

PARTNERS FOR BISPECIFICS: COMBINATORIAL APPROACHES TO AUGMENT T-CELL FUNCTION AND MITIGATE EXHAUSTION

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Background T-cell engaging bispecific antibody molecules (BsAb) redirect T cells towards antigen-expressing cells and have shown efficacy in various B-cell malignancies.¹⁻³ The CD19xCD3 BsAb blinatumomab was the first in class to be approved in the setting of R/R BCP-ALL with a response rate of 43%.⁴ Evaluating mechanisms of resistance to bispecifics has been hampered by a lack of an appropriate model to mimic the clinical application of continuous BsAb exposure over several weeks to months. We developed an *in vitro* model system to simulate the clinical administration of blinatumomab as continuous infusion over 28 days and demonstrated that continuous exposure to a BsAb induces T-cell exhaustion.⁵ To identify partners for bispecifics to mitigate exhaustion, a diverse toolbox of off-the-shelf immunomodulating agents was evaluated. Using our *in vitro* model system, we explored the potential of BsAb treatment in combination with i) the tyrosine kinase inhibitor dasatinib, ii) the immunomodulator lenalidomide and iii) aPD-1/aPD-L1 checkpoint blockade to enhance T-cell function (figure 1).

Methods Healthy donor T cells were cocultured with CD19⁺ OCI-Ly1 cells and continuously stimulated with a CD19xCD3 BsAb alone or in combination with dasatinib (12.5 nM), lenalidomide (10 µg/ml), nivolumab (10 µg/ml) or atezolizumab (10 µg/ml) for 28 days. Every 7 days, T cells were isolated and characterized in functional assays: (1) Expression of inhibitory receptors (IRs; PD-1, Tim-3, LAG-3), (2) BsAb-mediated cytotoxicity against hCD19-Ba/F3 cells after 72h, (3) T-cell expansion as fold change of CD2⁺ counts, (4) BsAb-mediated cytokine secretion measured via intracellular staining or in supernatants.

Results Dasatinib reduced IR co-expression and led to an increased proliferative and cytotoxic potential of T cells after 14 days of BsAb stimulation. Furthermore, it strongly increased granzyme B expression and secretion of IL-2 and IFN-g. Lenalidomide did not affect IR co-expression, but increased cytotoxicity of T cells after 14 and 21 days of BsAb stimulation. Additionally, it increased IL-2 and IFN-g secretion. Interestingly, both nivolumab and atezolizumab did not enhance T-cell function, suggesting a minor role of the PD-1/PD-L1 axis in the induction of exhaustion in our model system.

Conclusions Together, our findings highlight that T-cell function during BsAb stimulation *in vitro* strongly improves by combinatorial treatment with dasatinib or lenalidomide. Albeit checkpoint inhibitors can boost T-cell responses, they could not ameliorate BsAb-induced T-cell exhaustion. Our data supports combinatorial approaches of BsAbs and small molecules to achieve durable T-cell responses in patients.

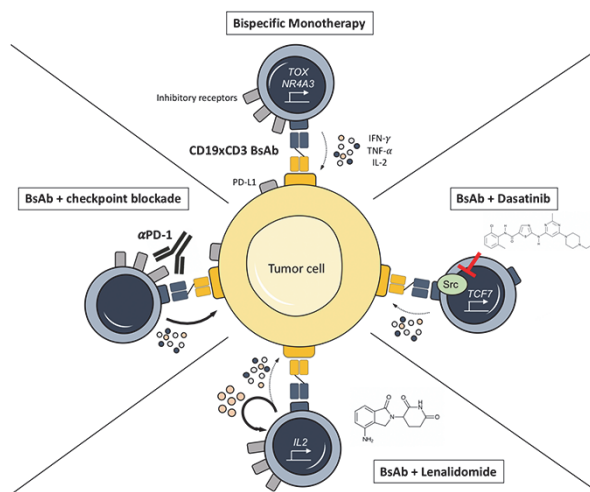
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Ethics Approval Peripheral blood (PB) were collected from healthy donors (HDs) after written informed consent was received in accordance with the Declaration of Helsinki and approval was granted by the Institutional Review Board of the Ludwig-Maximilian-Universitaet (Munich, Germany; ID 216-08).



Abstract 897 Figure 1 Partners for Bispecifics

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