Simultaneous costimulatory T-cell engagement and checkpoint inhibition by PRS-344/ONC0055, a 4-1BB / PD-L1 bispecific compound for tumor localized activation of the immune system

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Background

Multiple lines of evidence show that 4-1BB (CD137), a key costimulatory immunoreceptor, is a highly promising therapeutic target in cancer. Current antibody-based approaches showed immune cell activation not only in tumor tissues but also in the periphery, associated with dose-limiting on-target toxicity and a limited therapeutic window. To overcome this limitation, we generated PRS-344/ONC055, a 4-1BB/PD-L1 bispecific Anticalin®/antibody fusion protein. PRS-344/ONC055 is designed to promote 4-1BB clustering on 4-1BB-positive T cells only in presence of PD-L1 expressing cells. PD-L1, the primary ligand of the T-cell receptor PD-1, is widely expressed in the tumor microenvironment resulting in an inhibitory interaction with PD-1. Combining 4-1BB-induced T-cell co-stimulation and expansion with anti-PD-L1 mediated immune checkpoint blockade may overcome the limitation of single agent therapy and offer benefit to ICP-resistant or non-responsive patients. PRS-344/ONC0055 not only merges the potential of a combinatorial therapy in one molecule but also favors the localized activation of antigen-specific T cells in the tumor microenvironment, potentially reducing peripheral toxicity.

Here we provide a preclinical dataset demonstrating that PRS-344/ONC0055 is capable of providing strong 4-1BB-mediated T-cell co-stimulation that is strictly PD-L1 dependent and requires simultaneous TCR signaling thereby restricting T cell activation to antigen-specific, tumor-localized T cells. PRS-344/ONC0055 provides good target binding properties and pharmacokinetics supporting further development of this drug. This program is part of the strategic alliance between Pieris and Servier.

Concept: Tumor-localized co-stimulatory T cell activation combined with checkpoint blockade

PRS-344/ONC0055 clusters 4-1BB only in the presence of PD-L1^{high} expressing tumor and/or antigen-presenting cells in the tumor microenvironment or tumor-draining lymph node. At the same time, blocking the PD-1/PD-L1 interaction further increases T cell responsiveness. However, no clustering of 4-1BB is expected in the periphery where PD-L1 expression levels are low.

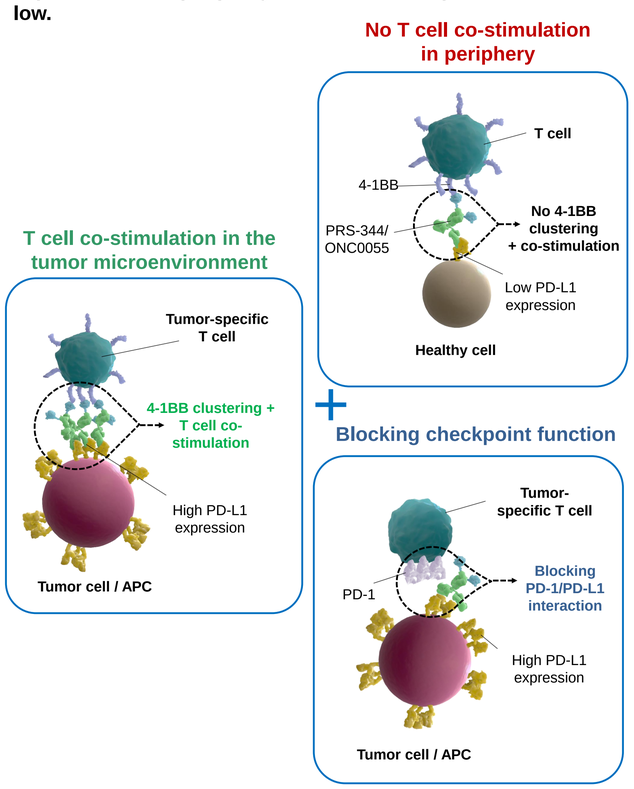


Figure 1. Concept of tumor-localized co-stimulatory T cell activation combined with immune checkpoint blockade. A) Low PD-L1 expression in the periphery is not able to sufficiently cluster 4-1BB which is required to ensure 4-1BB signaling. This results in a reduced risk of peripheral toxicity. B) High PD-L1 expression in the tumor microenvironment, presented on tumor cell and/or APCs, leads to sufficient 4-1BB clustering resulting in a tumor-localized T cell co-stimulation, further enhancing TCR signaling of tumor-specific T cells. C) At the same time, the co-inhibitory PD-1/PD-L1 pathway is efficiently blocked, abrogating suppression of tumor-specific T cells.

PRS-344/ONC0055 is capable of robust target engagement

PRS-344/ONC0055 bispecific demonstrates comparable target binding properties to 4-1BB and PD-L1 as the respective single building blocks and is capable to bind both targets simultaneously.

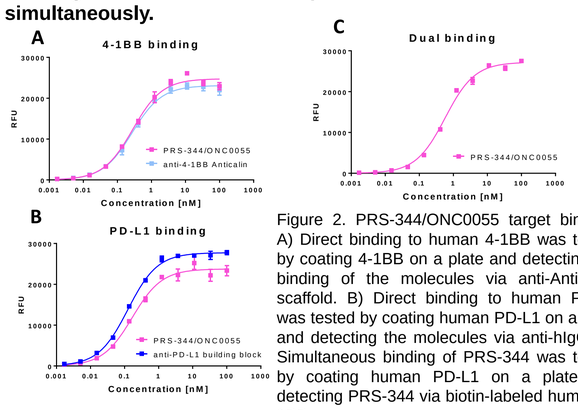


Figure 2. PRS-344/ONC0055 target binding. A) Direct binding to human 4-1BB was tested by coating 4-1BB on a plate and detecting the binding of the molecules via anti-Anticalin-scaffold. B) Direct binding to human PD-L1 was tested by coating human PD-L1 on a plate and detecting the molecules via anti-hIgG. C) Simultaneous binding of PRS-344 was tested by coating human PD-L1 on a plate and detecting PRS-344 via biotin-labeled human 4-1BB.

PRS-344/ONC0055 recognizes functional relevant epitopes

PRS-344/ONC0055 bispecific effectively competes with PD-1 /PD-L1 binding and shares an overlapping 4-1BB binding epitope with clinically active anti-4-1BB benchmark mAb.

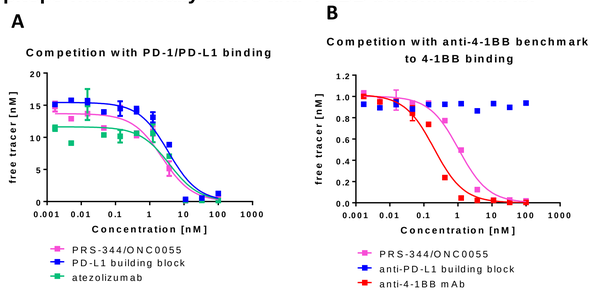


Figure 3. A) Competition to PD-1/PD-L1 binding was assessed in an ELISA based format using coated human PD-1 and human PD-L1-Fc as a tracer. Detection was performed with anti-hIgG. B) Competition with an anti-4-1BB benchmark mAb was assessed in an ELISA based format using coated anti-4-1BB benchmark mAb and human 4-1BB-biotin as a tracer. Detection was performed via ExtrAvidin-HRP.

PRS-344/ONC0055-mediated costimulation is highly effective and PD-L1 dependent

4-1BB clustering and downstream signaling mediated by PRS-344/ONC0055 in presence of a PD-L1-positive cell line are significantly stronger than those of the benchmark anti-4-1BB mAb and are strictly PD-L1 dependent

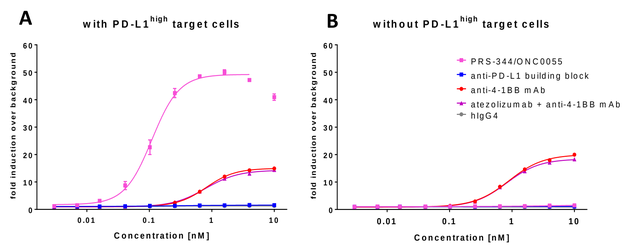


Figure 4. PRS-344/ONC0055-mediated co-stimulatory activity was measured in a Jurkat-4-1BB-NF-κB reporter cell line. A) in presence or B) absence of PD-L1-positive colon cancer cell line RKO.

PRS-344/ONC0055 bispecific retains full checkpoint blockade capacity

PRS-344/ONC0055 retains checkpoint blockade activity similar to anti-PD-L1 mAb building block and atezolizumab

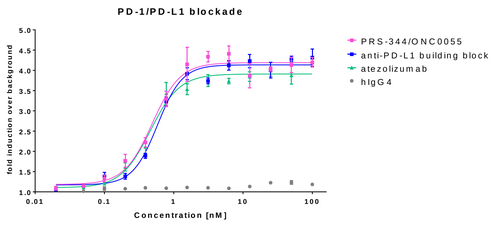


Figure 5. PD-1/PD-L1 checkpoint blockade activity was assessed in a Jurkat-PD-1-NFAT reporter cell line co-cultured with PD-L1 expressing CHO cells.

PRS-344/ONC0055 demonstrates synergistic effect in T cell activation

The combination of atezolizumab and anti-4-1BB benchmark demonstrates the strong synergistic effect of T cell co-stimulation and checkpoint blockade in T cell activation. With PRS-344/ONC0055, this synergistic effect is massively increased.

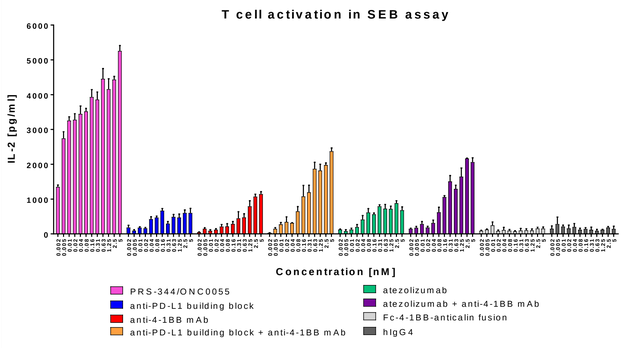


Figure 6. PBMCs from healthy blood donors were stimulated with 0.1 ng/ml SEB in presence of various concentrations of constructs. After 3 days, IL-2 secretion levels were measured from the supernatant. Exemplary data is shown. Background IL-2 levels was 35 pg/ml (PBMC + SEB without constructs). No increase in IL-2 secretion observed when PBMC were not activated with SEB (not shown).

PRS-344/ONC0055-mediated T cell activation is PD-L1 dependent and only occurs in combination with TCR activation

PRS-344/ONC0055-mediated co-stimulation is strictly PD-L1 dependent, reducing the risk of peripheral toxicity. In addition, co-stimulation only occurs in combination with simultaneous TCR signaling, further restricting PRS-344/ONC0055-mediated co-stimulation to antigen-specific T cells.

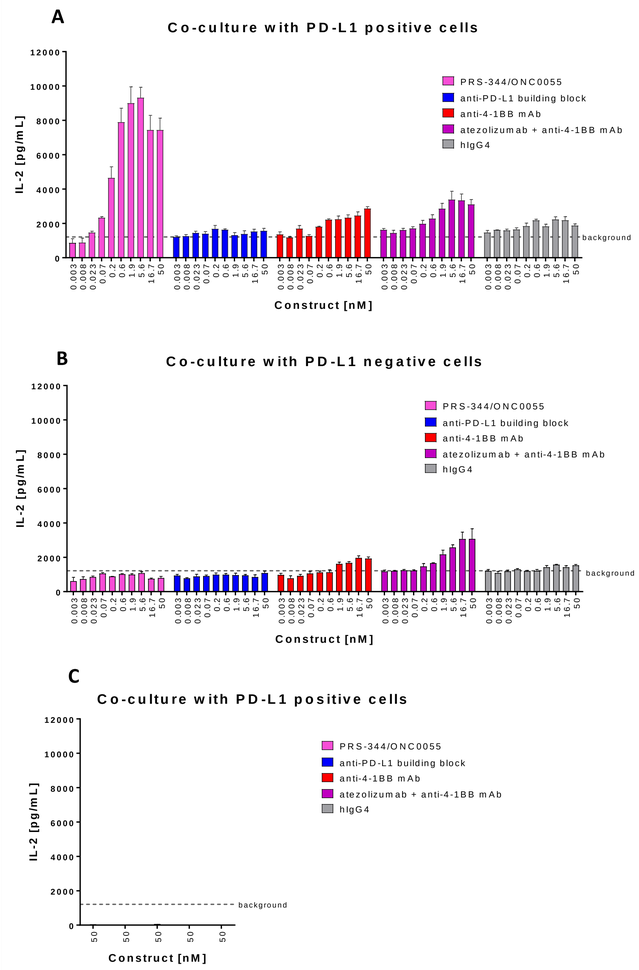


Figure 7. Pan T cells from healthy blood donors were co-cultured in 0.25 µg/ml anti-CD3 mAb coated plates in presence of various concentrations of constructs with A) PD-L1 transfected CHO cells or B) mock transfected CHO cells. C) 50 nM of each construct were added to Pan T cells co-cultured with PD-L1 transfected CHO cells in absence of anti-CD3 mAb which is activating TCR signaling. Background = Pan T cells + anti-CD3 mAb + target cell line.

PRS-344/ONC0055 induces an effective CD8 T cell response in a mixed lymphocyte reaction

PRS-344/ONC0055 induces an effective CD8 T cell response in MLR shown by secretion of several cytokines and cytotoxic molecules which is superior to combination of benchmarks.

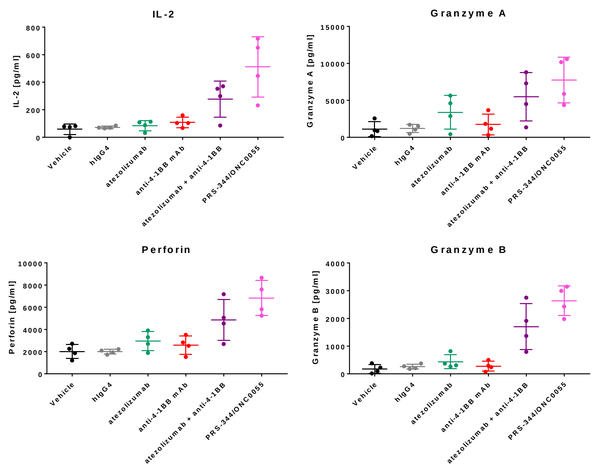
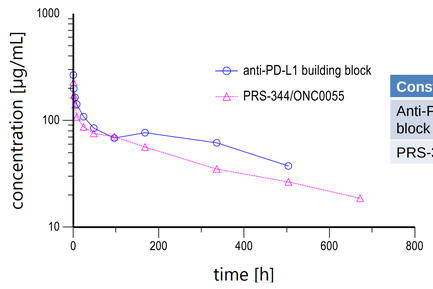


Figure 8. CD8 T cells were co-cultured for 6 days with mature monocyte-derived dendritic cells from another healthy blood donor. Cytokine secretion was measured from the supernatant. Results are shown for IL-2, Granzyme A, Granzyme B and Perforin. Similar results were obtained for IFNγ, TNFα, GM-CSF, IL-13, IL-5, soluble FasL, MIP-1α and MIP-1β. No change in secretion levels observed for IL-6. Graphs show results of 4 different donors.

PRS-344/ONC0055 displays antibody-like pharmacokinetics in mice

The mab-like half-life of the anti-PD-L1 mAb building block is preserved within PRS-344/ONC0055.



Construct	t1/2 [h]
Anti-PD-L1 building block	390
PRS-344/ONC0055	295

Figure 9. PK was analyzed in male CD1 mice of about 6 weeks of age. Animals were injected with 10 mg/kg of the respective construct and plasma samples taken at the indicated timepoints. ADA-positive samples were removed and a non-compartmental analysis performed.

Conclusion

- **PRS-344/ONC0055 is a 4-1BB/PD-L1 bispecific based on the genetic fusion of a high-affinity 4-1BB-binding Anticalin® moiety and an anti-PD-L1 mAb.**
- **Target binding is retained in the bispecific format and both arms of the PRS-344 bispecific are functional.**
- **PRS-344/ONC0055-mediated 4-1BB activation is strictly PD-L1 dependent potentially reducing the risk of peripheral toxicity. Furthermore, 4-1BB co-stimulation only occurs in combination with simultaneous TCR signaling further reducing the risk of peripheral toxicity by limiting co-stimulation to antigen-specific T cells.**
- **PRS-344/ONC0055 induces an effective CD8 T cell response by secretion of several cytokines and cytotoxic molecules.**
- **PRS-344/ONC0055 demonstrates strong synergistic effect in T cell activation which is more pronounced than the combination of benchmarks.**
- **In mice, PRS-344/ONC0055 displays antibody-like pharmacokinetics.**

The here-reported preclinical data support proceeding to further development of PRS-344/ONC0055.