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IMMUNOBIOLOGY AND IMMUNOTHERAPY

Comment on Zhao et al, page 1790

Optimized CD19/CD22/CD3 antibody

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In this issue of *Blood*, Zhao et al[1] presented an optimized CD19/CD22/CD3 trispecific antibody and demonstrated its antitumor potential in immune escape and patient derived xenograft (PDX) mouse models of B-cell malignancy.

Bispecific antibodies retargeting T cells to tumor cells have become a recognized tool for cancer therapy, especially for hematological malignancies. Blinatumomab (CD19/CD3) is a prominent example.[2] This development has spurred the engineering of bispecific antibodies with diverse formats.[3] However, tumor heterogeneity and antigen loss, as part of the tumor escape and resistance mechanism, remain major challenges.[4,5] The generation of trispecific antibodies, targeting two different tumor-associated antigens, is an extension of the concept, expected to overcome these problems by improving tumor selectivity and specificity.[6] However, the design of trispecific antibodies forming immunological synapse is challenging, due to the individual differences in target size and epitope position. In consequence, there is no "one best format," and individual solutions are required.

Zhao et al reported here the generation of a novel (CD19/CD22/CD3) trispecific antibody with optimized configuration by a site-specific fusion approach. Furthermore, the therapeutic advantage of the trispecific antibody over corresponding bispecific antibodies was demonstrated in xenograft tumor mouse models with varied CD19/CD22 expression profiles. In the first part of the study, Zhao et al generated bi- and trifunctional antibodies composed of a CD3-directed Fab fragment, a CD19-directed single-chain Fv (scFv), and a CD22-directed nanobody. The scFv and the nanobody were fused to the heavy and light chain of the Fab fragment, respectively. Fusion either to the N-terminus or C-terminus of the Fab chains led to bi- and trispecific antibody variants differing in the relative position and proximity of the binding units. In vitro, all variants with one exception performed as intended, mediating targeting dependent cytotoxicity of activated T cells. Fusion of the CD22- and CD19-binding units to the C-terminus of the Fab fragment (ie, opposing the CD3-binding site) appeared most favorable. Quantitative analysis of immunological synapses and cytokine release (interleukin 2 [IL-2]/interferon γ [IFN-γ]/tumor necrosis factor α [TNF-α]) from retargeted T cells supported this finding. Interestingly, the authors opted for a design without Fc region, combining diverse small antibody formats, where linker design becomes a crucial factor. Linker length and configuration can be expected to influence the distance and orientation of the binding units and impact the stability, expression, and bioactivity of the molecule.[7] For N-terminal fusion a rigid peptide linker from pyruvate dehydrogenase was used, and for C-terminal fusion a flexible $(G_4S)_3$ linker was used. The rationale of different linker usage was not stated. Comparative analysis of the optimized trispecific antibody with the corresponding bifunctional antibodies showed similar binding and cytotoxic activity on single-target expressing cell lines and superior retargeting and stimulatory activity on dual target expressing cell lines. Thus, the trispecific antibody not only showed the capacity to replace the bispecific antibodies, but also added value by enhancing the retargeting potency on tumor cells expressing both antigens. Importantly, the in vitro studies were conducted with a panel of tumor cell lines and primary B-cell acute lymphoblastic leukemia (B-ALL) tumor cells with different target expression levels. Thus, the retargeting-activity of the antibodies was shown in a broad range of target densities.

Next, the antitumor activity of the optimized trispecific antibody was assessed in humanized tumor mouse models. Immunodeficient NCG mice were injected with

tumor target cells and human T cells. Antibodies were administrated daily for a week, and tumor burden and survival were determined. In the model with Nalm6-luc cells ($CD19^+/CD22^+$), treatment with the trispecific antibody was more effective than the treatment with the individual or combined bispecific antibodies. The effect was even more pronounced in an immune evasion model with a mixture of Nalm6-KO19 and Nalm6-KO22 cells (ie, tumor cells expressing only CD22 and CD19, respectively, mimicking tumor heterogeneity and antigen loss). Comparison of the trispecific antibody and an in-house made blinatumomab confirmed the superior efficacy of the trispecific antibody, although the antitumor effect was less pronounced, probably due to the use of freshly isolated peripheral blood mononuclear cells instead of activated T cells. Finally, the evaluation was completed in a PDX model with primary B-ALL cells ($CD19^{medium}/CD22^{low}$). Also, here, strongest effects in delaying tumor cell regrowth and prolonging the survival were obtained by the trispecific antibody. These are impressive results corroborating for the first time the trispecific antibody concept for the CD19/CD22/CD3 constellation. The focus on the CD19/CD22 expression profile makes the data particularly interesting. Further studies will be needed to investigate the pharmacokinetics and pharmacodynamics. Treatment-induced T-cell stimulation was shown by an increase in IL-2/IFN-γ/TNF-α serum levels. The trispecific antibody induced significantly higher cytokine levels than the combination of bispecific antibodies. There was no weight loss observed in mice treated with the trispecific antibody. However, analysis of toxicity was limited, and the cytokine release syndrome is a major concern for this treatment strategy.[8] Thus, further studies will be needed. Current developments in the bispecific antibody field show that by decreasing the affinity of the CD3-specific antibody, the cytokine release of targeted T cells can be reduced without compromising their cytotoxic activity.[9] This might be considered as an option for further development.

Overall, the study of Zhao and colleagues provides an important contribution to the field of trispecific antibodies and presents a potential therapeutic option for B-ALL with heterogenous CD19 expression.

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CLINICAL TRIALS AND OBSERVATIONS

Comment on Soumerai et al, page 1822

Tackling ALK-positive LBCL

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In this issue of *Blood*, Soumerai et al present the first patient-derived xenograft (PDX) mouse models of anaplastic lymphoma kinase (ALK)-positive large B-cell lymphoma (LBCL) to investigate novel therapeutic approaches for this rare aggressive lymphoma subtype.[1] The authors show that the next-generation ALK inhibitors alectinib and lorlatinib have promising activity in these preclinical in vivo PDX models and in intensively pretreated patients with relapsed/refractory ALK-LBCL.

ALK-LBCL is a very rare aggressive B-cell lymphoma subtype with immunoblastic-plasmablastic morphology.[2] The lymphoma cells are characterized by plasmacytic differentiation lacking expression of classical B- and T-cell markers. Thus, ALK-LBCLs belong to the rare group of $CD20^-$ B-cell lymphomas.[3] ALK-LBCLs are further characterized by strong granular cytoplasmic expression of ALK, caused by oncogenic *ALK* gene fusions, with the t(2;17) translocation being the most frequently detected genetic aberration.[4] Overall, only a few cases and small retrospective series of patients with ALK-LBCL have been reported in the literature. Following conventional cyclophosphamide, hydroxydaunorubicin, oncovin, and prednisone-based front-line chemotherapy, patients with ALK-LBCL frequently relapse with dismal outcomes.[3] Treatment of patients with relapsed/refractory disease with the ALK inhibitor crizotinib was reported to induce only short-term remissions.[5] Therefore, a significantly better understanding of the biology of this entity is required to develop novel therapeutic approaches for these high-risk patients.

Soumerai et al succeeded in creating the first PDX models of ALK-LBCL by implanting lymphoma cells from ALK-LBCL refractory patients in nonobese diabetic scid γ mice. Engrafted lymphomas maintained the same oncogenic