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LYMPHOID NEOPLASIA

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BCL11B, the Cerberus of human leukemia

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In this issue of *Blood*, Di Giacomo et al provide support for *BCL11B* as an important oncogene for early human acute leukemia with both myeloid and lymphoid features.[1]

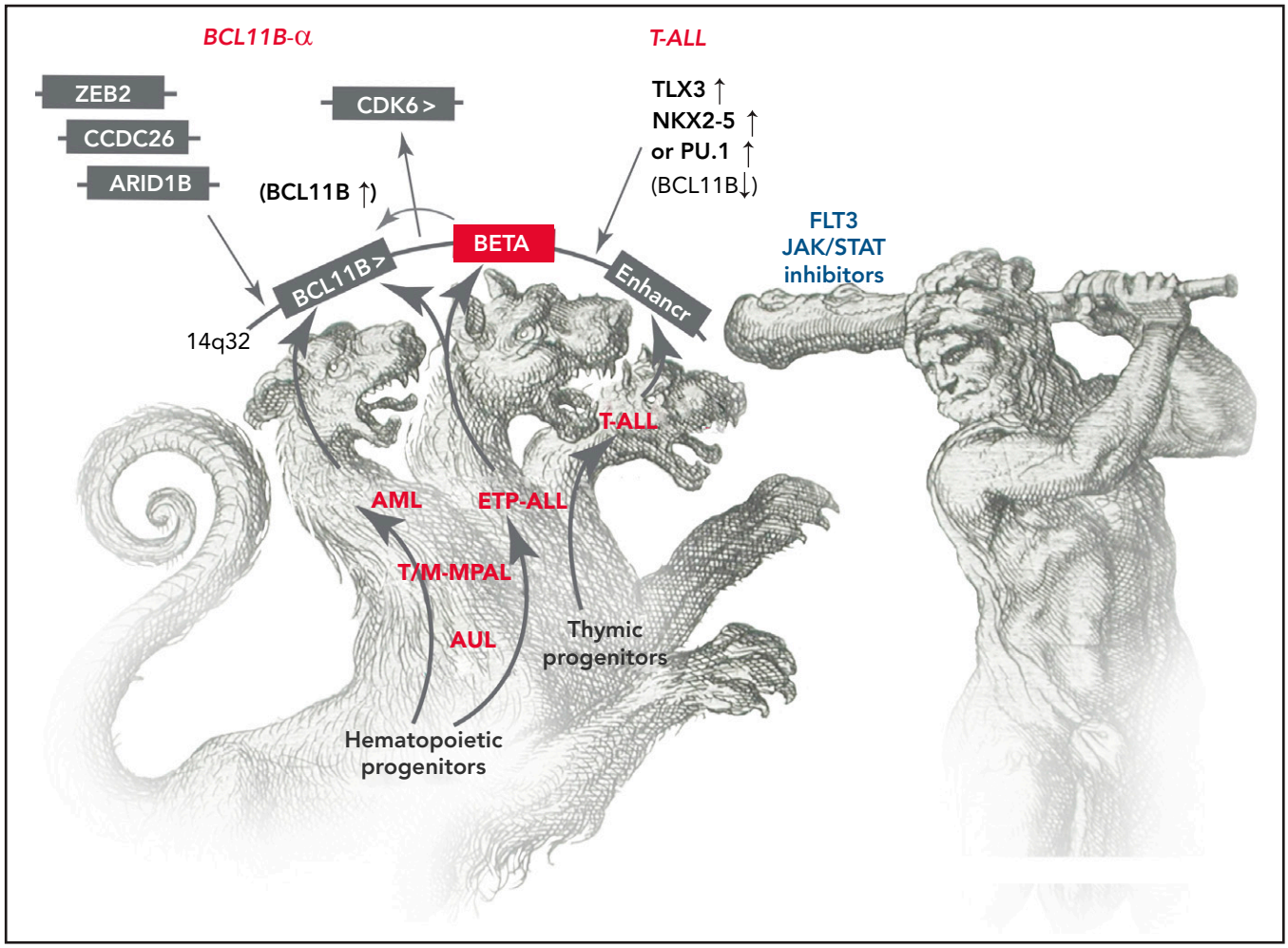
In Greek mythology, Cerberus (in Greek, κερβερος; Kérberos) is the 3-headed watchdog who protects the dead from leaving the Underworld. Recent findings on lineage-promiscuous acute leukemia reveal the *BCL11B* locus as a multiheaded beast that can drive a variety of immature leukemias, including undifferentiated acute leukemia (AUL), minimally differentiated acute myeloid leukemia (AML M0/M1), mixed-phenotype acute leukemia with T myeloid features (T/M MPAL), early thymic progenitor (ETP) acute lymphoblastic leukemia ALL (ALL), and T-cell ALL (T-ALL) by virtue of different pathogenic mechanisms. T/M MPAL and ETP ALL are closely related, lineage-promiscuous diseases, both on the (epi)-genetic and transcriptomic levels, that may originate from a common precursor cell.[2] T/M MPAL and ETP ALL tumors are frequently characterized by *ETV6*, *NUP214-ABL1*, or *CALM-AF10* fusion products and mutation rates of *WT1*, *FLT3*, *RAS*, and *JAK/STAT*, which are estimated to be the same, whereas NOTCH1-activating mutations and *CDKN2A/B* loss-of-heterozygosity aberrations are nearly absent, unlike in patients with T-ALL.[2,3]

Since its initial discovery as a translocation partner, recurrent chromosomal rearrangements between the T-cell lineage commitment factor *BCL11B* and the *TLX3* oncogene or, less frequently, *NKX2-5*, *NKX2-1*, or *PU.1* have been found in patients with T-ALL. These rearrangements highjack the *BCL11B* enhancer that is normally activated during early T-cell development in the thymus. This enhancer, located 1 Mb distal of the *BCL11B* gene, drives ectopic expression of these oncogenes as initiating events of T-ALL leukemogenesis. These rearrangements thereby inactivate 1 functional *BCL11B* allele. Approximately 16% of T-ALL patients accumulate inactivating mutations in *BCL11B*, strongly implying that *BCL11B* acts as an important tumor-suppressor gene during early T-cell pathogenesis.[4,5]

In recent studies, novel t(2;14)(q22;q32) translocations that affect the *BCL11B* locus were identified in patients with lineage-promiscuous AUL, T/M MPAL, or ETP ALL, as well as in patients with poorly differentiated AML, in whom T-cell markers may also be expressed.[2,6-9] In contrast to the *BCL11B* enhancer highjacking by oncogenes, as seen in patients with T-ALL, the t(2;14) translocation yields an in-frame *ZEB2-BCL11B* fusion product that is driven by the *ZEB2* promoter.[2,6,8] It leads to the misexpression of *BCL11B* in early progenitor cells where the *BCL11B* enhancer is not normally active. Although ZEB2 activation in an early T-cell context drives T-cell pathogenesis with enhanced interleukin-7 signaling in mice,[9] *ZEB2-BCL11B* fusions in lineage-ambiguous leukemia patients are chiefly found in combination with activating *FLT3* mutations.[2,8]

Di Giacomo et al provide further support for an oncogenic role of *BCL11B* in a subgroup of patients with lineage-ambiguous leukemia. Novel recurrent rearrangements were identified in which transcriptional regulatory sequences of various loci were highjacked by the *BCL11B* gene itself in nearly 4% of AML M0/M1 and 3.6% of patients with T-ALL, including those with T/M MPAL, ETP ALL, and rare cases of T-ALL.[1] In addition to (2;14)(q22.3;q32), other rearrangements that were identified included t(6;14)(q25.3;q32), t(8;14)(q24.2;q32), and t(7;14)(q21.2;q32), which repositioned *ARID1B*, BENC-c*MYC*, or *CDK6* regulatory sequences upstream or downstream of the *BCL11B* gene, respectively. All these rearrangements result in high expression of *BCL11B*. These patients with so-called *BCL11B*-activated acute leukemia share a unique expression signature, including a JAK/STAT signature, that is different from that of other patients with AML, ETP ALL, and T-ALL. Leukemia cells from *BCL11B*-activated patients express stem cell antigens, including HLA-DR, CD117, and/or CD34, together with T-cell and myeloid markers, such as CD2, CD7, CD13, and/or CD33. In addition to previously reported findings,[2,8] all *BCL11B*-activated patients carried mutations in *FLT3* in the absence of NOTCH1-activating mutations. Recurrent mutations in *WT1*, *DNMT3A*, and *TET2* were found in 44%, 33%, and 22% of *BCL11B*-activated patients, respectively.

This study integrates the data presented by Montefiori et al[10] at the late-breaking abstract session during the 2020 meeting of the American Society of Hematology. Genome-wide RNA and whole-exome sequence analyses of a large pan–acute leukemia cohort comprising 2573 patients led to the identification of 60 patients who had a similar profile (typically $CD7^+$, $CD2^+$, $CD5^-$, $CD1a^-$, $cCD3^+$, $CD8^-$, $cMPO^{+/-}$, and myeloid/stem cell marker positive). These 60 patients included 25 T/M MPAL, 20 ETP ALL, 8 AML, and 2 UAL, 80% of whom harbored *FLT3* alterations. Ninety-three percent of the patients carried rearrangements that produced *ZEB2-BCL11B* or *RUNX1-BCL11B* fusion transcripts or activated *BCL11B* by rearrangements to



BCL11B, the Cerberus of human leukemia. The *BCL11B* locus resembles the 3-headed mythological creature Cerberus, because it is involved in various initiating oncogenic events for lineage-promiscuous AUL, T/M MPAL, AML M0/M1, ETP ALL, and T-ALL patients, although by virtue of different mechanisms. In patients with T-ALL, the *BCL11B* enhancer is highjacked by various oncogenes during early thymopoiesis. In a subtype of lineage-ambiguous patients whose disease expresses high levels of *BCL11B* or *BCL11B*-containing fusion transcripts and who are categorized as *BCL11B*-activated (*BCL11B*-a) patients, *BCL11B* acts as an oncogene because of its activation in early hematopoietic progenitor cells upon the chromosomal reposition of transcriptional regulatory sequences of specific loci as indicated. Alternatively, *BCL11B* can be driven from a 2.5-Kb region amplification located 700 Kb downstream of the *BCL11B* gene (denoted as *BCL11B* enhancer tandem amplification [BETA]). Most *BCL11B*-a patients are characterized by activating *FLT3* mutations or have high JAK/STAT signaling activities that provide sensitivity to FLT3 or JAK/STAT inhibitors. The figure has been adapted from an etching by Antonio Tempesta (Florence, Italy, 1555-1630).

regulatory sequences of *ARID1B*, the BENC distal *cMYC* enhancer at CCDC26, *CDK6*, *ETV6*, or *SATB1*. Another 21% of the patients identified had multicopy tandem duplications of a 2.5-Kb region, denoted as the *BCL11B* enhancer tandem amplification, that is located 700 Kb distal of the *BCL11B* gene that activates *BCL11B* transcription.[10]

These combined studies show that these novel *BCL11B* gene aberrations can drive oncogenesis, which yields lineage-promiscuous acute leukemias with myeloid and T-cell lymphoid features, including UAL, T/M MPAL, AML, and ETP ALL in patients (denoted as *BCL11B*-activated patients; see figure). *BCL11B* therefore seems an essential oncogene for these *BCL11B*-activated patients that may originate from early hematopoietic progenitor cells, in contrast to T-ALL, which originates from recent thymic progenitor cells or its offspring cells in the thymus. In patients with lineage-ambiguous acute leukemia, *BCL11B* is activated upon its repositioning in close proximity to promoters or enhancers that are active at the precursor cell stage and that remain active during early myeloid/lymphoid stages. Although *BCL11B*-activated patients in general were more resistant to genotoxic agents, FLT3 tyrosine kinase inhibitors or JAK/STAT inhibitors may provide the clinical power of Hercules to slay the deranged *BCL11B* Cerberus locus in these lineage-promiscuous leukemias.[1]

Conflict-of-interest disclosure: The author declares no competing financial interests. ■

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DOI 10.1182/blood.2021011856

MYELOID NEOPLASIA

Comment on Imgruet et al, page 790

The crux of Cux1 in myeloid neoplasms

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In this issue of *Blood*, Imgruet and colleagues investigate how loss of the tumor suppressor gene *Cux1* modulates DNA repair activity in the hematopoietic compartment and how this contributes to the pathogenesis of therapy-related myeloid neoplasms (tMNs).[1]

The blood system has tremendous regenerative capacity, which is called upon in response to blood loss, severe infections, and exposure to toxins, including systemic chemotherapy. Frequently, the blood system will reemerge from the ashes, but unlike the mythical phoenix, which rises reborn, the blood system comes back altered, and the fire will sometimes rage out of control. One way that this manifests is in higher rates of clonal hematopoiesis, myelodysplasia, and acute leukemia in the years following cancer treatment. This is a significant problem, because hematological malignancies that arise after therapy are difficult to treat and generally have poor outcomes.

CUX1 is a multifunctional protein, implicated in gene regulation, cell-cycle control, cell signaling, apoptosis, and the DNA damage response, and its role in cancer is predictably complex.[2] CUX1 is typically impaired in myeloid neoplasms; it is lost in around half of all cases of tMN, mostly through loss of chromosome 7, but sometimes through focal deletions or other mutations.[3] Mice engineered to have low *Cux1* expression are predisposed to myelodysplasia.[4] In line with earlier work in cell lines, the investigators show loss of Cux1 impairs the DNA damage response in primary murine hematopoietic stem and progenitor cells. Cux1-deficient progenitors expand after treatment with the alkylating agent *N*-ethyl-*N*-nitrosourea, and the stress encourages the rapid outgrowth of erythroleukemia, providing a new way to model this aggressive disease.

The investigators employ various functional assays to assess DNA repair activity in Cux1-deficient cells. They probe the response to DNA damaging agents, stain for DNA damage markers, and survey DNA strand breaks with COMET assays. None of these assays is perfect, but together they build a case that implicates Cux1 in modulating the DNA damage response. How does this occur? Again, it seems CUX1 acts at multiple levels. Imgruet et al suggest Cux1 recruits histone-modifying complexes to sites of damage and that this helps nucleate DNA repair. Some suggest a broader role, coordinating the expression of multiple DNA repair components, particularly in the ATM/ATR pathway.[5] Others suggest CUX1 directly modulates the activity of glycosylases, like OGG1, that repair oxidative damage.[6] More work is required to determine which of these activities is most crucial, or whether they work in concert.

The question then becomes, are CUX1-deficient cells accumulating more DNA damage? It is possible, but the answer is not yet definitive. By pulling together exome data from patients with various myeloid neoplasms, it appears *CUX1*-mutated samples have a slightly higher total mutation burden.[1,7] However, the difference is modest, and these comparisons are complicated by the low number of cases, the diverse disease spectrum, and differences in age, treatment history, and lifestyle factors. One way to answer this question would be to perform whole-genome sequencing on clonal cultures of blood cells to reveal the mutation burden associated with CUX1 deficiency, either in the mouse model or in material from patients.[8] Studying the resulting mutational signatures, during steady state and in response to stress, will help reveal any underlying DNA repair defect.

If there is more DNA damage, are these mutations driving disease progression and poor outcome? The prevailing view is that DNA damage provides more fuel for the fire. Here, the authors reveal the power of their mouse model, which allows them to transiently lower *Cux1* expression.[4] They show that reintroducing Cux1 rescues erythroid differentiation and prevents myeloid transformation, suggesting that any DNA damage that has accumulated is not enough to drive disease progression. This is exciting, because it suggests that drugs that restore CUX1 function, or that act downstream of this multifunctional regulator, may offer a way to treat the disease. Indeed, targeting altered signaling and survival pathways in CUX1-deficient cells seems to be a promising strategy.[9] Although encouraging, it will be important to pursue these questions in more relevant clinical models that mirror the complexity of the disease.

We are just beginning to appreciate the influence of cancer therapies on the blood system.[10] This understanding will grow as