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

molecular alterations detected in the primary lymphoma tissue. Treatment with the next-generation ALK inhibitors lorlatinib and alectinib resulted in significant tumor inhibition when compared with mice treated with vehicle only. Remarkably, these encouraging results were directly translated into the clinic by consecutively treating four refractory ALK-LBCL patients with alectinib. Intriguingly, 3 of 4 patients achieved a complete remission, with 2 patients maintaining response following allogeneic stem cell transplantation. Notably, the 1 patient who showed progressive disease after treatment with alectinib, monotherapy with the third-generation ALK inhibitor lorlatinib induced a complete remission.

This manuscript impressively illustrates how PDX models can be used to test novel therapeutic strategies for rare diseases and how these results can successfully be translated into clinical practice. The promising effect of the ALK inhibitors lorlatinib and alectinib, first discovered in the established PDX models, induced complete remissions in refractory ALK-LBCL patients. Although tumors may quickly evolve when passaged several times, comprehensive analyses have previously shown the reproducibility and translatability of PDX models.[6] Particularly for rare cancer entities, PDX models provide an important tool to explore the underlying biology and to evaluate novel targeted therapies.

At this stage, the molecular mechanisms that cause acquired crizotinib resistance and how the next-generation ALK inhibitors alectinib and lorlatinib overcome this resistance are still being explored. A genome-wide screen in ALK-positive anaplastic large-cell lymphomas recently revealed that loss of the phosphatases PTPN1 and PTPN2 leads to crizotinib resistance by activating SHP2, JAK-STAT, and MAPK signaling.[7] Accordingly, combining ALK- and SHP2-inhibitors synergistically inhibited wild-type as well as PTPN1/PTPN2 knockout ALK-positive anaplastic large-cell models.[7] Whether the same molecular mechanisms also are involved in crizotinib resistance in ALK-LBCLs is currently unknown. Thus, a better molecular understanding will be key to further improve targeted therapies in ALK-LBCLs.

Although the findings hold great potential to improve outcome of patients with ALK-LBCL, these results need to be further confirmed in larger patient cohorts, ideally within prospective clinical trials. This, however, will only be possible with a major international effort due to the rarity of the disease. Therefore, international networks and close collaborations are crucial to overcome this obstacle and to further improve both our understanding of the molecular pathogenesis of ALK-positive LBCL as well as treatment strategies for affected patients.

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