A novel multi-specific antibody targeting PD-L1-overexpressing cancers that stimulates antigen-committed CD8+ T cells through concomitant engagement of a T cell costimulatory receptor

Tea Gundel¹, Matthias Brock², Stefan Warnmuth³, Alexandre Simonin⁴, Christian Hess⁵, Eva Oswald⁶, Julia Tietz⁶, Julia Zeberer⁶, Dana Mahler⁶, Simone Muntwiler⁶, Benjamin Kärtner⁶, Belinda Wickhalden⁶, Antonia Pölderl⁶, Daniia Diem⁶, Teddy Beltrametti⁶, Robin Hoff⁶, Sebastian Meyer⁶

¹Numb Innovation AG, Einsektrastrasse 34, 8205 Waldenburg, Switzerland; ²Numb Therapeutics AG, Quarerstrasse 8B, 8808 Pfäffikon, Switzerland; ³Charles River Discovery Service Germany GmbH, Am Flughafen 12-14, 79106 Freiburg, Germany

Targeting PD-L1-overexpressing cells with therapeutic antibodies is a clinically validated strategy for the treatment of various solid tumors. In order to increase efficacy, PD-L1/PD-1 blocking agents are currently being applied in combination with additional immune checkpoint modulators (ICMAs). However, such combination therapies are often associated with considerable treatment-related adverse events, resulting in a severe therapeutic window and thereby limiting treatment efficacy. To maximize potency and improve the safety of ICM combination approaches, we sought to design a multi-specific molecule bearing two ICM domains that depletes PD-L1-overexpressing cancer cells via selective recruitment and stimulation of tumour-reactive effector T cells in the tumor microenvironment (TME). The molecule consists of three monovalent antibody variable domain fragments (Fv's) specific for PD-L1, human serum albumin, and a T cell costimulatory receptor fused in a single chain (PD-L1:HNAAcostim:His6:scDb:scFv). The monovalent antibody variable domain structure of the molecule ensures that clustering of the costimulatory receptor on effector cells can only arise when the molecule costimulatory binds to PD-L1 on the surface of target cells. The addition of a half-life-extending anti-HSA domain, meanwhile, not only enables convenient dosing but will also promote delivery of the molecule to the TME. Here, we demonstrate the successful production of a novel multi-specific antibody that potently blocks PD-L1/PD-1 signaling and elicits T cell costimulation solely in the presence of cells that overexpress PD-L1, as confirmed using a transgenic Jurkat reporter T cell line in co-culture with HT29 and HCC827 cancer cell-lines. We show that, in vitro, the molecule costimulates T cells considerably more selectively and potently than a first-generation, clinical-stage, multi-specific molecule that selectively forms immunological synapses between cancer cells and activated effector cells.

Figure 1: A) Cross-linking of T cell costimulatory receptors (Costims) with bivalent anti-PD-L1 IgG-C and Costim IgG-C results in Costim signaling exclusively in proximity of PD-L1 expressing tumor cells and thereby directs its activity to PD-L1 expressing tumor tissue. B) The PD-L1xCostim scDb-scFv induces Costim signaling more selectively and potently than the combination of PD-L1 IgG-C and Costim IgG-C. C) The PD-L1xCostim scDb-scFv more potently induces IL-2 production in PMBCs when compared to the combination of PD-L1 IgG-C and Costim IgG-C. D) Pharmacokinetic analysis to quantify PD-L1xCostim scDb-scFv in serum samples from animals in HCC827 xenograft models. E) The PD-L1xCostim scDb-scFv-2 more potently induced IL-2 production and IFNγ secretion than PD-L1 IgG-C, the Costim-IgG-C, or the combination of the two. In absence of anti-CD3 stimulation, scDb-scFv-2 demonstrated superior binding affinity to human PD-L1 compared to PD-L1xCostim scDb-scFv-1. B) The PD-L1xCostim scDb-scFv-2 more potently induced IL-2 production and IFNγ secretion than PD-L1 IgG-C, the Costim-IgG-C, or the combination of the two. In absence of anti-CD3 stimulation, scDb-scFv-2 demonstrated superior binding affinity to human PD-L1 compared to PD-L1xCostim scDb-scFv-1. C) The PD-L1xCostim scDb-scFv-2 more potently induced IL-2 production and IFNγ secretion than PD-L1 IgG-C, the Costim-IgG-C, or the combination of the two. In absence of anti-CD3 stimulation, scDb-scFv-2 demonstrated superior binding affinity to human PD-L1 compared to PD-L1xCostim scDb-scFv-1. D) The PD-L1xCostim scDb-scFv-2 more potently induced IL-2 production and IFNγ secretion than PD-L1 IgG-C, the Costim-IgG-C, or the combination of the two. In absence of anti-CD3 stimulation, scDb-scFv-2 demonstrated superior binding affinity to human PD-L1 compared to PD-L1xCostim scDb-scFv-1. E) The PD-L1xCostim scDb-scFv-2 more potently induced IL-2 production and IFNγ secretion than PD-L1 IgG-C, the Costim-IgG-C, or the combination of the two. In absence of anti-CD3 stimulation, scDb-scFv-2 demonstrated superior binding affinity to human PD-L1 compared to PD-L1xCostim scDb-scFv-1.

Conclusions

• The PD-L1xCostim scDb-scFv induces Costim signaling exclusively in the proximity of PD-L1 expressing cells and thereby directs its activity to PD-L1 expressing tumor tissue.
• The requirement for a secondary signal through T cell receptor (TCR) stimulation was demonstrated by the PD-L1xCostim scDb-scFv in anti-tumor T cells.
• PD-L1xCostim scDb-scFv therapy in humanized mouse models of NSCLC resulted in stronger reduction of tumor growth than therapy with PD-L1 IgG and Costim IgG. This correlated with a higher degree of CD8+ T cell infiltration (as shown).
• In humanized NOD mice, the PD-L1xCostim scDb-scFv showed dose-dependent in vivo efficacy with a terminal half-life of approximately 40 hours.

PD-L1xCostim scDb-scFv-2 therapy resulted in stronger reduction of tumor growth and higher response rates than therapy with PD-L1 IgGs or Costim IgGs (not shown).

Costim signaling only occurs in combination with TCR stimulation and thereby directs its activity to PD-L1 expressing tumor tissue. This is further supported by the data shown.

Costim signaling occurs exclusively in proximity of PD-L1 expressing tumor cells and thereby directs its activity to PD-L1 expressing tumor tissue.