Background: DuoBody®-PD-L1x4-1BB (GEN1046) is an investigational, potential first-in-class bispecific immunomodulatory antibody designed to elicit an anti-tumor immune response by simultaneous and complementary blockade of PD-L1 on tumor or immune cells and conditional 4-1BB stimulation on T cells and NK cells. Here we utilized a multi-omics approach to evaluate whether DuoBody-PD-L1x4-1BB could reverse T-cell exhaustion in vitro.

Methods: An in vitro mixed lymphocyte reaction (MLR) assay was developed, optimized, and validated, where healthy donor T cells were exhausted by repeated stimulation with anti-CD3/CD28 beads prior to co-culturing with allogeneic LPS-matured dendritic cells. Publicly available single cell RNA sequencing (scRNAseq) datasets were harmonized across multiple solid-tumor indications (including treatment-naïve and anti-PD-1 and/or anti-CTLA-4 pretreated samples) and analyzed for co-expression of PD-1 and 4-1BB on various immune-cell subsets based on their transcriptome signatures.

Results: In the T-cell exhaustion MLR assay, T cells showed increased expression of inhibitory receptors (e.g., TIM-3, LAG-3) and exhibited hyporesponsiveness for both proliferation and cytokine secretion upon restimulation with anti-CD3/CD28 beads, which was partially reversed by PD-1 blockade. DuoBody-PD-L1x4-1BB reinvigorated the exhausted T-cell response in vitro, as shown by restored IFNγ secretion. The effect of DuoBody-PD-L1x4-1BB in this assay was roughly two-fold higher to that of PD-1 blockade. When combined, DuoBody-PD-L1x4-1BB showed the potential to synergize with anti-PD-1 antibody treatment as cytokine secretion was further potentiated compared to each agent alone. More extensive molecular profiling from the T-cell exhaustion MLR assay will be presented. Using solid tumor public scRNAseq datasets, we demonstrated co-expression of 4-1BB and PD-1 on exhausted CD8+ T cells in the tumor microenvironment. Furthermore, in patients treated with agents that block the PD-1 pathway, an increase in exhausted CD8+ T cells expressing PD-1 was observed.

Conclusions: PD-1 and 4-1BB are co-expressed on exhausted CD8+ T cells within the tumor microenvironment in solid tumors and T cell dysfunction may represent a potential resistance mechanism to checkpoint inhibitors (CPI). DuoBody-PD-L1x4-1BB restored IFNγ secretion by exhausted T cells in vitro more potently than PD-1 blockade, which could be further potentiated by the combination with an anti-PD-1 antibody. Together, these results support evaluation of DuoBody-PD-L1x4-1BB in the post-CPI setting and the combination of tumor-targeted 4-1BB co-stimulation with PD-1 checkpoint blockade for the treatment of solid tumors. DuoBody-PD-L1x4-1BB is currently being investigated in an ongoing phase 2 clinical trial in NSCLC patients who have progressed on prior CPI therapy (NCT05117242).

Acknowledgements: These experiments were funded by Genmab A/S and BioNTech SE.