

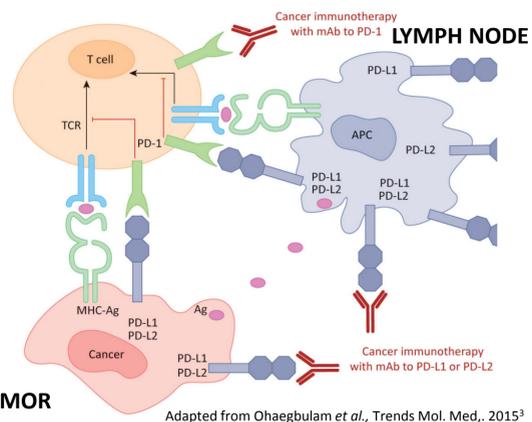
Abstract

Background: The recognition of tumors by the immune system has long been appreciated and the presence of tumor associated lymphocytes has been reported as a positive prognostic indicator in multiple tumor types. However, despite evidence of immune reactivity, tumors are still able to grow suggesting a sub-optimal response. Programmed cell death-1 (PD-1) is an immune checkpoint receptor expressed by T-cells that, through interactions with its ligands, PD-L1 and PD-L2, has been demonstrated to suppress cancer-specific immune responses. Clinical evaluation of anti-PD-1 and anti-PD-L1 antibodies is ongoing in multiple tumor types, including non-small cell lung cancer, melanoma and bladder cancer and to date, two anti-PD-1 antibodies and one anti-PD-L1 antibody have been approved for the treatment of human cancers. Here, we describe the identification of TSR-042, a novel investigational anti-PD-1 antibody. **Materials and Methods:** TSR-042 is a potent, selective, IgG4 humanized monoclonal antibody generated from a mouse hybridoma using the SHM-XEL™ system. It was characterized in a number of *in vitro* and *in vivo* studies and completed IND-enabling preclinical activities. **Results:** TSR-042 displays high affinity to both human and cynomolgus monkey PD-1, as assessed by surface plasmon resonance to recombinant PD-1 and flow cytometry using cell lines overexpressing recombinant PD-1 or binding to native protein on peripheral blood mononuclear cells. TSR-042 does not cross-react to the mouse species orthologue. In addition to binding to PD-1, TSR-042 also inhibits the interaction of both PD-L1 and PD-L2 with PD-1. The functional antagonist activity of TSR-042 to augment *in vitro* responses of primary human CD4+ T-cells was assessed in a human CD4+ mixed lymphocyte reaction (MLR) assay. TSR-042 was found to be a potent functional antagonist in this system, resulting in increased IL-2 production. Furthermore, TSR-042 was also found to have increased activity in the presence of either anti-TIM3 or anti-LAG3 antibodies. TSR-042 was also shown to not induce significant stimulation of cytokine release from human PBMCs when incubated as a single agent. Owing to its lack of cross-reactivity to rodent PD-1, a comprehensive safety program in cynomolgus monkey was performed. Results from single dose and repeat-dose intravenous toxicology studies indicated that TSR-042 was well-tolerated and displayed a profile that supported assessment of the molecule in the clinic. **Conclusion:** Taken together, these data demonstrate that TSR-042 is a potent anti-PD-1 receptor antagonist with pre-clinical properties that support its clinical investigation in cancer patients. TSR-042 is currently being assessed in a Phase 1 clinical trial (NCT02715284).

Introduction

- PD-1 is one of several checkpoint receptors that have been implicated in T-cell exhaustion and limiting the activity of T-cells in tumors¹. Blocking the PD-1 pathway has yielded promising results with the approval of several agents.
- TSR-042 is an anti-PD-1 immunoglobulin G4 (IgG4) humanized monoclonal antibody (mAb) generated using SHM-XEL™ technology² that binds with high affinity to PD-1 and is currently in clinical testing.

Figure 1. Blocking the PD-1 pathway



TSR-042 binds with high affinity to PD-1

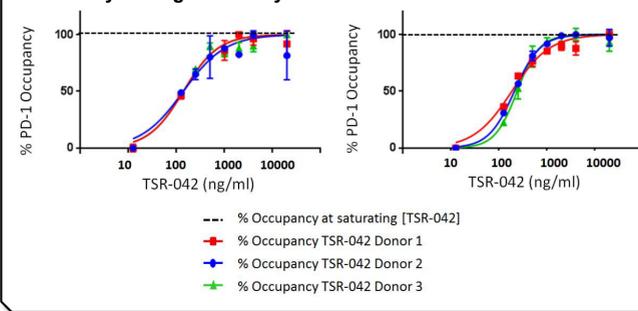
- Binding of TSR-042 to PD-1 across species was assessed by surface plasmon resonance to recombinant PD-1-Fc and flow cytometry to CHO-K1 cells over-expressing recombinant PD-1 (Table 1) and native PD-1 expressed on cynomolgus monkey or human donor peripheral blood mononuclear cells (PBMCs) (Figure 2).

Table 1: Binding of TSR-042 to recombinant PD-1

Species	Kinetic Parameters (SPR)			PD-1 expressing CHO cells EC ₅₀ (nM)
	K _{assoc} (Ms) ⁻¹	K _{dissoc} (s ⁻¹)	K _D (nM)	
Human PD-1	5.7x10 ⁵	1.7x10 ⁻⁴	0.30	2.0
Cyno PD-1	4.3x10 ⁵	2.3x10 ⁻⁴	0.53	3.4

cyno = cynomolgus monkey, K_{assoc} = association rate constant; K_{dissoc} = dissociation

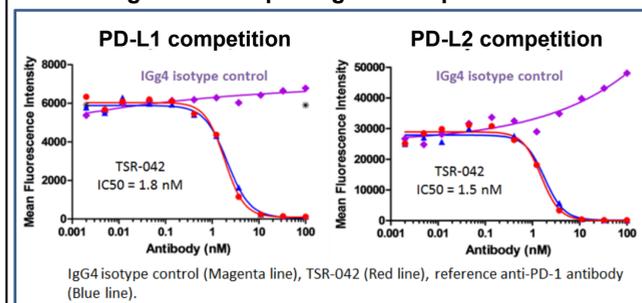
Figure 2. TSR-042 binds to native PD-1 on PBMCs



TSR-042 inhibits the interaction of PD-L1 and PD-L2 with PD-1

- TSR-042 is a potent antagonist of PD-1/PD-L1 or PD-L2 receptor-ligand interactions as assessed by a flow cytometry assay that measured the binding of DyLight 650 (DyL650)-labeled PD-L1 and PD-L2 to recombinant PD-1-expressing CHO-K1 cells (Figure 3).

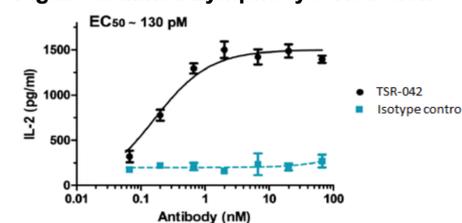
Figure 3. Receptor-ligand competition



TSR-042 enhances the activity of T cells in a mixed lymphocyte reaction

- The functional antagonist activity of TSR-042 was tested in an MLR assay in which primary human CD4+ cells were mixed for 48 h with human monocyte-derived dendritic cells from a different donor in the presence of TSR-042 or isotype control. Antagonism of PD-1 by TSR-042 was demonstrated by increased IL-2 production (Figure 4).

Figure 4: Mixed Lymphocyte Reaction



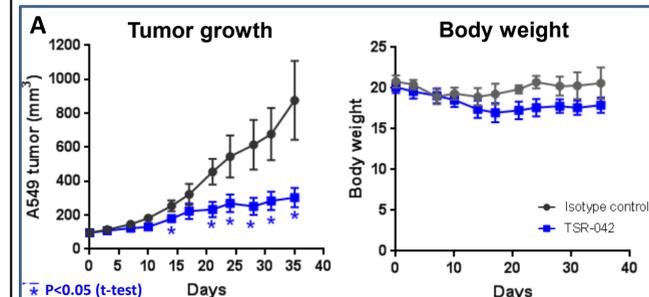
Nonclinical toxicology

- A comprehensive preclinical toxicology assessment was performed to evaluate the safety profile of TSR-042. There was no evidence of related local irritation and no compound-related adverse changes observed for any parameter during the dosing phase or recovery phase at doses up to 100 mg/kg.

TSR-042 exhibits anti-tumor activity in a humanized tumor xenograft model

- TSR-042 does not cross-react to mouse PD-1 and the *in vivo* activity was evaluated using in a humanized mouse model, huNOG-EXL (Taconic).
- A549 cells (5E6 cells per mouse) were implanted subcutaneously into huNOG-EXL mice and tumors were grown to 80-120 mm³ before randomization (N=8 per group) for treatment with either TSR-042 or isotype control (10 mg/kg, twice per week). Mice were sacrificed on day 36 and pharmacodynamic changes of immune cells in the tumor were assessed. In this model, TSR-042 had a significant anti-tumor effect that was not associated with body weight loss (Figure 5A). Furthermore, this anti-tumor activity was associated with increased CD8 cell infiltrate in the tumors (Figure 5B).

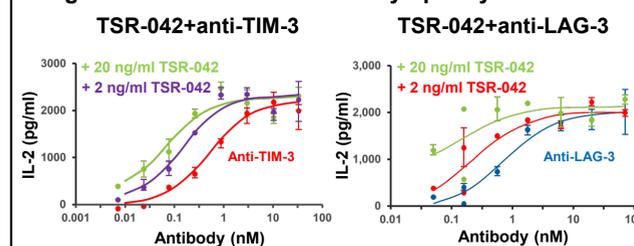
Figure 5. huNOG-EXL Mouse Model



TSR-042 can be combined with anti-TIM-3 or anti-LAG-3 to potentiate T cell activation in a mixed lymphocyte reaction

- The potential for TSR-042 as a combination partner was tested in an MLR assay in which primary human CD4+ cells were mixed for 48 h with human monocyte-derived dendritic cells from a different donor.
- In this system, TSR-042 was found to enhance the effect of both anti-TIM-3 and anti-LAG-3 agents (Figure 6).

Figure 6: Combination Mixed Lymphocyte Reaction



Summary

- TSR-042 is a humanized IgG4 monoclonal antibody that binds to PD-1 with high affinity and potentially blocks PD-1/PD-L1 and PD-L2 receptor-ligand interactions.
- TSR-042 was a potent functional antagonist in an MLR assay system and demonstrated anti-tumor activity in a xenograft model in humanized mice.
- TSR-042 was well tolerated in IND-enabling pre-clinical toxicology studies and is currently being assessed in a Phase 1 clinical trial (NCT02715284).

References

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2. Bowers *et al.*, (2011). Coupling mammalian cell surface display with somatic hypermutation for the discovery and maturation of human antibodies. *Proc. Nat. Acad. Sci.* 108: 20455-20460
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