The Anticalin-antibody bispecific PRS-352/S095025 strongly stimulates human CD4+ T cells in a PD-L1–dependent manner

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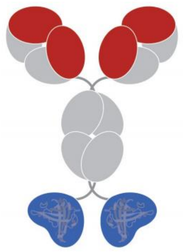
Background

- OX40 is a costimulatory receptor promoting survival and cytokine release by effector T cells and inhibiting human regulatory T cells.
- Agonistic anti-OX40 antibodies under development rely on Fc gamma receptor (FcγR)-mediated crosslinking and have shown only limited antitumoral efficacy in clinical settings.
- One hypothesis is that FcγR crosslinking of these antibodies may not allow optimal activation of the OX40 pathway.
- PRS-352/S095025 was designed to maximize OX40's via a targeted approach.
- PRS-352/S095025 is a bispecific fusion protein of OX40-targeting Anticalin proteins with a PD-L1-targeting monoclonal antibody. The modified IgG4 backbone allows optimal activation of OX40 in the presence of PD-L1 but not FcγR.

PRS-352/S095025

PD-L1 binding antibody



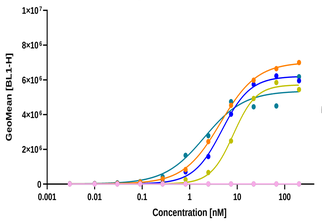
OX40 agonist Anticalin® proteins

Results: PD-L1 and OX40 binding

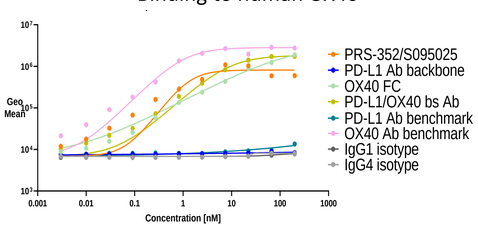
The bispecific PRS-352/S095025 binds to PD-L1 and OX40 in cell based assays with similar affinity to parental building blocks and benchmarks

PRS-352/S095025 binding to hPD-L1 or hOX40, expressed on the surface of engineered Flp-In-CHO cell line, has been analysed by flow cytometry

Binding to human PD-L1



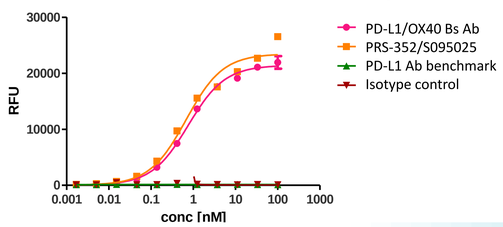
Binding to human OX40



The bispecific PRS-352/S095025 simultaneously engages both targets

PRS-352/s095025 binding to both targets was analysed by ELISA using recombinant PD-L1 for coating and recombinant OX40 for detection.

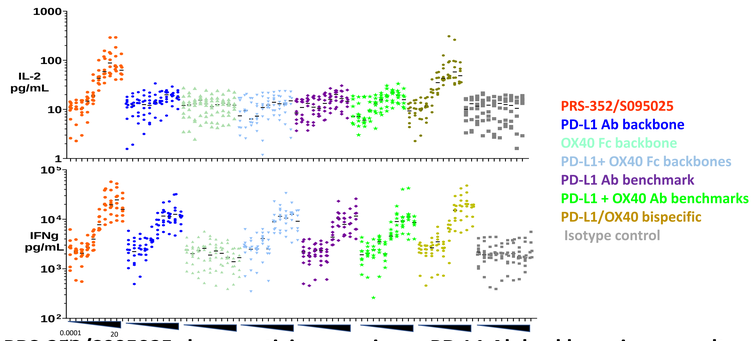
Simultaneous binding ELISA



Results: in vitro activity on human primary immune cells

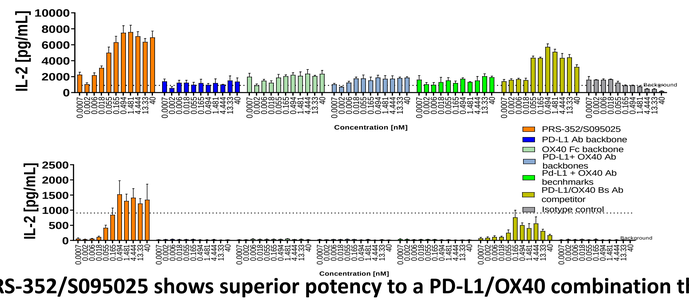
PRS-352/S095025 stimulates human CD4 T cells *in vitro* and is superior vs benchmarks

Human primary CD4 T cells were cultured with monocyte derived dendritic cells in mixed lymphocyte reaction assay, and treated with increasing doses of tested items for 6 days. IL-2 was measured in the supernatants (S/N) using Luminex technology. Individual donors shown, N=6.



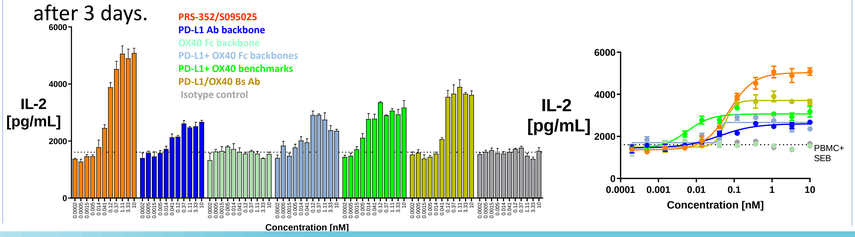
PRS-352/S095025 shows activity superior to PD-L1 Ab backbone in a co-culture assay

Human PBMCs and the PD-L1+ tumor cell line MDA-MB-231 were co-cultured in presence of dose response of test items, and IL-2 was measured in S/N after three days as a readout of T cell stimulation.



PRS-352/S095025 shows superior potency to a PD-L1/OX40 combination therapy in a staphylococcal enterotoxin B assay

Human peripheral blood mononuclear cells were activated using sub-optimal dose of SEB and incubated with increasing doses of test items. IL-2 was quantified in S/N after 3 days.

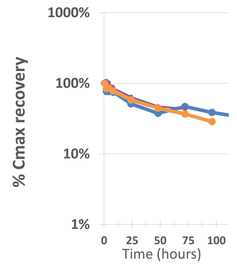


Results: exposure and ex vivo activity in monkey

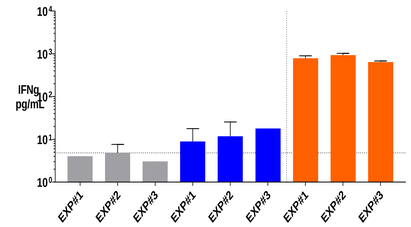
A) PRS-352/S095025 bispecific shows exposure similar to the reference anti-PD-L1 antibody in Non Human Primates (NHP's)

Exposure in cynomolgus monkeys after single IV injection (7.7 mg/kg) of PRS-352/S095025 (orange) and PD-L1 backbone (blue) has been quantified in plasma using an ELISA assay.

A) Exposure in NHP



B) Activity ex vivo on monkey PBMC



B) PRS-352/S095025 bispecific stimulates ex vivo monkey immune cells

IFNg secretion has been analysed in a 2-way mixed lymphocyte reaction assay using cynomolgus monkey PBMCs treated with PRS-352/S095025 (orange) compared to PD-L1 (blue) and an isotype control (grey). Each bar represent a 2-way MLR.

Conclusions

PRS-352/S095025 is a novel bispecific designed to promote PD-L1 specific agonism of OX40 coupled to PD1/PD-L1 pathway inhibition. The results show that PRS-352/S095025 :

- specifically binds to PD-L1 and Ox40 with similar affinity to parental building blocks
- inhibits the PD-1/ PD-L1 pathway with comparable potency to anti-PD-L1 antibodies
- stimulates human CD4 T cells
- show superior potency to anti-PD-L1 or combination therapy benchmarks in different in vitro assays
- drives T cell stimulation (IFNg) in NHP's
- has a PK profile comparable to the parental PD-L1 antibody in NHPs

These in vitro and in vivo data support further development of PRS-352/S095025.

Acknowledgements

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