Discovery of TSR-022, a novel, potent anti-TIM-3 therapeutic antibody

Haley Laken[1], Kristen McEachern[1], Anwar Murtaza[1], Jean da Silva Correia[2], Patricia McNeeley[2], John Zhang[1], Paul Vancutsem[1], Keith Wilcoxen[1] & David Jenkins[1]

[1]TESARO, 1000 Winter Street, Waltham, MA 02451; [2]AnaptysBio, 10421 Pacific Center Court, San Diego, CA 92121

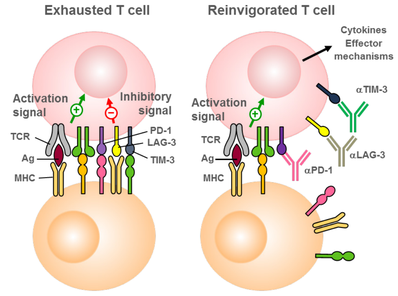
Abstract

Background: The augmentation of an anti-tumor immune response through multiple therapeutic approaches has become one of the more promising areas of oncology research and development. For example, clinical activity has been demonstrated through immune checkpoint blockade with several molecules, including those targeting cytotoxic T-lymphocyte-associated antigen-4 (CTLA-4), programmed death-1 (PD-1) and one of its ligands, PD-L1. However, despite successes reported with agents directed against these targets, many patients do not receive benefit, suggesting that there are additional mechanisms of immune evasion that may prevent effective anti-tumor immunity. T-cell immunoglobulin and mucin-domain containing-3 (TIM-3) is a novel immune checkpoint initially identified on interferon-gamma producing CD4+ T-helper 1 and CD8+ cytotoxic T-cells and has been implicated in the exhaustion of T-cells. More recently, TIM-3 expression has also been identified on other immune cell types, although its functional role in this context remains to be fully elucidated. Here, we describe the identification of TSR-022, a novel IgG4 anti-human TIM-3 therapeutic antibody. **Materials and Methods:** TSR-022 is a potent and selective humanized monoclonal antibody, generated through the use of SHM-XEL™ technology. It was characterized in a number of *in vitro* and *in vivo* studies and completed IND-enabling preclinical activities. **Results:** TSR-022 binds to recombinant human and cynomolgus monkey TIM-3 with pM affinity and does not bind appreciably to mouse TIM-3. Binding of TSR-022 to TIM-3 enhances T-cell activation, for example, increasing cytokine generation from activated CD4+ T-cells and by increasing IL-2 production in a CD4+ T-cell/dendritic cell mixed lymphocyte reaction assay. Importantly, TSR-022 also increased IL-2 secretion not only alone but also in combination with anti-PD-1 antibodies, including the novel anti-PD-1 antagonist, TSR-042. As a single agent, TSR-022 did not induce cytokine release from human peripheral blood mononuclear cells and when evaluated for binding to C1q and CD16a, TSR-022 displayed properties typical for a human IgG4 antibody, suggesting it is unlikely to mediate appreciable complement-dependent cytotoxicity or antibody-dependent cell cytotoxicity. Owing to its lack of cross-reactivity to rodent TIM-3, a comprehensive safety program in cynomolgus monkey was performed. Results from single dose and repeat-dose intravenous toxicology studies indicated that TSR-022 was well-tolerated and displayed a profile that supported progressing the molecule into clinical studies. **Conclusion:** Taken together, these data demonstrate that TSR-022 is a potent anti-TIM-3 receptor antibody with pre-clinical properties that support its clinical investigation in cancer patients.

Introduction

- In spite of the clinical success of immunotherapies, many patients do not respond, suggesting that there are additional mechanisms of immune evasion preventing effective anti-tumor immunity[1]. Beyond PD-1, several additional immune checkpoints, including TIM-3 and LAG-3 have been proposed to play a role in T-cell exhaustion and limiting the anti-tumor immune response (Figure 1).
- TSR-022 is a humanized Immunoglobulin G4 (IgG4) anti-TIM-3 antibody generated using SHM-XEL[2] technology that enhances T-cell activation and is currently being evaluated in the clinic.

Figure 1. TIM-3 as immunotherapy target



TSR-022 binds with high affinity to recombinant TIM-3

- TSR-022 was characterized by surface plasmon residence (SPR) for binding to human and cynomolgus monkey recombinant TIM-3 (Fc fusion protein). SPR measurements demonstrated that TSR-022 binds human and cynomolgus TIM-3 with high affinity (Table 1). In addition, further studies demonstrated that TSR-022 did not bind to human TIM-1 or PD-1 (data not shown). Binding of TSR-022 to human or cynomolgus monkey TIM-3 expressed on CHO-K1 cells was also determined by flow cytometry (Table 1).

Table 1: Binding of TSR-022 to recombinant TIM-3

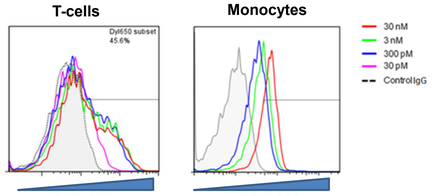
	Kinetic Parameters (SPR)			TIM-3 expressing CHO cells	
	K_{assoc} $(Ms)^{-1}$	K_{dissoc} (s^{-1})	K_D (pM)	EC_{50} (nM)	EC_{90} (nM)
Human	$1.5x10^7$	$1.1x10^{-4}$	7	0.17	1.67
Cynomolgus Monkey	$1.1x10^7$	$1.9x10^{-4}$	17	0.27	1.47

[a] Kassoc = association rate constant; Kdissoc = dissociation rate constant; KD = dissociation constant

TSR-022 binds to native TIM-3 on activated T-cells and monocytes

- TIM-3 expression has been reported to be low on resting T cells and increased after T cell activation.[3,4] . TIM-3 expression has also been reported on monocytes. To confirm binding of TSR-022 to T cells, isolated T cells were activated on anti-CD3/anti-CD28 coated plates for 48 h to induce TIM-3 expression, cells were stained with TSR-022 at various concentrations and binding was evaluated by flow cytometry. (Figure 2A). In similar studies, TSR-022 binding to monocytes that were purified from peripheral blood mononuclear cells (PBMCs) was confirmed (Figure 2).

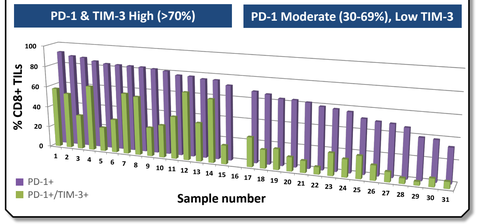
Figure 2: TSR-022 binding to native TIM-3



Expression profile of TIM-3

- The expression of TIM-3 and PD-1 in CD8+ tumor infiltrating lymphocytes was evaluated by flow cytometry in non-small cell lung cancer (NSCLC) cancer samples using a method that we previously developed[5]. Consistent with a potential role in the negative regulation of T-cell function, TIM-3 expression was identified across the samples evaluated (N=31; Figure 5).

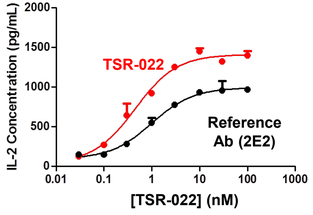
Figure 5. TIM-3 expression in NSCLC



TSR-022 enhances the activity of T cells

- The ability of TSR-022 to enhance the activity of CD4+ T-cells was determined. In these studies, CD4+ T-cells were isolated from healthy donor PBMCs, prior to being stimulated with anti-CD3/anti-CD28 for 48 h prior to addition of TSR-022 or control IgGs for an additional 48 h. In this system, TSR-022 dose-dependently enhanced IL-2 release from the activated CD4+ T-cells (Figure 3).

Figure 3: Activated CD4+ T-cell assay



Non-clinical toxicology

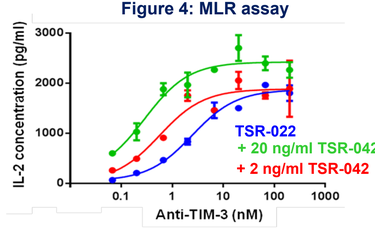
- A comprehensive preclinical toxicology assessment was performed to evaluate the safety profile of TSR-022. There was no evidence of related local irritation and no compound-related adverse changes observed for any parameter during the dosing phase or recovery phase at doses up to 100 mg/kg.

Summary

- TIM-3 is an emerging immunotherapy target that has been implicated in limiting the anti-tumor immune response.
- TSR-022 is a humanized antibody that binds to TIM-3 with high affinity and acts to enhance T-cell function alone and in combination with PD-1 blockade.
- TSR-022 was well tolerated in IND-enabling pre-clinical toxicology studies and is currently being assessed in a Phase 1 clinical trial (NCT02817633).

TSR-022 can be combined with anti-PD-1 to potentiate T cell activation in an MLR assay

Figure 4: MLR assay



- The functional activity of TSR-022 was also evaluated in a mixed lymphocyte reaction (MLR) assay, in which primary human CD4+ T-cells were mixed with monocyte-derived dendritic cells from a different donor. In these studies, dendritic cells and allogeneic CD4+ T cells were incubated in the presence of TSR-022 or isotype control for 48 h and activation of T cells determined by the level of IL-2 secretion. TSR-022 dose-dependently increased IL-2 production, an effect that was further enhanced in combination with an anti-PD-1 antibody at a concentration of 2 or 20 ng/ml. These data demonstrate that antagonizing TIM-3 by TSR-022 alone or in combination with anti- PD-1 can result in potent enhancement in T cell activation (Figure 4).

References

1. Le Mercier *et al.,* (2015). Frontiers in Immunology 6: 1-15.
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.
3. Freeman *et al.,* (2010). Tim genes: a family of cell surface phosphatidylserine receptors that regulate innate and adaptive immunity. Immunol. Rev. 235: 172-189.
4. Monney, L. *et al.*, (2002). Th1-specific cell surface protein Tim-3 regulates macrophage activation and severity of an autoimmune disease. Nature 415: 536-541.
5. Zhang *et al.,* (2016). Optimization of a method for FACS analysis of checkpoint receptor expression on tumor infiltrating lymphocytes in Chinese cancer patients. #3823. Proceedings of the AACR, Vol 57, April 2016.