

A tetravalent anti-PVRIG×PD-L1 bispecific antibody demonstrates dual checkpoint blockade and preclinical anti-tumour activity.

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Introduction

PVRIG (CD112R) is an inhibitory receptor in the DNAM-1/TIGIT/CD96 network. PVRIG binding to PVRL2 (CD112) suppresses CD8+ T-cell function. Human tumours show increased PVRIG expression on T and NK cells relative to normal tissue, while tumour-cell PVRL2 can limit anti-tumour immunity¹.

PD-L1 on cancer cells binds to PD-1 on activated T cells and suppresses T-cell activation and effector function. Inflammatory signals, including interferon- γ , can induce PD-L1 expression, providing a feedback mechanism that tumours can exploit to evade immune attack.

Preclinical studies have shown PVRIG blockade increases T-cell cytokine production and cytotoxic activity. Combining PVRIG and PD-(L)1 blockade further enhances T-cell activation². These findings provide a rationale for investigating simultaneous PVRIG–PVRL2 and PD-1–PD-L1 blockade to address complementary mechanisms of immune suppression.

Here, we evaluate a tetravalent anti-PVRIG×PD-L1 IgG1 bispecific antibody designed to block both receptor–ligand interactions. We assess its functional activity and anti-tumour efficacy in preclinical models.

Figure 1. PVRIG×PD-L1 bispecific antibody design and mechanism of action

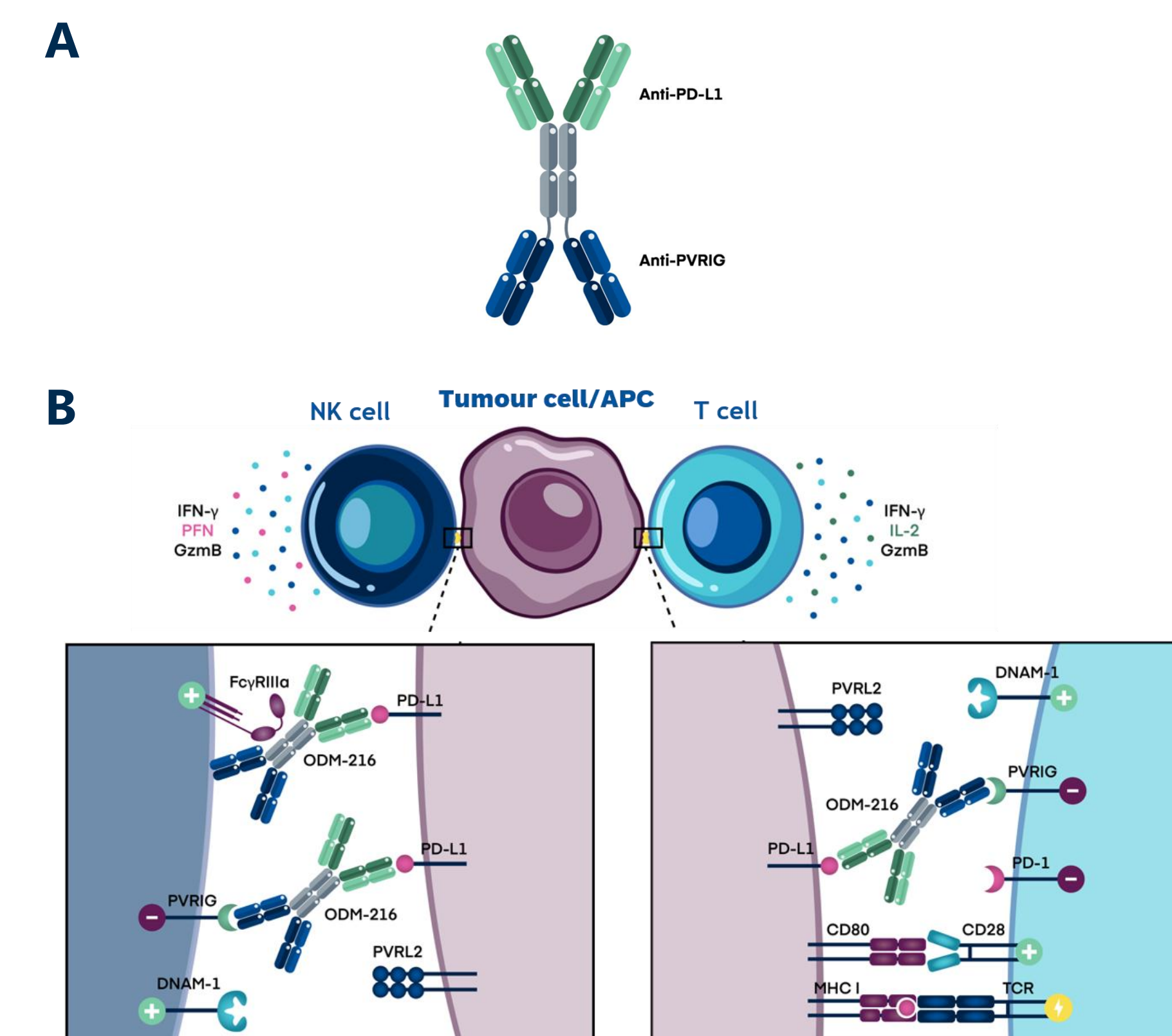


Figure 1. PVRIG×PD-L1 bispecific antibody proposed mechanism of action. (A) Tetravalent anti-PVRIG×PD-L1 bispecific antibody design. (B) Proposed dual checkpoint blockade and FcγRIIIa engagement supporting T-cell and NK-cell activity and clustering of PVRIG+ and PD-L1+ cells.

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Declaration of Interests:

Financial Interests: Full-time employment, Head of Immuno Oncology, Orion Pharma.

Stocks/shares: Orion Pharma, AstraZeneca, milestone pharma.

Study is sponsored by Orion Pharma.

ODM-216 is an investigational medicinal product. Its safety and efficacy have not been established

RESULTS

Figure 2. Receptor–ligand inhibition assay

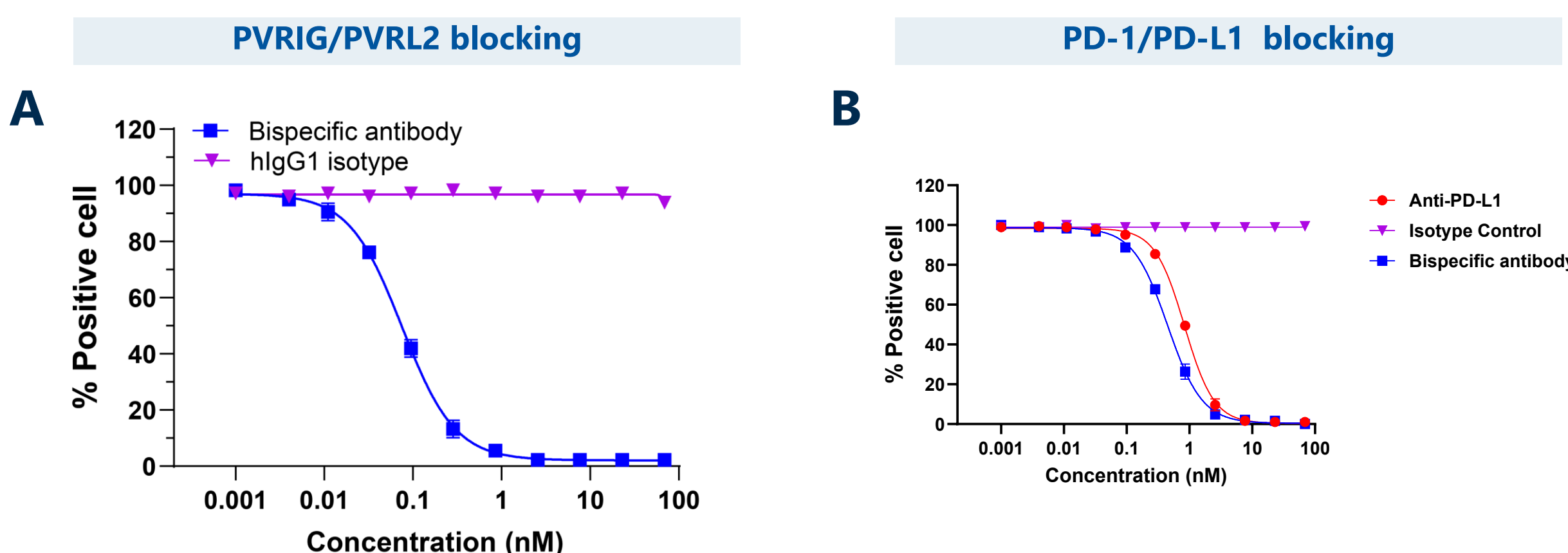


Figure 2. CHO cell dependent ligand receptor inhibition. PVRIG×PD-L1 bispecific antibody demonstrated concentration-dependent competitive inhibition of (A) PVRIG–PVRL2 and (B) PD-1–PD-L1 interactions. These data demonstrate concentration-dependent inhibition of PVRIG–PVRL2 and PD-1–PD-L1 interactions

Figure 3. FcγRIIIa engagement –ADCC reporter assay

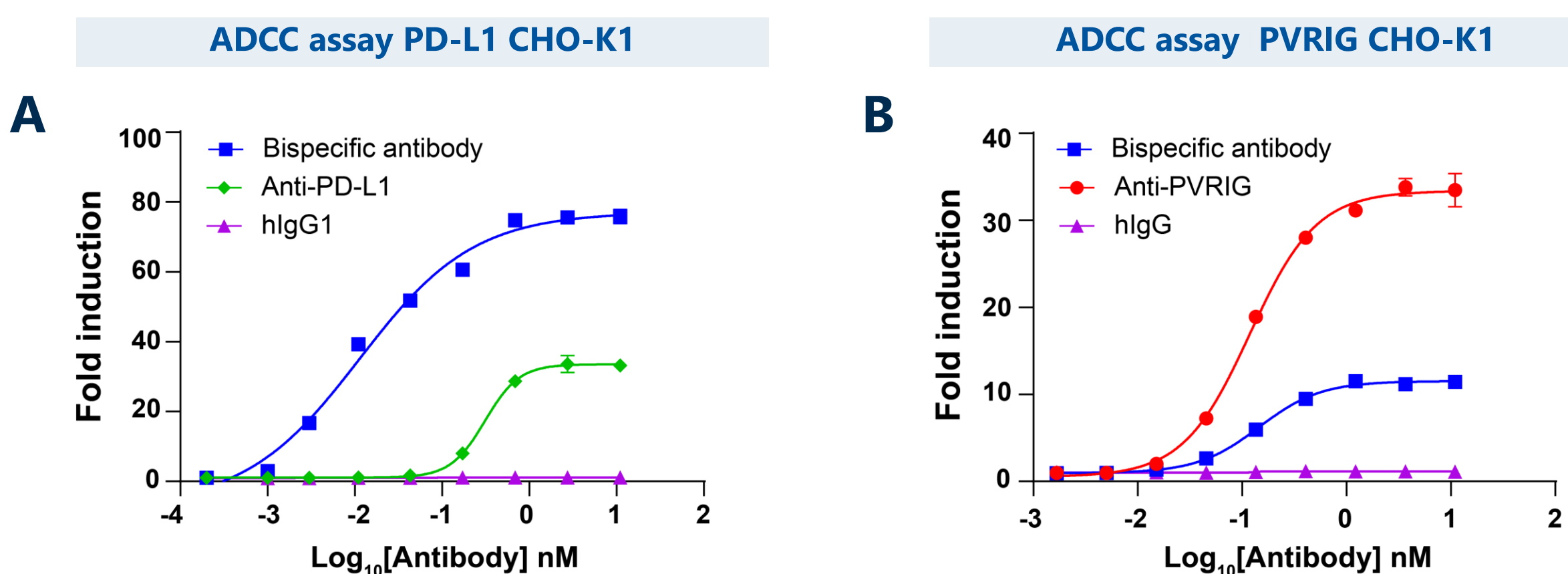


Figure 3. ADCC reporter assay activity. (A) Assay performed with PD-L1-expressing CHO cells (B) Assay performed with PVRIG-expressing CHO cells. PD-L1 arm of the bispecific antibody demonstrated superior FcγRIIIa engagement whereas PVRIG arm demonstrated lower FcγRIIIa engagement.

Figure 4. T-cell activation in a tumour cell- T cell co-culture assay

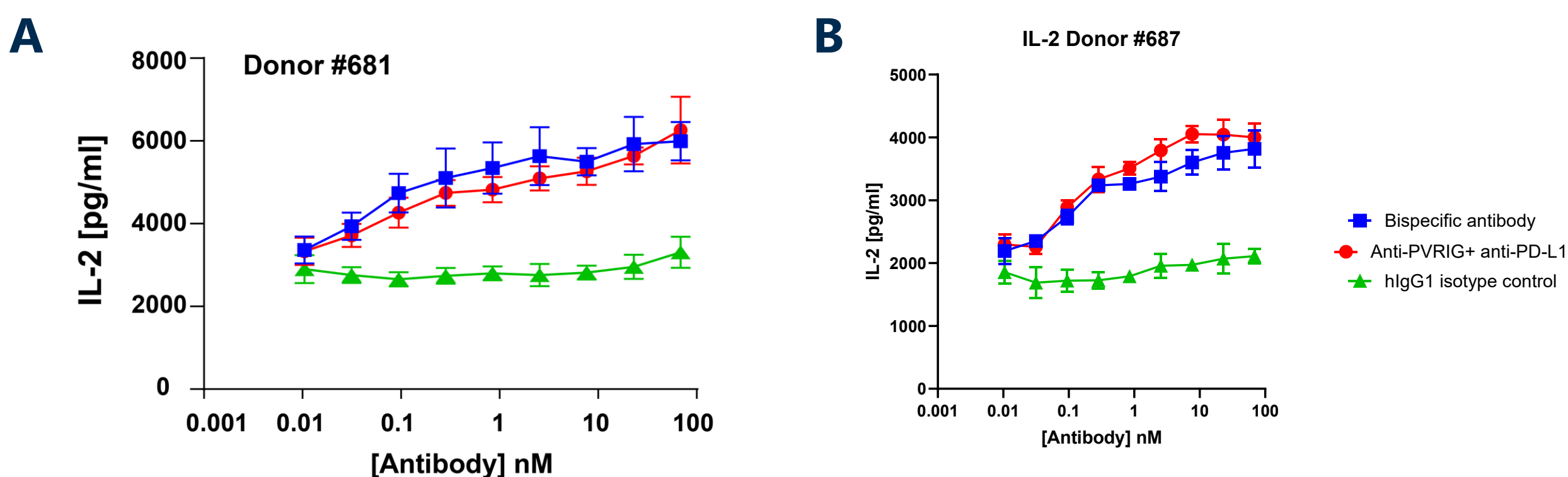


Figure 4. T-cell activation. Anti-PVRIG×PD-L1 bispecific antibody induced dose-dependent T-cell activation, as measured by IL-2 secretion, in a co-culture assay comprising OKT3-expressing tumor cells and primary T cells. Results from two independent donors as shown: (A) donor 681 and (B) donor 687.

Figure 5. PVRIG×PD-L1 bispecific antibody mediate PVRIG+ / PD-L1+ cell clustering

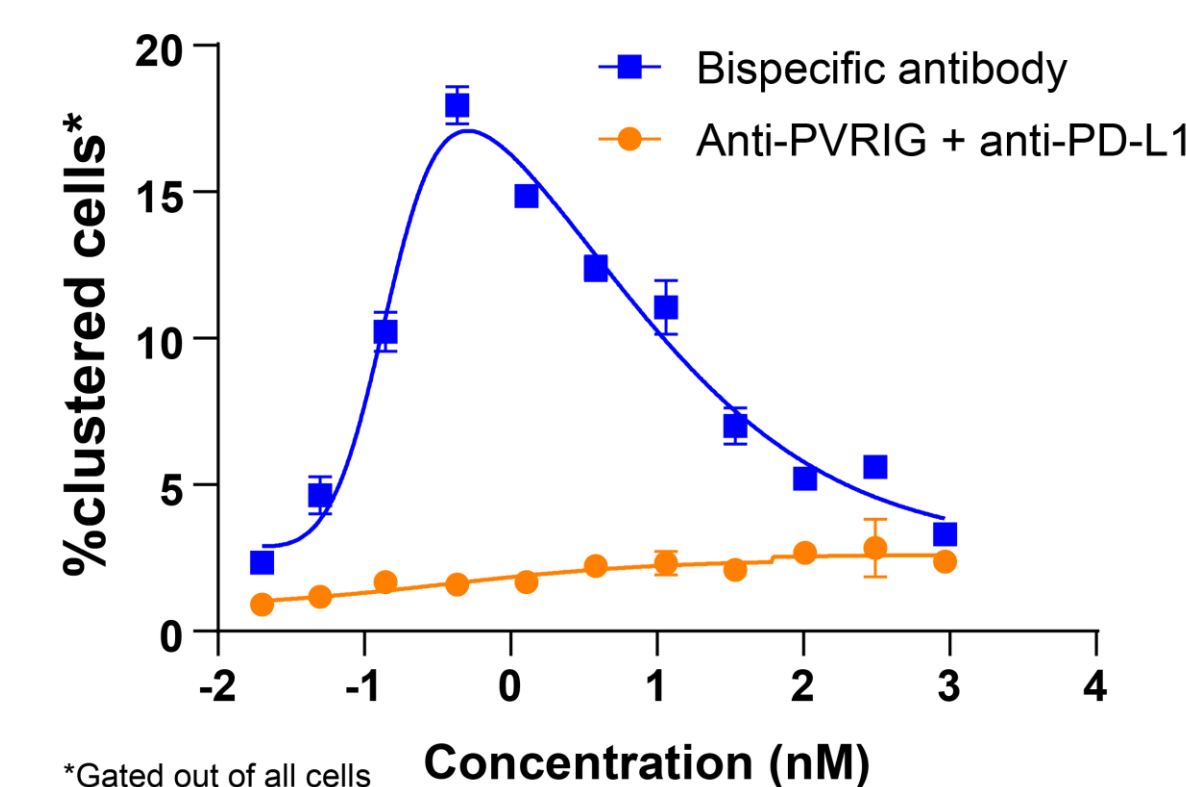


Figure 5. PVRIG×PD-L1 bispecific antibody-mediated cell clustering. The bispecific antibody induced clustering of engineered PVRIG+/PD-L1+ CHO cells. Cell clustering reached a maximum at intermediate antibody concentrations and decreased at higher concentrations.

Figure 6. In-vivo anti-tumour responses in humanized mice

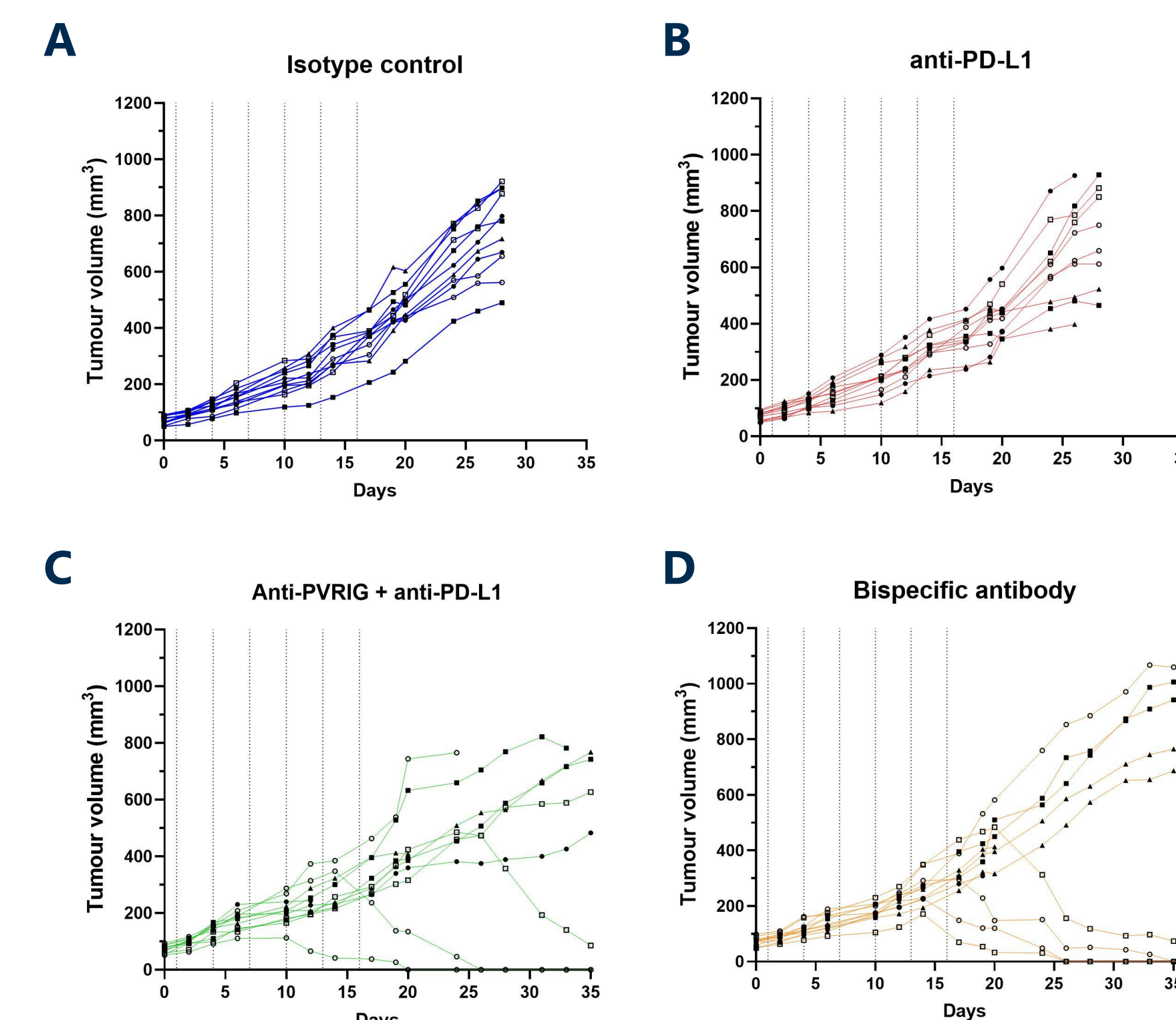


Figure 6. In vivo tumour response. CD34+ humanized mice were, n=8/9 per group, subcutaneously injected with a cancer cell line expressing both PD-L1 and PVRL2. Once the tumour volume reached between 50-100mm³ tumour-bearing mice were intraperitoneally dosed with A) Isotype, (B) anti-PD-L1, (C) anti-PVRIG + anti-PD-L1 combination treatment or (D) bispecific antibody. Bispecific antibody demonstrated improved tumour growth control.

References:

- Whelan et al (2019) PVRIG and PVRL2 Are Induced in Cancer and Inhibit CD8+ T-cell Function. *Cancer Immunol Res* 1 February 2019; 7 (2): 257–268
- Vaena et al (2021) COM701 with or without nivolumab: Results of an ongoing phase 1 study of safety, tolerability and preliminary antitumor activity in patients with advanced solid malignancies. *J Clin Oncol* 39, 2504.

Figure 7. Immune-related pathway response

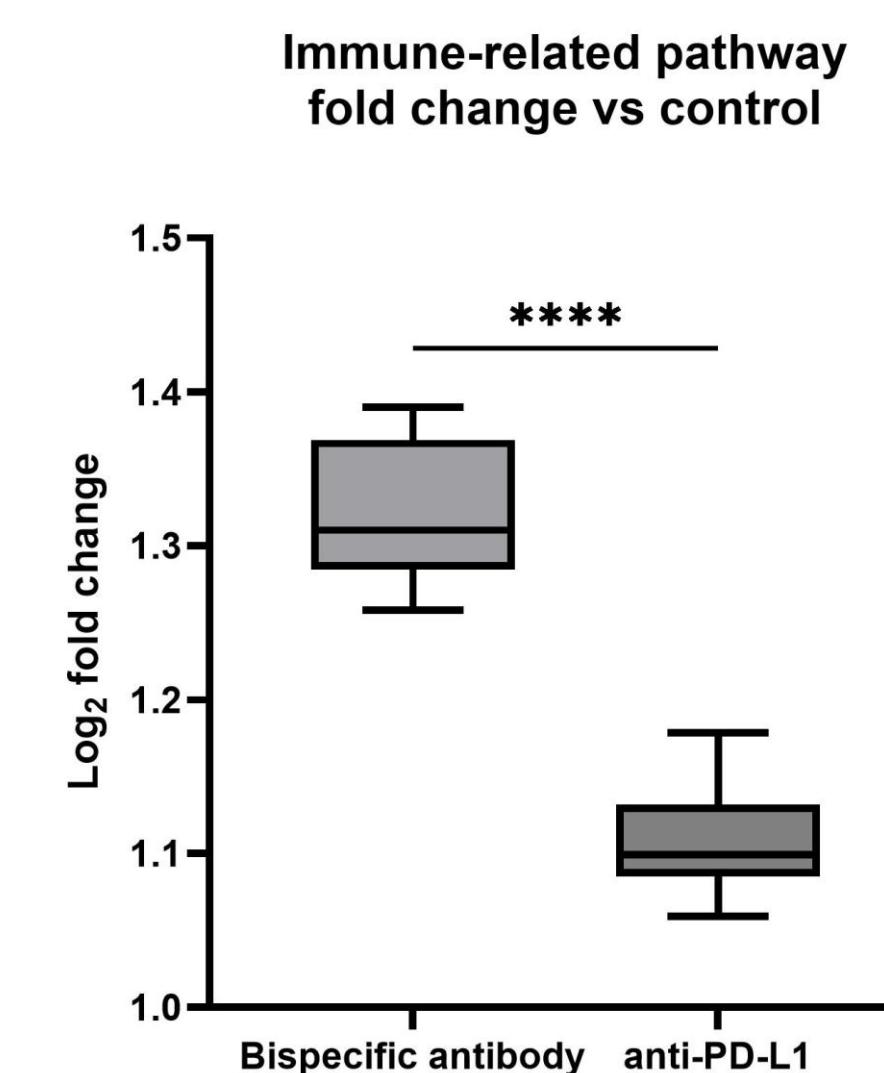


Figure 7. Bispecific antibody significantly upregulates immune-related pathways. Bulk RNA-seq was performed on tumours from CD34+ humanized mice (n=6) dosed three times with antibodies. Box plots show log₂ fold changes relative to control. The bispecific antibody demonstrated significant upregulation of genes associated with immune activation pathways compared to anti-PD-L1-treated mice.

Conclusions

- The novel tetravalent anti-PVRIG×PD-L1 bispecific antibody combines functional blockade of the PVRIG–PVRL2 and PD-1–PD-L1 interactions, supporting its proposed dual-checkpoint mechanism of action.
- The in vitro data demonstrated immune-activating potential including ADCC-associated activity and increased IL-2 secretion.
- The PVRIG×PD-L1 bispecific antibody induced greater cell clustering than the corresponding combination of monospecific antibodies, supporting the proposed ability to promote T cell-APC immune synapse formation.
- The bispecific antibody showed preclinical anti-tumour activity, including tumour regressions in a subset of animals and a significant upregulation of genes associated with immune activation.
- Together, these findings support the clinical development of anti-PVRIG×PD-L1 bispecific antibody.
- A Phase 1/2 clinical study (BICIDES), NCT07805252, has been initiated in Spain. For additional clinical data, see poster 2043eTiP.