the presence of a proline residue, in addition to the cysteine, as in the original mutant, resulted in signaling (see figure). This could explain why no simple cysteine substitutions or insertions have been reported as yet.

The TSLP receptor is a different story. It shares the IL-7Rα chain, but uses CRLF2 as a second chain rather than γc. Unlike the insertions in IL-7Rα in T-ALL, overexpression of CRLF2 in B cell-derived acute lymphoblastic leukemia (B-ALL) is a frequent pattern that is created by chromosomal rearrangements.[7-10] Overexpression confers a modest ligand-independent signal in vitro and may require TSLP in vivo to mediate its leukemic effects. CRLF2 can also display a gain-of-function mutation, F232C, that gives a stronger ligand-independent signal and is found in a subset of CRLF2 overexpressors in B-ALL. In the current report,[1] a noncysteine mechanism is analyzed in mutations of CRLF2 in B-ALL. The study examined one such CLRF2 mutant, which, like the atypical IL-7Rα mutants, occurred within the transmembrane region and, in BaF3 cells induced homodimerization. This CRLF2 mutant required coexpression of IL-7Rα to signal and grow as leukemia in mice, suggesting it may also heterodimerize with IL-7Rα in an orientation that activates their associated Janus kinases.

These studies point to new leukemogenic signaling mechanisms and reinforce the IL-7/TSLP axis as therapeutic targets in ALL.

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● ● ● MYELOID NEOPLASIA

Comment on Placke et al, page 13

CDK6, a new target in MLL-driven leukemia

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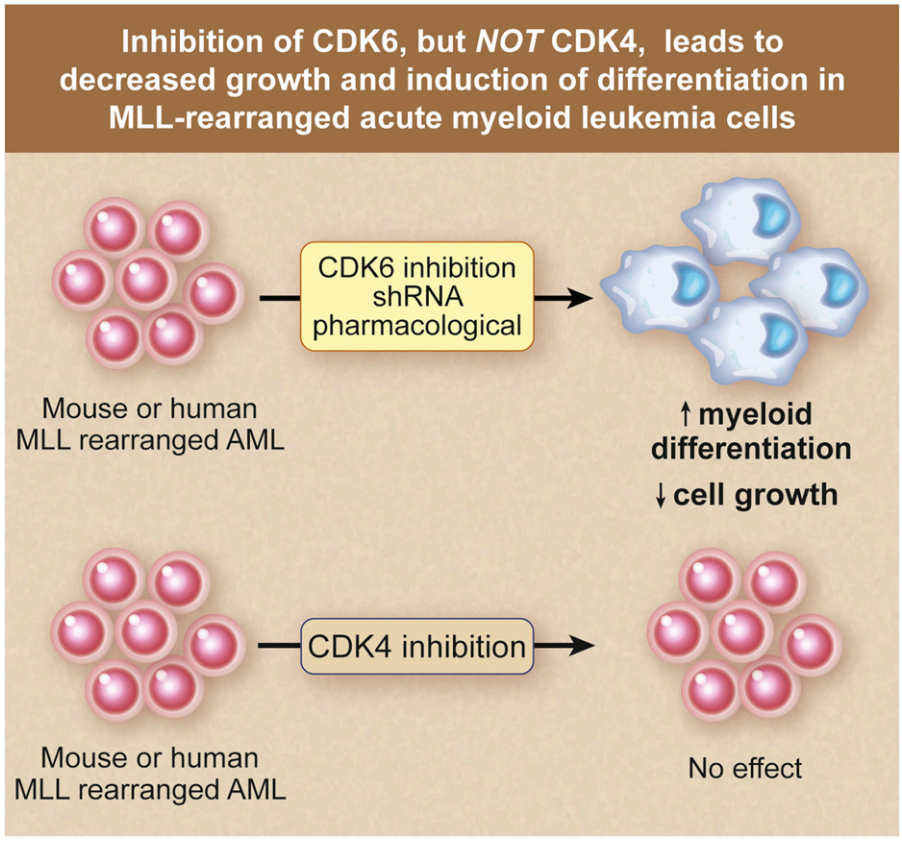
In this issue of *Blood*, Placke et al identify the cell-cycle regulator CDK6 as a promising new target in mixed lineage leukemia (MLL)-rearranged acute myeloid leukemia (AML) and show that its downregulation or pharmacological inhibition leads to growth inhibition and differentiation of MLL-driven leukemic cells.[1]

Chromosomal rearrangements involving the *MLL* gene, located on chromosome 11q23, are found in 5% to 10% of pediatric and adult AML and are usually associated with a poor prognosis. More than 50 fusion partners have been described, with frequent



Inhibition of CDK6, but not CDK4, leads to decreased growth and induction of differentiation in MLL-rearranged AML cells. Professional illustration by Debra T. Dartez.

implication of, for example, AF9, ENL, ELL, AF6, and AF10.[2] *MLL* fusion genes present a common feature of disruption: the N-terminal portion of MLL is fused to the C-terminal portion of its partner. Leukemogenic mechanisms mediated by MLL translocations are diverse and include the loss of the C-terminal H3K4 methyltransferase domain of MLL, followed by silencing of tumor suppressors; the binding/recruitment of protein complexes and transcriptional regulators to the N-terminal fraction of MLL; and the recruitment of aberrant transcriptional regulators by some fusion partners. For instance, the N-terminal portion of MLL interacts with the menin protein and leads to upregulation of target genes such as the HOX-A gene cluster.[3] Fusion partners of MLL interact with several proteins, including the H3K79 methyltransferase DOT1L.[4] These oncogenic interactions could be promising therapeutic targets, and small molecule inhibitors targeting interactions with menin,[5] DOT1L,[6] or BRD4[7,8] are being developed. However, although promising, such approaches are not yet available in clinical practice, and the therapy of MLL-rearranged AML continues to be very challenging and associated with poor outcome. The identification of pharmacologically immediately actionable, critical mediators of the leukemia-promoting effects of MLL fusions is of high relevance for the improvement of therapeutic strategies in this particular subtype of AML.

To find new pathways deregulated in MLL-translocated leukemia, Fröhling and colleagues used a functional genetic RNA interference screening approach and identified the cyclin-dependent kinase CDK6 as selectively implicated in the growth of MLL-AF9–, MLL-AF4–, and MLL-AF6–positive cell lines, in striking contrast to MLL wild-type cell lines (see the schematics in the figure). The downregulation of CDK6 by 3 different short hairpin RNAs (shRNAs) followed by rescue experiments confirmed that MLL-rearranged human leukemia cells indeed rely on CDK6 expression. Similar results were obtained with primary murine cells transduced with MLL-AF9. Interestingly, Placke et al found that CDK6 downregulation affected cell cycling of AML cells only very modestly; however, they observed a striking induction of myeloid differentiation in MLL-rearranged AML cells upon CDK6 inhibition. Rescue with wild-type CDK6 abolished this differentiation-inducing effect, contrary to the rescue with a CDK6 kinase-dead mutant, or with wild-type CDK4, a functional homolog of CDK6 with regards to its cell cycle-regulatory function. These findings demonstrate that the effects on differentiation of MLL-rearranged AML cells are indeed highly specific to CDK6 and are mediated by its kinase activity. In further mechanistic studies, Placke et al found that MLL-AF9 binds the CDK6 locus, and that inhibition of MLL-AF9 (in MLL-AF9–positive cells) led to a decrease of CDK6 expression, and forced expression of MLL-AF9 in wild-type cells increased CDK6 expression levels, strongly suggesting that CDK6 is a direct target of truncated MLL.

The findings by Placke et al have immediate translational implications. Pharmacologic inhibition of their newly identified target is possible via an oral inhibitor of CDK4/CDK6 (PD-0332991; Palbociclib), which is currently being tested in clinical trials for treatment of a variety of cancers, including breast cancer[9] and mantle cell lymphoma.[10] Importantly, similar to the results obtained with knockdown of CDK6 by shRNAs, Placke et al found that PD-0332991 decreases the growth and increases the differentiation of MLL-rearranged AML cell lines. This substantial inhibitory effect was confirmed in primary human AML cells harboring different MLL translocations. Finally, the authors explored the impact of CDK6 inhibition in an in vivo transplantation model of tertiary MLL-AF9–induced AML (ie, a very aggressive and rapidly progressing model of the disease). Downregulation of CDK6 led to abrogation of the differentiation block of MLL-AF9 AML cells and to strikingly prolonged survival. These findings provide in vivo proof of concept for the leukemia-inhibitory and differentiation-inducing effects of CDK6 suppression in MLL-rearranged AML and suggest targeting of CDK6 as a novel approach in patients with this subtype of AML. Because monotherapy of MLL-rearranged AML with CDK6 inhibitors is a clinically rather unlikely scenario, further preclinical studies are warranted to assess the efficacy of CDK6 in the context of treatment with chemotherapeutic or other agents used in the treatment of AML.

In summary, through a series of highly complementary approaches comprising shRNA-mediated and pharmacological inhibition, mouse and human systems, and different in vitro and preclinical in vivo models, the work by Placke et al establishes a critical role of CDK6 in MLL-rearranged AML. Of note, this study highlights the potential of functional genetic approaches to uncover important pathomechanisms in leukemia that may evade detection by other strategies, such as DNA sequencing or transcriptional profiling. The finding that inhibition of CDK6 leads to differentiation of MLL-rearranged AML cells renders it an attractive novel target and provides a strong rationale for the clinical testing of CDK6 inhibitors in MLL-rearranged AML.

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