Runx1 blocks CBFβ-SMMHC–driven leukemia development. Given the block in leukemia development, the investigators hypothesized that LICs were negatively affected by loss of *Runx1*. To test this hypothesis, they quantified a previously described LIC population termed abnormal myeloid progenitors (AMPs; Lin^- $c\text{-}Kit^+Sca1^-CD34^-FC\gamma II/III^+$).[8] Importantly, the investigators found that loss of *Runx1* hindered the development and maintenance of the AMP population, implying that *Runx1* is required for CBFβ-SMMHC–driven LICs (see figure panel A).

Given the prior paradigm that CBFβ-SMMHC prevents RUNX1 from binding DNA, the investigators performed a series of detailed molecular studies to delineate the requirement for CBFβ-SMMHC and RUNX1 in the LIC population. Using bulk and single-cell RNA-sequencing, the investigators identified 2 hallmarks of LICs generated by CBFβ-SMMHC but lacking RUNX1. First, these LICs showed reduced expression of genes known to be critical drivers of the cell cycle, including the G2M checkpoint and targets of c-Myc and E2F. Second, they showed increased expression of genes required for normal myeloid differentiation. Collectively, these data imply that CBFβ-SMMHC works collaboratively with RUNX1 to mediate the proliferative signal and differentiation block, which are hallmarks of the Gilliland model for AML development.

Next, to definitively establish how CBFβ-SMMHC and RUNX1 cooperate to control gene expression, the investigators demonstrate that RUNX1 recruits CBFβ-SMMHC to chromatin, and they work in concert to activate gene expression, including genes that control proliferation (see figure panel B). This is in sharp contrast to prior literature that reported that CBFβ-SMMHC operates predominantly to repress gene expression. These important mechanistic studies connect how CBFβ-SMMHC and RUNX1 work collaboratively to directly regulate genes critical to LICs. It is important to note that these data represent 1 of the important ways that CBFβ-SMMHC operates during leukemia development, but it may be that the existing model of CBFβ-SMMHC sequestering RUNX1 occurs in parallel and is also critical to leukemogenesis. As such, it may be that, although CBFβ-SMMHC sequesters most RUNX1 during leukemia development, a small amount of active RUNX1 is required for transformation.

Perhaps most importantly, this work has the potential to inform targeted therapies. Although current treatment regimens rely on high-dose chemotherapy, an ongoing concern remains that conventional therapeutics are not effective at targeting LICs, which can cause relapse even when present in miniscule amounts. Prior studies have identified a small molecular inhibitor of the CBFβ-SMMHC:RUNX1 protein:protein interaction as a potential therapy for inv(16) AML.[9,10] Zhen et al elevate this potential approach by demonstrating that it could directly target LICs, an important driver of relapse. In doing so, the investigators provide a path forward to develop more effective and less toxic therapies for patients with inv(16) AML.

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CLINICAL TRIALS AND OBSERVATIONS

Comment on Bartlett et al, page 2401

Hodgkin lymphoma: outsmarting HRS cells

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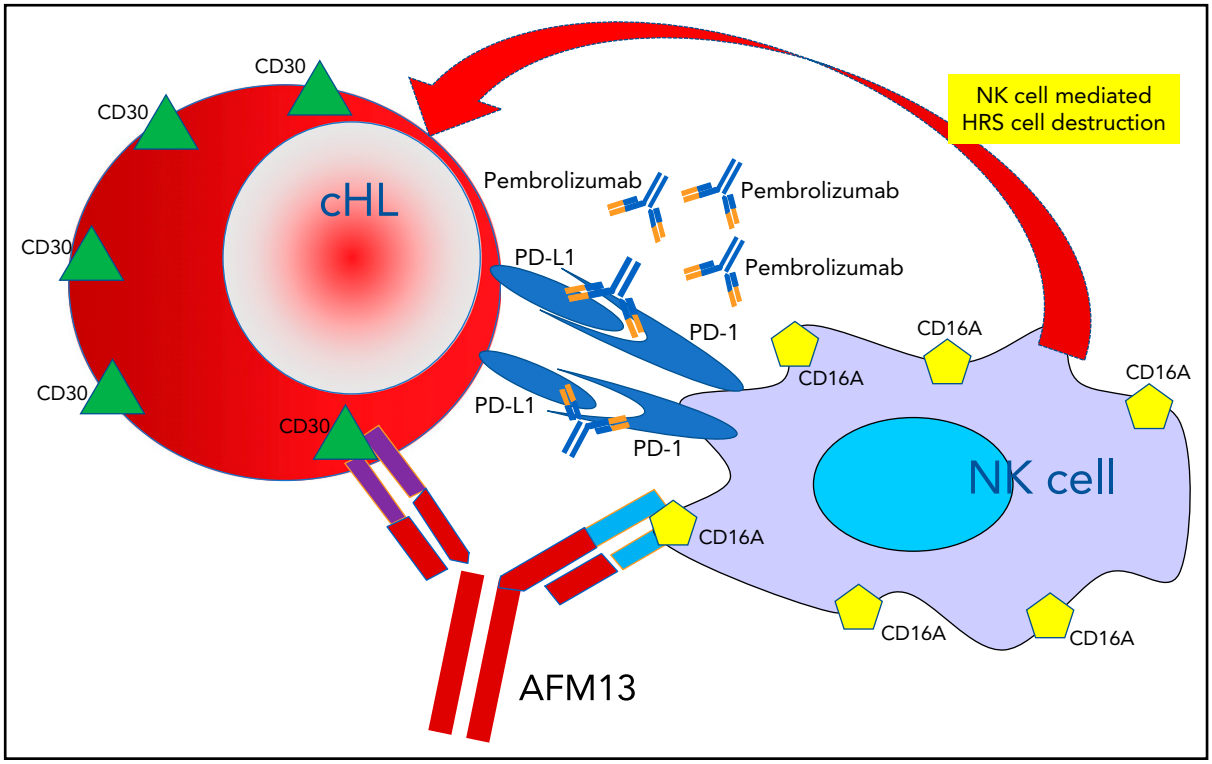
In their article in this issue of *Blood* describing the use of a new bispecific antibody, AFM13, plus a checkpoint inhibitor, pembrolizumab, for patients with recurrent or refractory classic Hodgkin lymphoma (cHL), Bartlett and her coauthors provide dramatic evidence of the treatment potency that can be achieved with a 3-pronged therapeutic attack employing 2 biologic agents targeting specific immunologic characteristics of cHL Hodgkin and Reed-Sternberg (HRS) cells.[1] They show how to forcefully recruit the patient's immune system's killer cells while at the same time enhancing their cytodestructive potency. Their work nicely demonstrates how recent progress in the treatment of cHL has been carefully built on an increasingly exquisite understanding of the molecular biology underlying the immunology of malignant HRS cells and their dynamic interaction with both specific and nonspecific effector cells in the immune system.

The universal, persistent expression of the CD30 antigen on the surface of cHL cells provides an attractive target for specially crafted antibodies, a property that has been effectively exploited by the development of the antibody-drug conjugate



One arm of the bispecific antibody AFM13 binds to CD30 surface antigens on cHL HRS cells, and the other arm binds to CD16A surface antigens on NK cells, leading to NK cell–mediated destruction of the HRS cells. This therapeutic attack is dramatically enhanced by adding pembrolizumab, which blocks the protection from immune attack that HRS cells employ by elaborating PD-L1.

brentuximab vedotin, which employs a CD30 targeted antibody to deliver the microtubule disrupting effector toxin monomethyl aurostatin E to the target HRS cells.[2,3] The novel bispecific antibody AFM13 targets that same CD30 antibody with 1 arm of a monoclonal antibody, while at the same time, the other arm binds to the CD16A cell surface antigen expressed on natural killer (NK) cells (see figure), thus, dragging the NK cells into tight proximity with HRS cells. Once there, however, the NK cells have only limited capacity to injure the HRS cells, presumably at least in part because the HRS cells express program death-ligand 1 (PD-L1), which blunts the NK cell's ability to disrupt the HRS cells, accounting for the limited activity of AFM13 as a single agent (overall and partial response rates only 12%).[4] By adding pembrolizumab, a checkpoint inhibitor, to the treatment mix, Bartlett and her coinvestigators cleverly overcome this NK cell blunting, releasing the destructive potential of the NK cells, resulting in impressive overall and complete response rates of 88% and 42%, respectively, in 30 patients with advanced, recurrent, or refractory cHL that had proven resistant to standard therapy, including brentuximab vedotin in 43% and autologous hematopoietic stem cell transplant in 40% of the patients. Gratifyingly, reflecting the biologic specificity of the treatments and resultant ability to avoid off target toxicity, this therapeutic potency is achieved with modest toxicity, primarily consisting of infusion reactions, reaching grade 3 in only 13% of patients.

Progress in the treatment of advanced stage cHL has unfolded in 3 major therapeutic eras. First, multidrug combinations of cytotoxic chemotherapeutic agents, such as ABVD (doxorubicin, bleomycin, vinblastine, and dacarbazine), demonstrated that even far advanced disease can be cured.[5,6] Next, highly intensified chemotherapy was shown to be able to increase cure rates either when employed for disease in relapse despite primary chemotherapy[7,8] or in first-line treatments, such as escalated BEACOPP (bleomycin, etoposide, doxorubicin, cyclophosphamide, vincristine, prednisone, and procarbazine).[9] In both eras increased efficacy came with the high price of increased short- and long-term toxicity. Now, in the third major era, that of active immunologic treatment of cHL, additional progress is being achieved by exploiting insights into the basic biology of HRS cells and their complex interaction with immunologic effector cells in the tumor microenvironment. Results achieved with checkpoint inhibitors, such as nivolumab and pembrolizumab, which target and block the inhibition of immune cell destruction of HRS cells by cytotoxic effector cells induced when HRS cells produce programmed death ligands, document the high level of effectiveness that can be achieved by unleashing immune effector cells to attack HRS cells. A remaining obstacle to further progress, however, consisted of how to bring the effector NK cells into close proximity with the target HRS cells and then, once these cells are in position, how to overcome the inhibition posed by HRS cells' innate ability to block destruction of target cells by elaborating programmed death inhibitory molecules. The AFM13 investigators settled on the clever 3-pronged approach of using a bispecific antibody, AFM13, to pull NK cells into tight proximity with HRS cells and at the same time flooding the system with pembrolizumab, to eliminate the programmed cell death-1/PD-L1 enforced blockage of NK cell–mediated target cell destruction (see figure). This approach works nicely, producing clinically useful responses in most patients, including complete metabolic responses in almost half, and does so with minimal toxicity, a direct consequence of employing biologically selective agents.

The full potential that can be realized in this third major therapeutic era of the treatment of cHL will only be reached when novel immunologic approaches such as AFM13 plus a checkpoint inhibitor are integrated into front-line treatment. Guided and encouraged by the evidence for a high level of efficacy provided by the AFM13 investigators, clinical trials can now be launched employing these biologically selective therapies in earlier and eventually front-line clinical trials. Much remains to be achieved, but the treatment of cHL with immunologically selective, largely nontoxic treatments is now well underway.

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HEMATOPOIESIS AND STEM CELLS

Comment on Hua et al, page 2410

BETting on stem cell expansion

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For decades, the expansion of hematopoietic stem cells (HSCs) has been an elusive goal. In this issue of *Blood*, Hua et al took a step toward reaching this goal by demonstrating that the addition of the small molecule bromodomain and extra-terminal motif (BET) inhibitor CPI203 to a cytokine cocktail results in expansion of serially transplantable long-term repopulating HSCs (LT-HSCs). They also showed enhancement of their potential to differentiate into megakaryocytes (MKs).[1]

Expansion of hematopoietic stem and progenitor cells (HSPCs) has been used to increase the low numbers of HSPCs in banked umbilical cord blood (UCB) units[2] and for ex vivo genetic engineering of HSPCs for gene therapy.[3] A wide range of approaches has been used for the ex vivo expansion of HSPCs, including cytokine cocktails (sometimes in combination with additional small molecules), ectopic expression of specific genes, and coculture with stromal cells.[4] One of the recurrent problems has been the progressive loss of stemness upon expansion. For example, the cocktail of stem cell factor (SCF), thrombopoietin (TPO), and Flt3L, often used in ex vivo gene-therapy protocols, was chosen because it minimizes the loss of stem cells. During the last few years, several small molecules have shown promising results in combination with cytokines in promoting HSPC expansion.[4]

Lysine acetylation is a fundamental posttranslational modification that plays an important role in the control of gene transcription in chromatin.[5] BET proteins (BRD2, BRD3, BRD4) are ubiquitously expressed proteins containing an N-terminal bromodomain (BRD) that binds to acetylated lysine residues in histones.[5] They can be regarded as epigenetic readers and transcriptional cofactors and are often found in superenhancers that direct cell-lineage fate decisions. CPI203 is a small molecule inhibitor that specifically targets BRD4 by competitive binding to the acetyl-lysine recognition pocket, thereby displacing BRD4 from chromatin. BRD4 marks the transcription start sites of cell-cycle genes. It also promotes progression of the cell cycle from G1 to S and G2 to M phase and has a critical role in hematopoiesis.[6]

BET proteins play an important role in regulating HSC stemness in the mouse. For instance, a BET inhibitor termed JQ1, which is structurally related to CPI203, increases HSC proliferation and mobilization in mice.[7] Based on these results, Hua et al from Oxford's Institute of Molecular Medicine, investigated 10 known small molecule inhibitors of these proteins, by adding them in increasing concentration to stem cell expansion cultures containing SCF, TPO, and Flt3L, which by itself cannot maintain or expand phenotypically or functionally defined human HSCs. Addition of only 1 of them, CPI203, resulted in significant expansion of human $Lin^{-}CD34^{+}CD38^{-}CD45RA^{-}CD90^{+}CD49f^{+}$ cells, designated as phenotypical HSCs (pHSCs). Cell viability, a problem that was seen with some other compounds, was not affected.

The investigators showed that although the total number of nucleated cells was lower, the numbers of immature pHSCs were higher and had actually expanded in the CPI203-supplemented cultures (see figure). In addition, they calculated that the total numbers of long-term culture-initiating cells in the pHSC subset was increased fivefold to 10-fold. Additional limiting dilution transplantation experiments in nonirradiated immune-deficient NSG and NSG-W mice indicated multilineage reconstitution and bone marrow engraftment levels up to 23% compared with <1% for control mice transplanted