

GENERATION OF AZD7789, A NOVEL PD-1 AND TIM-3 TARGETING BISPECIFIC ANTIBODY, WHICH BINDS TO A DIFFERENTIATED EPIOTOPE OF TIM-3

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Background PD-1 and TIM-3 are expressed on tumor infiltrating lymphocytes (TIL) across multiple indications where dual receptor expression is associated with diminished functionality.^{1,2} Studies have demonstrated that TIM-3 acts as a compensatory inhibitory mechanism post PD-1 blockade to reduce anti-tumor T-cell responses.³ We developed a novel anti-TIM-3 antibody, O13, which engages the TIM-3 IgV domain without blocking phosphatidylserine (PS) binding, eliciting differential functionality. O13 was incorporated into AZD7789, a monovalent bispecific antibody that binds PD-1 and TIM-3. Herein we show preclinical work to support clinical investigation.

Methods Co-crystals of anti-TIM-3 antibodies and recombinant human TIM-3 IgV were generated by standard methods. TIM-3+ Jurkat T-cells were stimulated and treated with anti-TIM-3 antibodies and assessed for IL-2 production. PD-1+ TIM-3+ virally-reactive human T-cells were co-cultured with tumor cell lines expressing viral peptides in the presence of AZD7789 and controls to assess cytokine production and cytotoxicity of T-cells *in vitro*, and in immunodeficient mice for tumor growth inhibition and survival data *in vivo*. *In vitro*, monocyte-derived dendritic cells (Mo-DC) were assessed for the ability of AZD7789 to modulate efferocytosis and cross-presentation.

Results Crystallography revealed that O13 binds an epitope of human TIM-3 outside of the PS-binding cleft. This binding site confers unique biology. While O13 elicited enhanced IL-2 production from TIM-3+ Jurkat T-cells, a PS-blocking anti-TIM-3 antibody diminished IL-2 secretion. Recognition of PS was critical for this response as mutation of the PS-binding site in TIM-3, or use of T-cells that cannot expose extracellular PS, did not elicit an antibody-mediated effect. O13 and AZD7789 enhanced efferocytosis via TIM-3 recognition of PS on dying tumor cells, and increased cross-presentation of tumor antigen to T-cells. AZD7789 also increased anti-tumor T-cell responses compared to anti-PD-1 and a PD-1/TIM-3 bispecific antibody that blocks PS-binding to TIM-3. Additionally, subsequent AZD7789 treatment prolonged *in vivo* growth inhibition in tumors that progressed on anti-PD-1 monotherapy, and AZD7789 enhanced IFN- γ secretion of *ex vivo* stimulated TIL derived from anti-PD-1 treated mice.

Conclusions AZD7789 enhanced primary human T-cell and Mo-DC anti-tumor responses over treatment with anti-PD-1 or a PD-1/TIM-3 bispecific antibody that blocks PS-binding to TIM-3. Our preclinical data shows AZD7789 modulates multiple immune functions, which may translate to clinical activity in both checkpoint inhibitor naïve and resistant tumors. AZD7789 is being tested in IO pretreated patients with NSCLC (NCT04931654) and cHL (NCT05216835).

Trial Registration NCT04931654; NCT05216835

REFERENCES

1. Thommen D, *et al.* A transcriptionally and functionally distinct PD-1+ CD8+ T cell pool with predictive potential in non-small-cell lung cancer treated with PD-1 blockade. *Nat Med.* 2018; **24**:994–1004.

2. Fourcade J, *et al.* Upregulation of TIM-3 and PD-1 expression is associated with tumor antigen-specific CD8+ T cell dysfunction in melanoma patients. *J Exp Med.* 2010; **207**: 2175–2186.
3. Koyama S, *et al.* Adaptive resistance to therapeutic PD-1 blockade is associated with upregulation of alternative immune checkpoints. *Nat Comm.* 2016; **7**:10501.

Ethics Approval All animal studies were conducted in accordance with IACUC protocol: AUP-21-15

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