD-L1 dependent and only occurs upon TCR engagement, reducing the risk of peripheral toxicity.**
- **PRS-344/S095012 stimulates the release of cytotoxic molecules and cytokines from activated antigen-specific CD8 T cells or polyclonal T cells.**
- **The in vitro functional activity of PRS-344/S095012 is superior to single agent anti-PD-L1 or benchmark anti-4-1BB mAb.**
- **Engagement of PDL1 and 4-1BB through PRS-344/S095012 bispecific is superior to combination of PD-L1 and 4-1BB mAbs.**

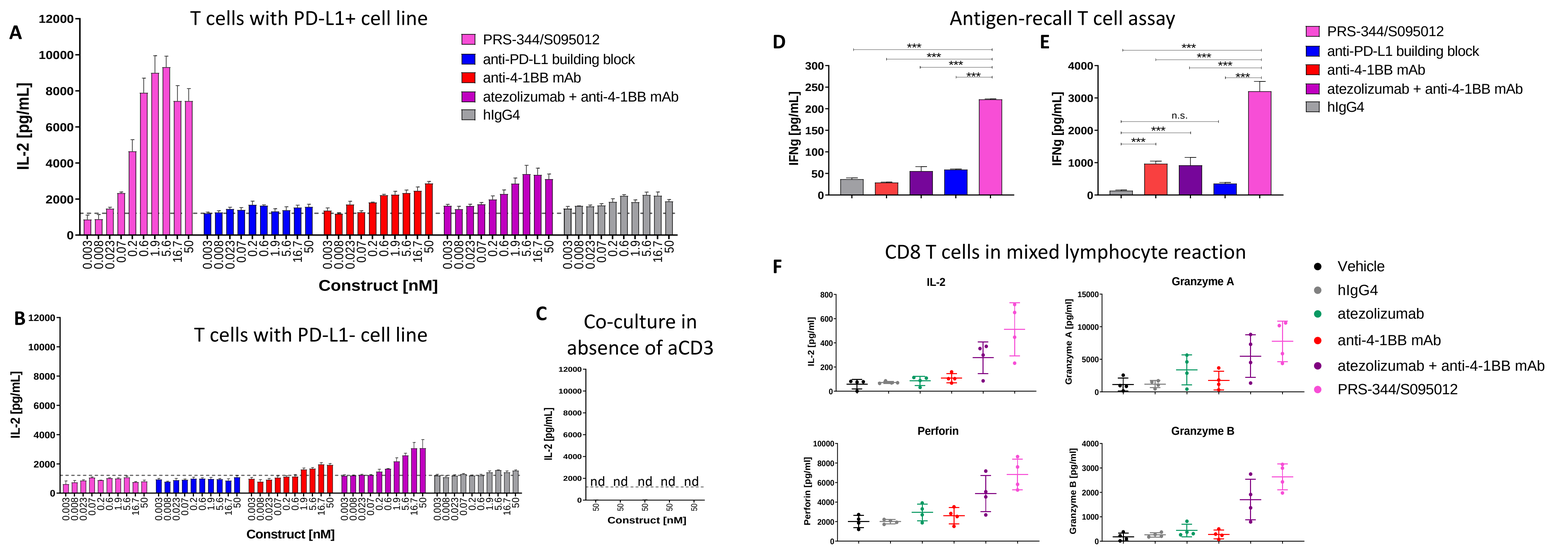
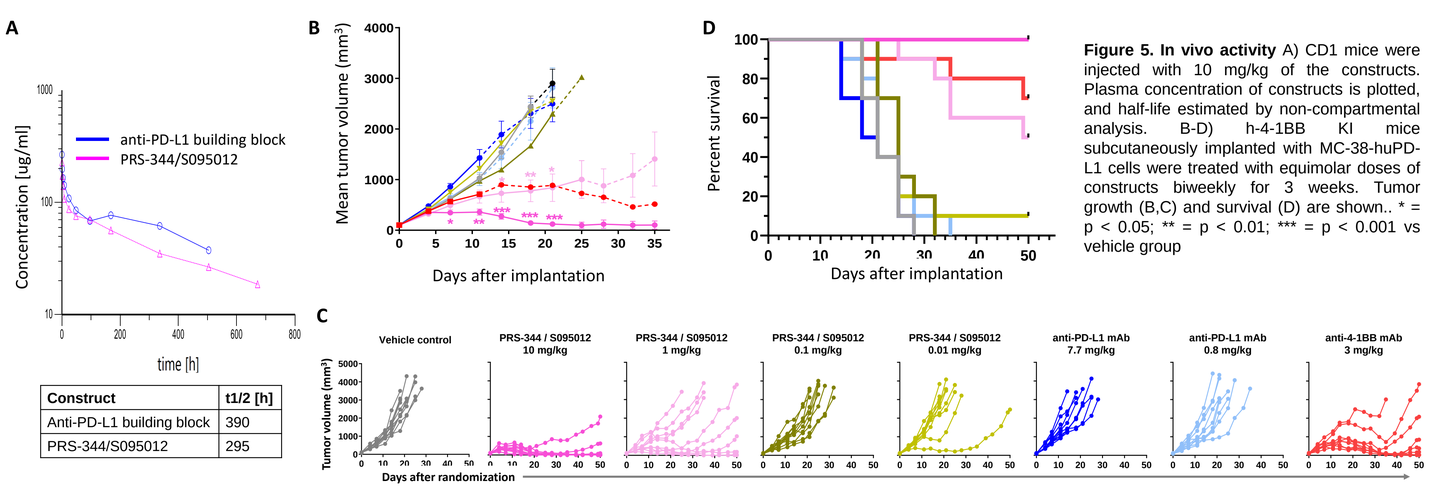


Figure 4. In vitro activity A-C) Co-culture assay: human T cells with coated anti-CD3 mAb and tested constructs co-cultured with A) hPD-L1 positive CHO cells or B) control CHO cells and C) W/O anti-CD3 as a negative control. n.d., not detected. D-E) Recall assay of human PBMCs stimulated with a peptide pool with the indicated constructs or (D), pre-expanded with a peptide pool and re-stimulated with the peptide pool plus the indicated constructs (E). Data is shown as mean ± SEM, n.s= non-significant, ***, P < 0.01. F) Mixed lymphocyte reaction: CD8 T cells from healthy blood donors co-cultured with monocyte-derived dendritic cells from another healthy blood donor.

PRS-344/S095012 displays Ab-like PK in mice and drives a strong anti-tumoral activity superior to anti-PD-L1 mAb

- **The mAb-like half-life of the anti-PD-L1 mAb building block is preserved within PRS-344/S095012.**
- **PRS-344/S095012 triggers a dose-dependent antitumoral response that leads to a significant extension of survival in a humanized KI model.**
- **Complete regression of implanted tumors is observed in 5 out of 10 mice treated with the highest dose of PRS-344/S095012.**
- **PRS-344/S095012 is superior to equimolar doses of anti-PD-L1 mAb treatment alone.**



Construct	t1/2 [h]
Anti-PD-L1 building block	390
PRS-344/S095012	295

Figure 5. In vivo activity A) CD1 mice were injected with 10 mg/kg of the constructs. Plasma concentration of constructs is plotted, and half-life estimated by non-compartmental analysis. B-D) h-4-1BB KI mice subcutaneously implanted with MC-38-huPD-L1 cells were treated with equimolar doses of constructs biweekly for 3 weeks. Tumor growth (B,C) and survival (D) are shown.. * = p < 0.05; ** = p < 0.01; *** = p < 0.001 vs vehicle group

Conclusions

- **PRS-344/S095012 is a 4-1BB / PD-L1 bispecific, generated by the genetic fusion of a 4-1BB-binding Anticalin® protein and an anti-PD-L1 mAb.**
- **PRS-344/S095012-mediated 4-1BB activation is PD-L1-dependent, potentially reducing the risk of peripheral toxicity. Furthermore, 4-1BB co-stimulation only occurs in combination with simultaneous TCR signaling, focusing co-stimulation to antigen-specific T cells.**
- **PRS-344/S095012 induces an effective CD8 T cell response, leading to secretion of inflammatory cytokines and cytotoxic molecules.**
- **PRS-344/S095012 displays mAb-like pharmacokinetics in mice.**
- **PRS-344/S095012 induces a dose-dependent anti-tumor response in a mouse model setup refractory to anti-PD-L1 and significantly extends the survival of mice.**
- **Preclinical data support clinical evaluation of PRS-344/S095012.**

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