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detailed results are shown for 4 of them, specifically, compounds 5, 6, 7, and 8. Using a kinome assay, the authors show that PROTAC modification leads to loss of tight specificity for ruxolitinib and baricitinib, which are normally JAK1/JAK2 specific. In addition to the 4 JAKs, PROTACs gained specificity for YSK4, MAP3K2, and MAP3K3 and other kinases.

The different PROTACs were then used in cytotoxicity and functional assays in ALL cell lines and xenograft cell lines from several Ph-like ALL patients. Unlike ruxolitinib, which failed to inhibit growth, compounds 5, 6, 7, and 8 induced complete inhibition at concentrations $<$100 nM. Toxicity in ALL cell lines correlated with the presence of rearranged *IGH-CRLF2* and *JAK2* mutant cells. Compound 8 was the best. Protein degradation was measured by western blots and the specificity varied among compounds between JAKs and the 2 targets of CRBN, namely G_1 to S phase transition 1 (GSPT1), which is involved in effective translation termination of nascent protein chains and the transcription factor IKZF1 (IKAROS family zinc finger 1). The significance of the degradation of this transcription factor in the tumor remains to be determined in vivo. In addition, whether baricitinib-derived PROTACs retain the ability to regulate Numb-associated kinases will also be of interest.

The activity of PROTACs appears to rely on a combination of the on-target effect on JAK2 and, for some of the compounds, the off-target effects of GSPT1. In the MHH-CALL-4 cell line modified to harbor a GSPT1 mutant (G575N) that is not sensitive to CRBN-mediated degradation, the effect of compound 6 was reduced, but the effect of compound 8, which does not target GSPT1, was not affected. Also, in MHH-CALL-4 cells, compounds 5, 6, and 7 inhibited JAK2-STAT5 phosphorylation, and this was more evident when cells were stimulated with TLSP. Compound 7 was assessed in an in vivo system where human primary *CRLF2* rearranged/*JAK2* wild-type ALL cells expressing YFP, and firefly luciferase were injected in immunodeficient mice. Compound 7 led to degradation of JAK1, JAK2, JAK3, TYK2, and GSPT1, as shown by western blots, and accordingly, led to a significant, but not spectacular, reduction of tumor growth. The tumor reduction in vivo occurred mainly in the peripheral blood and spleen and only to a lower extent in the marrow. Circulating tumor cells may require higher JAK2 signaling levels, or bone marrow penetration may play a role in this tropism.

Last, but not least, ex vivo assessment was performed on a series of xenograft cell lines with different mutations using the 4 compounds (5, 6, 7, 8) vs JAK2 inhibitors (CHZ868, ruxolitinib, and baricitinib) or the thalidomide analog lenalidomide. Interestingly, compound 8 is JAK2 specific and showed good results in some, but not all, xenograft cell lines from ALL patients. Stronger inhibition was exerted by the other compounds, which also degrade GSPT1.

The results presented here provide a pathway forward for the use of PROTACs against JAK-mutated malignancies and offer a new perspective on how current inhibitors can be rationally modified. This approach may be relevant for several subtypes of ALL, where multiple activating mutations in the *IL7R* and *CRLF2* have been reported that lead to activation of JAK1/JAK2.[10] Combining this approach with targeting other key events in oncogenesis holds real potential for engineering new therapies against some of the most difficult to treat malignancies.

Conflict-of-interest disclosure: The authors declare no competing financial interests. ■

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CLINICAL TRIALS AND OBSERVATIONS

Comment on Hitzler et al, page 2337

AraC: up for down

Shlomit Barzilai-Birenboim and Shai Izraeli[1] | Schneider Children's Medical Center of Israel

In this issue of *Blood*, Hitzler et al[1] demonstrate that high-dose cytarabine (HD-AraC) is required for the best likelihood for cure in the majority of children with Down syndrome with myeloid leukemia (ML-DS).

ML-DS is a unique subtype of leukemia occurring in children with DS younger than 4 years of age. It is always characterized by somatic mutations in the megakaryocytic-erythroid transcription factor *GATA1*, creating a GATA1 short protein (GATA1s) lacking its aminoterminal. Additional somatic mutations, mostly activating JAK-STAT signaling and inactivating chromatin modifiers, as well as additional unbalanced chromosomal copy number changes, are often observed in the leukemic cells.[2,3] Unlike acute myeloid leukemia (AML) in children

without DS, ML-DS is highly sensitive to chemotherapy, with excellent cure rates.[4,5] It is particularly sensitive to the nucleoside analog cytarabine. Research from Taub's laboratory suggests that the absence of the full-length GATA1 leads to higher intracellular concentration and activity of cytarabine.[6]

High toxicity from infection has been a limiting factor in the treatment of children with DS and leukemia (both AML and acute lymphoblastic leukemia). Children with DS and leukemia are particularly susceptible to respiratory viruses, in addition to being particularly susceptible to bacterial infections.[4,7] Many of these severe infections occurred during HD-AraC courses in the prior Children's Oncology Group AAML0431 protocol.[4] This observation and previous reports of the ultrasensitivity of ML-DS to lower doses of cytarabine[8,9] led Hitzler et al to eliminate the HD-AraC block from therapy for the majority of patients with ML-DS with an excellent response to induction therapy. This resulted in a reduction of the total dose of cytarabine from 27.8 g/m^2 in the AAML0431 to 3.8 g/m^2. In addition, because of the rarity of central nervous system (CNS) involvement by ML-DS, intrathecal (IT) cytarabine was reduced from 2 doses to a single dose.

The interim analysis revealed decreased survival compared with the previous protocol (2-year event-free survival [EFS] of 85.6% compared with 93.5% in AAML0431), highlighting the necessity of HD-AraC for the optimal outcome in ML-DS.[1] Although the reported percentages appear disappointing, it is important to note that EFS of 85% and overall survival of 91% for children with ML-DS, while receiving only an intermediate dose of cytarabine, is remarkable, and achieving so high a cure rate is a highly desirable goal for children with non-DS AML. Thus, children with ML-DS and severe comorbidities, for whom therapy for the AML is fraught with difficulty, might benefit from a treatment protocol lacking the more toxic HD-AraC. For other patients, additional work is needed to reduce the problem of infectious complications.

Relapse/refractory ML-DS, although rare, remains a major unmet need. In the current protocol, 12 standard risk (SR) patients (out of 114) experienced a relapse, 11 in the bone marrow and one in the CNS. Only 2 survived, despite intensive relapse treatment and stem cell transplant. CNS relapses are extremely rare in ML-DS, and the occurrence of isolated CNS relapse could suggest that the 1 IT cytarabine dose administered in the current protocol was insufficient. A complex karyotype and detectable GATA1s by next-generation sequencing were more frequent among SR patients who relapsed. Future studies should focus on the pathogenesis of relapse of ML-DS, on its early detection, possibly by next-generation sequencing quantification of GATA1s levels during remission, and on novel therapies for this extremely poor prognosis subgroup of ML-DS.

Conflict-of-interest disclosure: The authors declare no competing financial interests. ■

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HEMATOPOIESIS AND STEM CELLS

Comment on Kollman et al, page 2347.

STAT5B, the dominant twin, in hematopoietic stem cells

Yuhong Chen and Demin Wang | Versiti Blood Research Institute

In this issue of *Blood*, Kollmann et al used single-cell RNA sequencing (scRNA-seq) to identify a unique and dominant role for STAT5B in self-renewal of hematopoietic stem cells (HSCs) and leukemia stem cells (LSCs). Moreover, they found that the cell surface marker CD9 is an important STAT5B target gene, implicating CD9 as a novel therapeutic target for STAT5-driven leukemia.[1]

STAT5A and STAT5B are 2 of the 7 members of the STAT family, and both are activated by a broad spectrum of cytokines and growth factors.[2] STAT5A and STAT5B are 95% identical at the amino acid sequence level. They are commonly believed to have redundant roles in the hematopoietic system, because both are required for lymphocyte development and T-cell proliferation and function.[3-5] Both STAT5A and STAT5B are important for the repopulating potential of HSCs[6]; However, it is not known whether they play distinct roles in HSCs or uniquely activate target genes in rare HSC populations. Kollmann et al identified the unexpected predominant role of STAT5B in controlling the

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