Background Immune checkpoint blockade (ICB) therapies including anti-CTLA4 and anti-PD1/L1 antibodies have revolutionized cancer treatment landscape by eliciting durable remission. However, despite early response, most cancer patients eventually become resistant to the ICB therapies. The DNAM1 axis, an additional immunomodulatory pathway, is a potent regulator of innate and adaptive immunity. The immunoglobulin receptors TIGIT and PVRIG, which interact with their ligands PVR and PVRL2 in DNAM1 axis respectively, are reported to contribute to primary or acquired resistance to PD-1/L1 blockade. Here, we report the internally discovered D3L-002, an anti-TIGIT×PVRIG bispecific antibody (bsAb), bound to TIGIT and PVRIG simultaneously and effectively blocked the interaction between TIGIT/PVR and PVRIG/PVRL2. D3L-002 could restore the function of exhausted T/NK cells and result in potent anti-tumor activity.

Methods The affinity of D3L-002 was examined by surface plasmon resonance (SPR) method. Cellular binding and in vitro blocking assays were measured by FACS or ELISA. T cell activation was detected via TCR/NFAT signaling and IFN-γ cytokine secretion. NK cell function was examined by degranulation and target cell lysis. Antibodies’ efficacy was studied in syngeneic mouse tumor models.

Results D3L-002, a IgG1 tetravalent bsAb with symmetric IgG-single-chain variable fragment (scFv) structure, showed high binding affinity to the extracellular domain of human TIGIT (K_D < 1 nM) and PVRIG (K_D < 10 nM) respectively. D3L-002 bound to human TIGIT and PVRIG expressing cells with sub-nanomolar EC50 and was cross-reactive to respective cynomolgus homologs. Moreover, it demonstrated strong TIGIT/PVR and PVRIG/PVRL2 receptor-ligand interaction blocking activities in both cellular and protein level assays. This potent blocking translated well into enhanced functions in vitro. D3L-002 was able to activate TCR/NFAT signaling in Jurkat cells and showed better activity than anti-TIGIT and anti-PVRIG parental mAb alone, or their combination. D3L-002 could also enhance degranulation and cytotoxicity of primary NK cells and reinvigorate exhausted CD8+ T cells. In MC38 model, D3L-002 showed a trend of better anti-tumor efficacy than parental anti-TIGIT (P>0.05) and anti-PVRIG mAb (P<0.01). In addition, the combination of D3L-002 with anti-PDL1 mAb showed more potent efficacy than Atezolizumab monotherapy (P<0.01). MoA study indicated that D3L-002 monotherapy treatment depleted Treg, while combination with Atezolizumab induced CD8+ T cell proliferation in tumor microenvironment.

Conclusions D3L-002 is a novel TIGIT×PVRIG bsAb which demonstrated potent anti-tumor effect via TIGIT and PVRIG co-blocking in both in vitro and in vivo models. D3L-002 might provide a novel treatment approach for solid tumors and overcome resistance to current ICB therapies.

REFERENCES