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Review Article

BCOR gene alterations in hematologic diseases

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The BCL6 corepressor (BCOR) is a transcription factor involved in the control of embryogenesis, mesenchymal stem cells function, hematopoiesis, and lymphoid development. Recurrent somatic clonal mutations of the *BCOR* gene and its homolog *BCORL1* have been detected in several hematologic malignancies and aplastic anemia. They are scattered across the whole gene length and mostly represent frameshifts (deletions, insertions), nonsense, and missence mutations. These disruptive events lead to the loss of full-length BCOR protein and to the lack or low expression of a truncated form of the protein, both consistent with the tumor suppressor role of *BCOR*. *BCOR* and *BCORL1* mutations are similar to those causing 2 rare X-linked diseases: oculofaciocardiodental (OFCD) and Shukla-Vernon syndromes, respectively. Here, we focus on the structure and function of normal BCOR and BCORL1 in normal hematopoietic and lymphoid tissues and review the frequency and clinical significance of the mutations of these genes in malignant and nonmalignant hematologic diseases. Moreover, we discuss the importance of mouse models to better understand the role of *Bcor* loss, alone and combined with alterations of other genes (eg, *Dnmt3a* and *Tet2*), in promoting hematologic malignancies and in providing a useful platform for the development of new targeted therapies.

Introduction

The *BCL6 corepressor* (*BCOR*) is a tumor suppressor gene that was first identified in a 2-hybrid screen for interactors with the POZ domain of the transcriptional repressor BCL6.[1] Its product is a nuclear protein involved in lymphoid development[2] (potentiating BCL6 repression[1]), maintaining pluripotency of human embryonic stem cells,[3,4] and regulating mesenchymal stem cells function[5] and hematopoiesis.[6] The BCL6 corepressor-like protein 1 (BCORL1)[7] shares several features with BCOR but also shows distinctive characteristics, suggesting it may play different functions.[8]

In 2011, searching for new mutations in de novo adult acute myeloid leukemia (AML), we applied whole exome sequencing to AML patients with normal karyotype lacking *NPM1*, *CEBPA*, *FLT3*-ITD, *IDH1*, and *MLL*-PTD (the only known mutations at that time), which led us to discovery somatic clonal *BCOR* mutations in AML.[9] Another group simultaneously identified mutations of the *BCOR* homolog *BCORL1* in de novo and secondary AML.[10] Since then, mutations of these genes were increasingly reported in both malignant and nonmalignant hematologic diseases.

Germinal *BCOR* mutations also cause oculofaciocardiodental (OFCD) syndrome, a rare X-linked dominant disease that is embryonic lethal in males.[11] OFCD syndrome is characterized by congenital cataracts, abnormal facial traits, cardiac defects and dental anomalies, including canine teeth with extremely long roots.[11] *BCOR* mutations were also identified in Lenz microphthalmia syndrome.[11,12] The great variability in the severity of OFCD syndrome among females is likely due to differences in the proportion of cells (mosaicism) carrying a transcriptionally active X chromosome with *BCOR* mutation in various tissues.[13] In fact, *BCOR* is located on chromosome X, and X inactivation occurs randomly in the early embryo. Alternatively, a phenotype may not become manifest if cells harboring the *BCOR* mutation on the active X chromosome fail to survive (eg, leukocytes from OFCD syndrome patients show 96% to 100% allelic skewing in favor of cells expressing wild-type *BCOR*).[13] Germline *BCORL1* mutations cause the Shukla-Vernon syndrome (named SHUVER), an X-linked recessive disorder characterized by global developmental delay, variably impaired intellectual development and behavioral abnormalities.[14,15]

Although *BCOR* mutations are relatively uncommon, their occurrence has been increasingly reported in various hematologic diseases. The scope of this review is to bring all information in a single place, so that it can serve as reference for researchers and clinicians dealing with this issue. In particular, here we focus on the structure and function of normal *BCOR* in normal hematopoietic and lymphoid tissues and review the frequency and clinical significance of *BCOR* mutations in hematologic diseases. When available, data on *BCORL1* homolog are also provided. Moreover, we discuss the importance of mouse models to better understand the role of *Bcor* loss in promoting hematologic malignancies and providing a platform for developing new targeted therapies.

BCOR and BCORL1 genes and proteins

The characteristics of *BCOR* and *BCORL1* are summarized in Table 1. The *BCOR* gene locates at position 11.4 of the short arm of chromosome X and is made up of 15 exons[1] (supplemental

Table 1. Characteristics of BCOR and BCORL1 genes and proteins

Characteristics	*BCOR* gene	*BCORL1* gene
Location	Chromosome X (band Xp11.4)	Chromosome X (band Xp26.1)
No. of exons	15	13
Associated genetic syndrome	OFCD	Shukla-Vernon (SHUVER)
Mutations	Frameshifts, nonsense, and missense*	Frameshifts, nonsense, and missense*,†
Translocations	Rare (APL)*	No
ITD of PUFD (solid tumors)	Yes	No
Characteristics	**BCOR protein**	**BCORL1 protein**
Length	1755 amino acids	1711 amino acids
Subcellular location	Nucleosol and nuclear dots of various size	Speckle-like nuclear dots of consistent size
Major protein domains	BCL6-binding domain, PUFD motif, MLLT3-binding domain Tandem ankyrin repeats	CtBP1-binding site, PUFD motif, 2 LXXLL motifs, tandem ankyrin repeats
PUFD motif	Disordered	Ordered
Function	Transcriptional corepressor	Transcriptional corepressor
Interactors	BCL6, HDACs class I and II, MLLT3, FXBL10/JHDM1B, MLLT1/ENI, ZBTB5, SP1, ZBTB2, ZBTB7A/Pokemon, PCGF1, RING1A/B KDM2D	HDACs class I and II, CTBP1, PCGF1

*Sometimes may co-occur in AML and MDS.

†Rare variant of acute promyelocytic leukemia (APL).

Figure 1, available on the *Blood* Web site). BCOR shows a nuclear localization[16] that is driven by the interaction of its 2 nuclear localization signals[17] with the nuclear import proteins KPNA2, 4, and 6.[18] The BCOR protein is regarded to be widely expressed in the lympho-hematopoietic system,[19] but immunohistochemical studies with specific monoclonal antibodies are missing. Several alternative spliced transcript variants encoding 4 different isoforms (a, b, c, d) have been described.[1,20] The main isoform c uses 15 exons to generate a protein of 1755 amino acids (192 kDa).[1] Only a few isoforms retain known protein interactions, depending on the domains preserved by alternative splicing.

The BCOR protein contains 3 well-established functional domains, whereas the significance of the C-terminal tandem ankyrin (ANK) repeats remains unknown (Figure 1). The BCL6 binding site located at the N terminus of BCOR interacts with the POZ domain of BCL6[1,21] and with the α-helical region of interferon regulatory factor 8 (also targeting BCL6).[22]

The polycomb group RING finger (PCGF) Ub-like fold discriminator (PUFD) binding site is located at the C terminus of BCOR and interacts with the PCGF homolog 1 (PCGF1)[23] (Figure 1). BCOR, the PCGF1/RING enzymatic core, and KDM2B are critical components of the noncanonical polycomb repressive complex PRC1.1.[24-27] (Figure 2). The noncanonical PRC1.1 complex contains other proteins that may be tissue specific, for example, in germinal center B cells, BCOR forms a noncanonical PRC1.1 complex containing BCL6 and the CBX8 subunit.[2,24,26] This interaction allows recruitment of the complex to specific chromatin regions via mechanisms involving interaction with both chromatin marks and sequence-specific transcription factors. Finally, a BCOR-independent mechanism of recruitment of PRC2/canonical PRC1 complexes to nonresponsive targets that may counteract the gene activation because of BCOR loss has been previously described.[3]

The PUFD termini of BCOR, which are critical for binding to the ubiquitin-like RAWUL domain of PCGF1, are structurally disordered and become ordered only upon binding PCGF1[28] (Figure 1). In this way, the PCGF1/BCOR PUFD terminal residues are placed in conformations that are required to interact with the leucine-rich repeats of KDM2B (Figure 1).[28]

The PRC1.1 complex then is recruited to unmethylated cytosine guanine dinucleotide (CpG) islands that are frequently located around transcription start sites. Binding to unmethylated CpG islands occurs through the zinc finger-CxxC (ZF-CxxC) DNA-binding domain of histone demethylase KDM2B[3,29] (Figure 2) that specifically demethylates H3K36me2 via its jmjC domain. Binding of PCGF1-BCOR complex with KDM2B stimulates the E3 ligase activity of RING1B that in turn monoubiquitylates H2A on K119, promoting the accrual of canonical PRC2 complex[30] to monoubiquinated loci.[26] Conversely, *BCOR* loss results into a decrease of H2AK119ub1 at promoter regions of Hoxa and *Cebpa* family genes.[31] Thus, BCOR appears critical to couple the RING-PCGF1 enzymatic core to the chromatin bound KDM2B subunit. This function may be disrupted in case of BCOR loss or truncation. The tissue specificity of the PRC1.1 complex

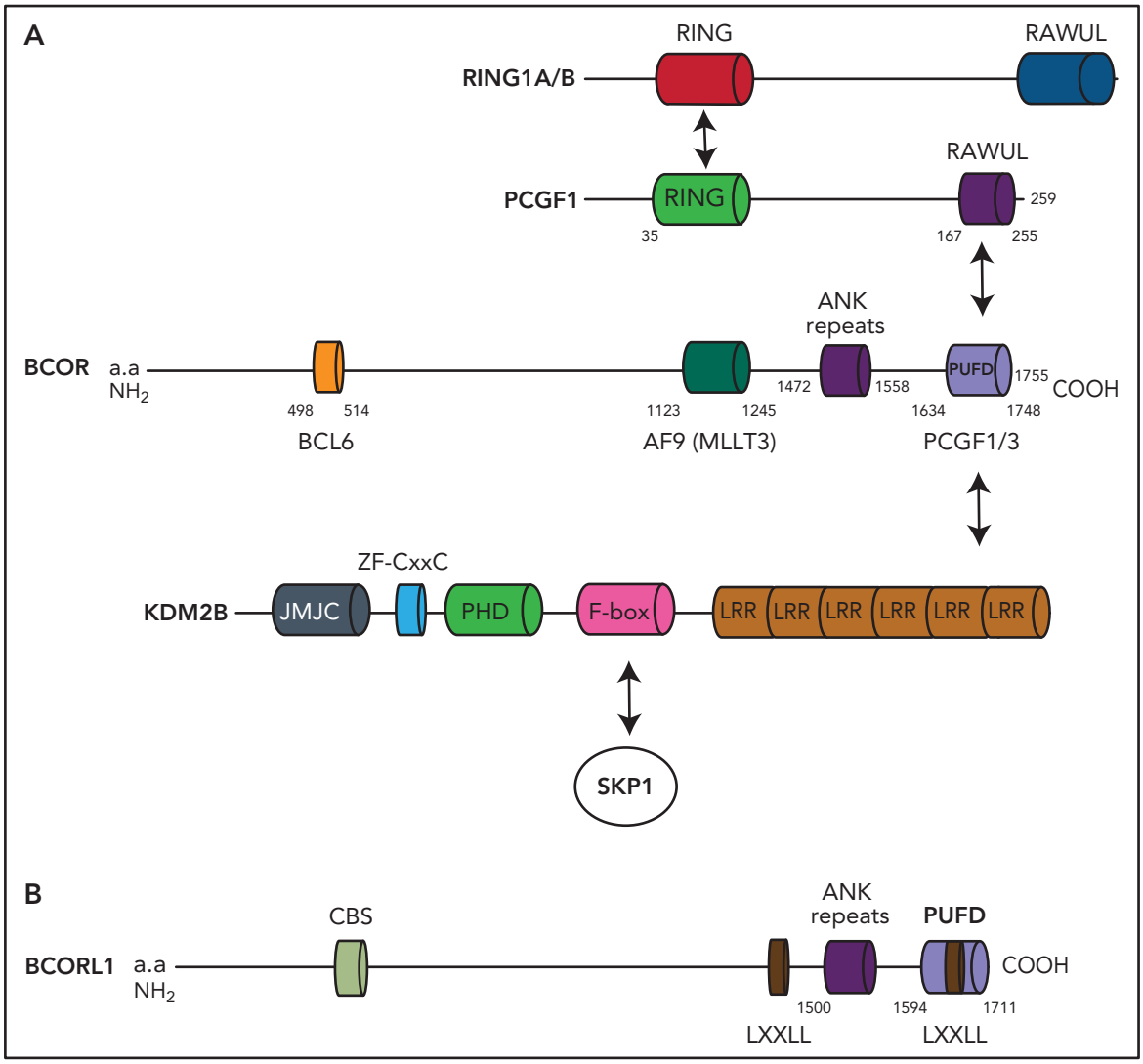

Figure 1. BCOR and BCORL1 proteins. (A) The BCOR protein is characterized by the BCL6 binding site, the AF9 (MLLT3) binding site, the ANK repeats, and the PUFD binding site capable to dimerize with PCGF1. When the BCOR PUFD domain binds to the RAWUL domain of PCGF1, the complex acquires stability and therefore BCOR is able to interact with the leucine-rich repeat domains of KDM2B. Other components of the multiprotein complex include the catalytic enzyme RING1A/B, RYPB, and SKP1. (B) The BCORL1 protein is characterized by the CtBP1 binding site (CBS), 2 LXXLL (nuclear receptor recruitment motifs), the ANK repeats, and the PUFD binding site.

may account for the localized rather than global effects of BCOR loss on H2A ubiquitination on K119.

EZH2, 1 of the members of the PRC2 complex, mediates the mono-, di-, and trimethylation of lysine 27 of histone H3 to generate H3K27me1/me2/me3 (Figure 2).[6] These events ultimately lead to repression of transcription by histone modifications in specific promoter regions (Figure 2). Conversely, the *Kdm2b* depletion (eg, in mouse embryonic stem cells) induces the derepression of lineage-specific genes and early differentiation.[32]

The third functional binding site of BCOR directly interacts with the transcriptional regulator AF9 (MLLT3),[33] the common fusion partner of mixed lineage leukemia (MLL) in leukemias.[34] In particular, AF9 binds the 2 BCOR isoforms with a unique 34 amino acid sequence in the midportion of the protein.[33]

The *BCORL1* gene maps to chromosome Xq25-q26.1.[7] The encoded nuclear protein seems to be expressed at higher levels in testis and prostate than in other tissues[7] (Table 1). BCORL1 is 1711 amino acids long and contains a PUFD domain necessary and sufficient to bind PCGF1 RAWUL and together bind KDM2B[35] (Figure 1). However, unlike BCOR, it lacks the BCL6 and MLLT3 binding sites and contains an LXXLL nuclear receptor recruitment motif and a PXDLS motif that interacts with the C-terminal binding protein (CtBP) corepressor, resulting in negative regulation of its target genes, including E-cadherin (Figure 1; Table 1). The repressive BCORL1 activity is mediated at least partially by class II histone deacetylases[7] (Table 1).

BCOR function in hematopoiesis and lymphoid development

The BCOR-containing PRC1.1 complex regulates hematopoiesis by opposing differentiation toward the myeloid lineage[6,31,36,37] through repression of HoxA and *Cebp* family genes.[27,38] Conversely, after depletion of *Pcgf1*[39] or *Kdm2b*,[40] hematopoietic

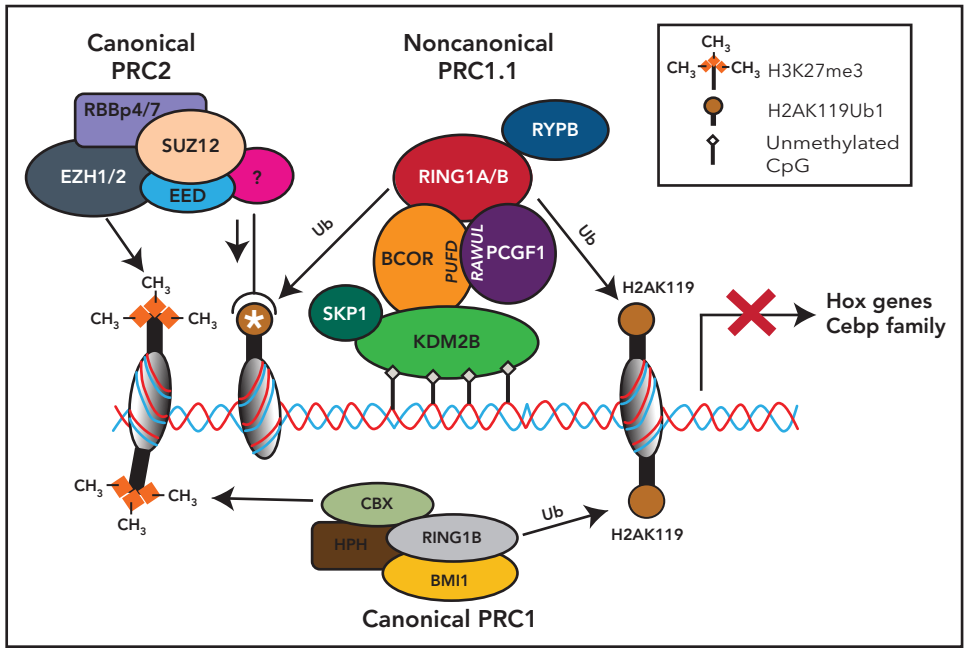

Figure 2. Noncanonical PRC1.1 complex and canonical PRC2 and PCR1 complexes in HSCs. The BCOR complex is recruited to the chromatin via binding of KDM2B to nonmethylated CpG islands, and it catalyzes the ubiquitination of the histone H2A at Lys119 (H2AK119ub) via the RING-PCGF1 enzymatic core. Ubiquinated loci (white asterisk) recruit the histone methyltransferase EZH2, one of the components of the polycomb repressor complex 2 (PRC2). PRC2 is then responsible for the histone H3 methylation at Lys27 (H3K27me3). All these histone modifications lead to the suppression of gene transcription. Canonical PRC1 complex through its components RING1B and CBX catalyzes both the ubiquitination of the histone H2A at Lys119 (H2AK119ub) and the histone H3 methylation at Lys27 (H3K27me3), also leading to the suppression of gene transcription.

stem cells (HSCs) are biased toward the myeloid lineage. Moreover, myeloid cells from mice lacking *BCOR* exons 9 to 10 and expressing a C terminus truncated BCOR unable to bind Pcgf1 show higher proliferation and differentiation rates in vitro.[37] Myeloid-biased hematopoiesis is also found in *Bcor*DE9-10/y progenitors.[31] Moreover, depletion of both *Runx1* and *Pcgf1* sustain the proliferative status and perturb the differentiation of HSCs because of increased expression of *Hoxa9*.[38] Thus, the BCOR-containing PRC1.1 complex is required to repress myeloid regulatory genes and to commit progenitors toward lymphopoiesis. Accordingly, loss-of-function *Bcor* leads to a selective disadvantage in B- and T-cell lineages.[40]

BCL6 is strongly expressed by the germinal center B cells of lymphoid follicles,[41,42] and by interacting with BCOR recruits the PRC1.1 complex that leads to the epigenetic transcriptional repression of BCL6 target genes (Figure 3). Specifically, genes controlling differentiation of B cells to plasma cells (PRDM1, IRF4) and cell cycle checkpoint (CDKN1A, CDKN1B) are transiently silenced to allow immunoglobulin affinity maturation[2] (Figure 3). This process is critical for the formation and function of germinal center B cells that physiologically shut off BCL6 immediately after they exit the germinal center. Aberrant persistence of this status because of deregulated BCL6 expression through translocations or activating mutations promotes lymphomagenesis.[43]

The Bcor-mediated recruitment of PRC1.1 complex by Bcl6 is also required for the differentiation of $CD4^+$ T cells into follicular helper T cells (helping B cells to become plasma cells and memory cells), through repression of genes promoting differentiation toward other lineages.[44,45] Independently by Bcl6, Bcor and Kdm2b in mice are both required to form $CD4^+$ T helper 17 (Th17) cells that protect from extracellular pathogens at mucosal surfaces.[46] Specifically, Bcor enhances Th17 development by repressing the *Lef1*, *Runx2* (runt-related transcription factor 2), and *Dusp4d* (dual-specificity phosphatase 4) genes, encoding proteins that inhibit the Th17 cell fate.[46]

No information is currently available on the BCORL1 function. Association of *BCORL1* hemizygous variants with the Shukla-Vernon syndrome suggests a potential role in neural development. Generation of a *BCORL1* targeted mouse model is warranted to address this issue.

BCOR and *BCORL1* gene alterations in human neoplasms

BCOR mutations mostly occur in hematologic malignancies and in mesenchymal tumors that curiously share histologic features (ie, small round blue cell appearance [Ewing-like sarcoma] or mixoid background and delicate capillary channels).[47,48] *BCOR* mutations are also detected in some central nervous system neoplasms and rare carcinomas.[49]

Unlike *BCOR* and *BCORL1*, other genes encoding components of the PRC1.1 complex are rarely mutated or deleted in hematologic malignancies. As far it concerns T cell malignancies, this may due to the fact that mice lacking the Pcgf1-binding domain of Bcor show a normal T lymphopoiesis,[31] thereby providing more opportunities for transformation than mice insufficient for *Kdm2b* (another component of PRC1.1) that display severely impaired lymphopoiesis.[40,50]

In both myeloid and lymphoid malignancies *BCOR* mutations are scattered throughout the *BCOR* coding sequence, more frequently exon 4 (52.2%), and resemble germline *BCOR* mutations causing the OFCD syndrome[11] (Figure 4A-B). The most common

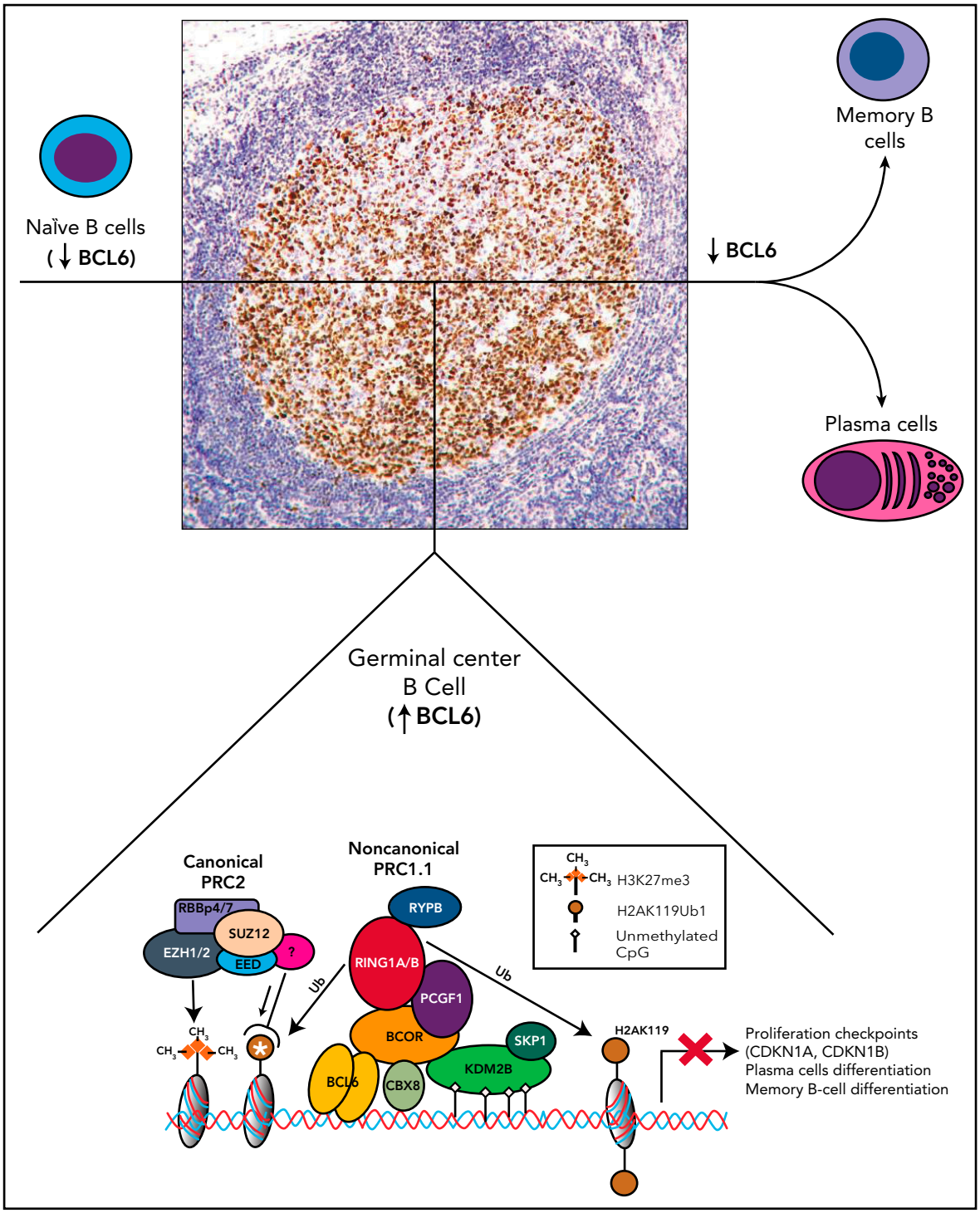

Figure 3. Role of BCOR and noncanonical PRC1.1 complex in the germinal centers of B-cell follicles. Mantle naïve B cells do not express BCL6. Germinal center B cells strongly express BCL6 (nuclear brown positivity at immunoperoxidase staining with monoclonal antibody PG-B6p[42]). BCL6 interacts with BCOR to recruits the PRC1.1 that leads to the epigenetic transcriptional repression of BCL6 target genes. CBX8 is also a component of the complex in the germinal center B cells.[2] The white asterisk indicates recruitment of PRC2 to the ubiquitinated loci. In addition to BCOR, the POZ domain of BCL6 also interacts with the SMRT and N-CoR corepressors that are part of the large multiprotein histone deacetylase-containing complexes and are also required for the repressive activity of BCL6. These events result into the temporary silencing of genes controlling differentiation of B cells to plasma cells and cell cycle checkpoint (CDKN1A, CDKN1B) to allow immunoglobulin affinity maturation. B cells that exit from germinal center downregulate BCL6 before giving raise to plasma cells and memory B cells.

mutations are frameshifts (deletions and insertions; 36.83%) followed by nonsense and missense mutations (20.49% and 20.24%, respectively; supplemental Table 1; supplemental Figure 2). *BCOR*-mutated AML samples show low *BCOR* mRNA levels (mean of 22%), likely because of nonsense-mediated *mRNA* decay.[9,51] *BCOR* mutations result into the absence of full-length BCOR protein (192 kDa) and the lack or low expression of a truncated form of the protein of lower molecular weight.[9] The

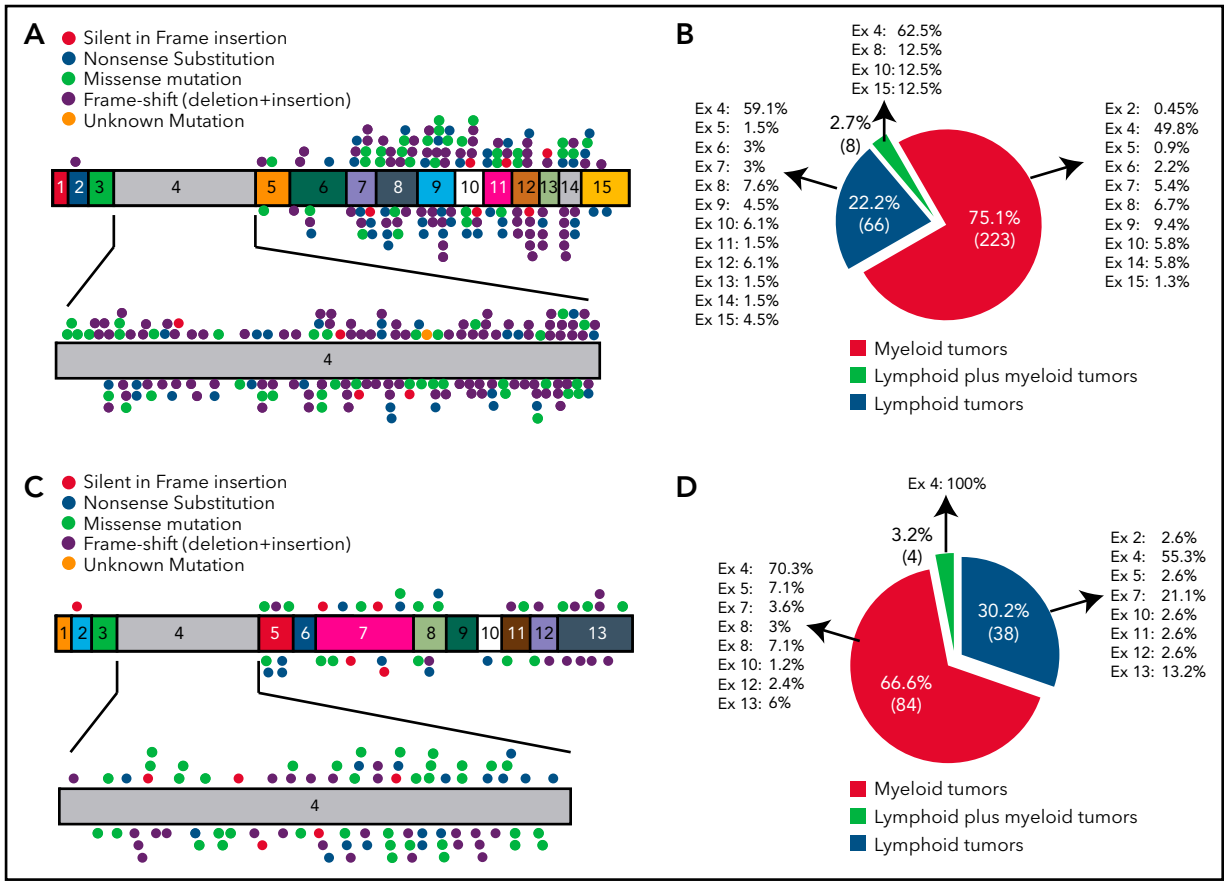

Figure 4. *BCOR* and *BCORL1* mutations in hematologic malignancies. (A,C) The numbers indicate the coding exon, whereas the plots indicate the mutations in BCOR (A) and BCORL1 (C). Red plot = nonsense mutation; orange plot = silent in frame insertion; green plot = missense mutation; black plot = frameshift mutation (deletion + insertion); pink plot = unknown mutation. (B,D) Frequency of mutations for each *BCOR* (B) or *BCORL1* (D) exon detected on myeloid neoplasms, lymphoid neoplasms, and in both of them.

disruptive nature of mutations is consistent with the tumor suppressor role of *BCOR*. *BCORL1* mutations show similar features (supplemental Table 2; Figure 4C-D). Next-generation sequencing[52] for copy number changes has also revealed *BCOR* deletions in AML.[53]

Mutations involving *BCOR* and other chromosome X genes (*PHF6*, *STAG2*, *ZRSR2*) were associated with male bias in AML.[54] The fact that, in females, the mutation of *BCOR* (or *PHF6*) has no deleterious effect if it occurs in the X chromosome inactivated by lionization could explain the male predominance.[54]

Internal tandem duplications (ITDs) of PUFD domain do not occur in hematologic malignancies but are detected in kidney clear cell sarcoma and central nervous system high-grade neuroepithelial tumor with BCOR alteration (CNS-HGNET-BCOR), a rare pediatric aggressive brain tumor.[55,56] Curiously, ITDs map to the structurally disordered *BCOR* PUFD termini, abrogating the binding to the PCGF1 RAWUL and preventing the formation of PRC1.1 complex.[28] Notably, such alterations were not reported for *BCORL1*.[28]

Translocations involving *BCOR* are mainly detected in undifferentiated round cell sarcoma, high-grade endometrial sarcoma, and ossifying fibromyxoid tumor.[47-49] Conversely, *BCOR* fusions were reported only in 2 patients with acute promyelocytic leukemia.[57,58] In both cases, *BCOR* was translocated with *the retinoic acid receptor α* (*RARα*) gene because of a rare t(X;17)(p11;q12).[57,58] Morphologically, 1 case showed Auer rods and Faggot cells, whereas the other did not. Both patients responded to all-transretinoic acid but experienced frequent relapses and were refractory to arsenic trioxide. The incidence (supplemental Table 1), significance, and clinical relevance of *BCOR* mutations in hematologicl diseases are discussed below.

BCOR and *BCORL1* mutations in myeloid neoplasms

AML

BCOR mutations are detected in 3.8% to 5.0% of adult de novo AML[9,59,60] and about 4% of AML with myelodysplasia-related changes.[61] Frequency is lower in pediatric AML[62] (*BCOR* 1.7%) and higher in secondary AML[52,63] (about 8%). We found that about 45% of *BCOR*-mutated AML had concomitant *DNMT3A* and/or *RUNX1* mutations and were mutually exclusive with *FLT3* and *NPM1* mutations.[9] The hierarchy of *BCOR*, *DNMT3A*, and *RUNX1* mutations in AML is poorly understood. *BCOR*-mutated AML patients also show a high rate of *N-RAS* and *K-RAS* mutations (36.8%).[59] *BCORL1* mutations occur at a frequency of 3.7% to

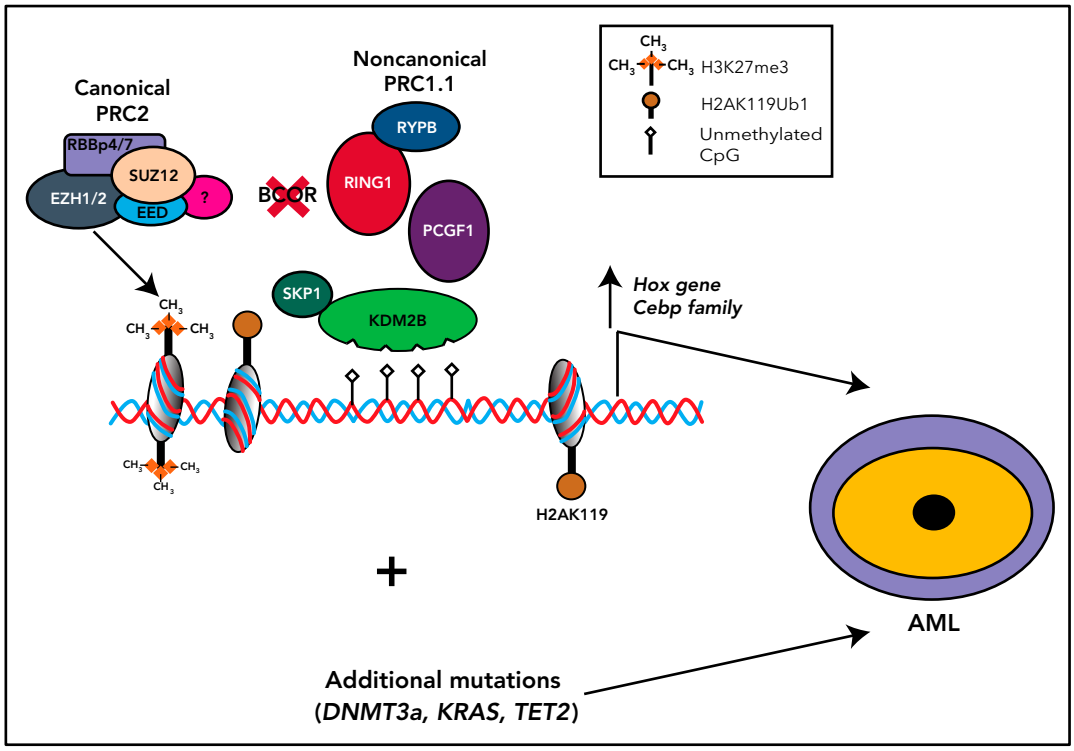

Figure 5. BCOR functional loss cooperates with other mutations to promote AML. Disruptive *BCOR* mutations that cause loss of the native protein or generate a truncated protein abrogate the capability of BCOR to bind PCGF1, thus preventing its interaction with KDM2B and the formation and recruitment to chromating of the enzymatic core. Thus, in hematopoietic stem and progenitor cells (HSPCs), the repressive activity of the complex is abrogated resulting into the expression of *Hox* and *Cebp* family genes. The occurrence of additional mutations (eg, *DNMT3A* and *RUNX1*) promotes the development of AML. Whether *BCOR* mutations precede or follow *DNMT3A* and *RUNX1* mutations remains to be established.

5.8% of AML adult patients[10,59] and 1.2% of pediatric AML patients.[62] Coincidental *BCOR* and *BCORL1* deleterious mutations were suggested to play a role (together with *PHF6* mutation) in the leukemic transformation of a patient with familial platelet disorder related to germinal C-terminal *RUNX1* mutation.[64] Thus, occurrence of *BCOR* and *BCORL1* mutations in the same tumor suggest the they may not be redundant, consistently with the likely different function of the 2 proteins.

Most *BCOR*-mutated AML patients show a normal karyotype.[9] Cases with abnormal karyotype include the following: trisomy 8, t(9;11), −7, and complex karyotype.[65] Trisomies 11 and 13, as well as inv(3)(q21q26)/t(3;3)(q21;q26), associate with high rate of *BCOR* mutations[65-68] (25%-38%). In AML with t(16;21)(p11;q22)/*FUS-ERG*, *BCOR* mutations are frequent and appear to precede chromosomal translocation.[69]

BCOR-mutated AML shows a lower remission rate after induction (47.4%).[59] In 422 de novo AML patients with normal cytogenetic, we found a shorter overall survival (OS) at 2 years in *BCOR*-mutated (25.6%) vs *BCOR*-unmutated (56.7%) patients.[9] In a Japanese cohort of 377 de novo AML patients, *BCOR* mutations were associated with lower 5-year OS and relapse-free survival, especially in patients ≤65 years of age, *FLT3-ITD* negative, and with intermediate cytogenetic prognosis.[59] Similarly, in 509 Chinese patients, *BCOR*-mutated cases showed an inferior 2-year OS and 2-year relapse-free survival compared with unmutated cases.[65] Hematopoietic stem cell transplantation seems to abrogate the adverse prognostic impact of *BCOR* mutations.[59,65]

The 2017 EuropeanLeukemiaNet risk stratification does not regard *BCOR* mutations as a prognostic predictor,[70] but their inclusion in the intermediate risk group was recently proposed.[71] Mutations of *BCOR*, other epigenetic modifiers, and RNA-splicing regulators also define a heterogeneous category (18% of AML) with intermediate/adverse prognosis of the genetic classification of AML.[60] *BCOR* mutations predicted complete response to venetoclax plus hypomethylating agents in AML.[72,73]

Myelodysplastic syndromes and myelodysplastic/myeloproliferative neoplasms

BCOR mutations occur in 4.2% to 5.0% of myelodysplastic syndromes (MDS) (vs 0.8%-2.0% of *BCORL1* mutations),[51,74] who usually carry a normal karyotype[75] and are comutated for *RUNX1* and *DNMT3A*.[51,74,76,77] *BCOR* mutations also associate with mutations of *ASXL1*, *NF1*, *ETV6*, *BCORL1*, *MECOM*, *RAD21*, *CEBPA*, and Cohesin genes.[74,78] *BCOR* mutations occur in all International Prognostic Scoring System (IPSS) risk groups and World Health Organization subtypes,[74] being more frequent in patients younger and with lower platelet counts at diagnosis.[51,74] Cryptic recurrent deletions at Xp11.4 (where *BCOR* is located) are detected in 2.8% MDS patients with normal or noninformative karyotype.[79]

The *BCOR* mutant-copy burden in flow-sorted $CD34^+/CD38^-$ early hematopoietic progenitors is lower than that of *RUNX1*, *STAG2*, and *ASXL1* mutations,[51] suggesting that *BCOR* mutations hierarchically occur at a later stage and define the clinical course rather than initiation of MDS.[51]

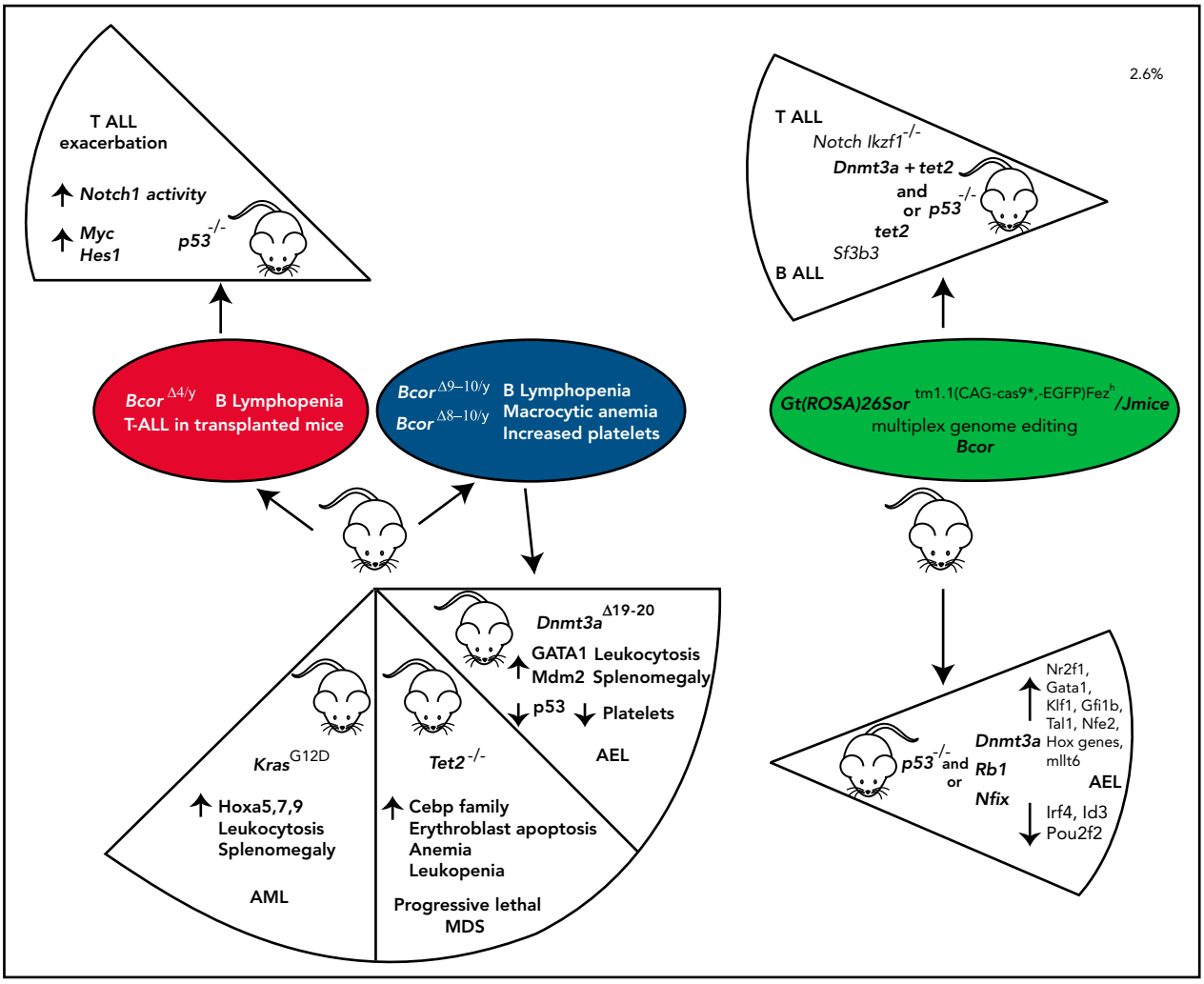

Figure 6. Schematic representation of *BCOR* knockout and double compound mouse models. The presence of different partner mutations in the *Bcor* conditional knockout mouse model (*Bcor*$^{\Delta 4/Y}$, *Bcor*$^{\Delta 9\text{-}10/Y}$, *Bcor*$^{\Delta 8\text{-}10/Y}$) variably affects the severity and penetrance of the disease phenotype. In particular, *Bcor*$^{\Delta 4/Y}$,*p53*$^{-/-}$ mice exacerbate the T-ALL developed in *Bcor*$^{\Delta 4/Y}$ mice. Compound mutant mice carrying *Bcor*$^{\Delta 9\text{-}10/Y}$,Tet2$^{-/-}$ mutations develop a progressive lethal MDS. Compound mice comutated for *Bcor*$^{\Delta 9\text{-}10/Y}$, and *Kras*^{G12D} develop AML, and mice comutated for *Bcor*$^{\Delta 8\text{-}10/Y}$ and Dnmt3a$^{\Delta 19\text{-}20}$ develop acute erythroid leukemia (AEL). An approach of multiplex genome editing of primary mouse hematopoietic stem and progenitor cell transplanted in a clustered regularly interspaced short palindromic repeat (CRISPR)-cas9 mice compound demonstrates that comutations of *Bcor*, *Trp53* plus *Dnmt3a* or *Rb1* or *Nfix* results in AEL. In contrast, the contemporary comutations of *Bcor*, *Dnmt3a*, *Trp53*, and *tet2* result in T-ALL, and the contemporary comutations of *Bcor*, *tet2* and *Sf3b3* lead to B-ALL.

The prognostic impact of *BCOR* mutations in MDS remains controversial.[51,74,76] Two studies on about 1000 patients showed inferior OS.[51,76] In another cohort of 621 patients, *BCOR* and *BCORL1* mutations did not impact OS, whereas the mutation type did. In particular, patients carrying frameshift mutations showed a median OS[74] lower than those with other types of mutations.[74] Moreover, mutations at the C terminus of *BCORL1* were associated with an OS shorter than mutations outside the C terminus.[74] However, these findings need to be confirmed.

Finally, MDS patients with isolated *BCOR* mutations showed a trend toward a prognosis poorer than cases comutated for *BCOR* and *TET2*, *ASXL1*, or *DNMT3A*.[80] This may be due to enrichment of poor cytogenetic in the *BCOR*-mutated-only group or to better response to hypomethylating agents when other epigenetic modulators are comutated.[80]

BCOR mutations occur in 3% to 10% of chronic myelomonocytic leukemia (CMML),[35,81,82] especially CMML-2, which is frequently comutated for *U2AF1* and *RUNX1*[51] or *ASXL1/EZH2*.[83] The prognostic value of *BCOR* mutations in CMML remains uncertain.[84,85] *BCOR* mutations also occur in 24% of MDS/myeloproliferative neoplasms with ring sideroblasts and thrombocytosis.[82,86] Notably, increased median corpuscular volume of erythrocytes and thrombocytosis was observed in our conditional knockout *Bcor* mice.[87]

Myeloproliferative neoplasms

BCL6-mediated repression of p53 is critical for leukemia stem cell survival in chronic myeloid leukemia (CML).[88] *BCOR* mutations are rare in chronic-phase CML and, when present, usually persist despite marked reduction of *BCR-ABL* transcript following tyrosine kinase inhibitors. Thus, they may originate from a preleukemic Philadelphia-negative (Ph$^-$) clone that existed independently of Ph1$^+$ clones.[89] *BCOR* mutations occur in about 16% of blastic-phase CML[90] and contribute driving CML transformation.[91] *BCOR* and *ASXL1* mutations also appear to be independent predictors for worse response to tyrosine kinase inhibitors of blastic-phase CML.[92]

A missense *BCOR* mutation was reported in 1 essential thrombocytemia patient triple negative for *JAK2*, *CALR*, and *MPL* and with a normal karyotype,[93] presenting with a high platelet count and resistant to therapy. Curiously, we observed thrombocytosis in our conditional knockout *Bcor* mice.[87] *BCORL1* and *RUNX1* mutations occurring concomitantly with a novel mutation of *JAK2* at serine 523 were detected in a patient with increased hematocrit.[94] Moreover, mutations of *BCORL1* (together with *TP53* and *NRAS*) have been associated with long-term (>21 years) leukemic transformation of polycythemia vera and essential thromcocytopenia.[95] However, this event is very rare.[96]

BCOR mutations in B-cell malignancies

The somatic mutation rate of B-cell chronic lymphocytic leukemia is low, including up to 2% of *BCOR* mutations.[97,98] Despite the low incidence of B-cell chronic lymphocytic leukemia in Asia, *BCOR* mutations tend to be more frequent in Korean than White patients.[99] Most *BCOR*-mutated cases are *IGHV* unmutated,[100] carry trisomy 12, and are *NOTCH1* mutated.[101,102]

B-cell prolymphocytic leukemia (B-PLL) usually carries a complex karyotype with frequent *MYC* translocations or gains and (del)17p.[103] About 25% of B-PLL patients harbor *BCOR* mutations[103] that are usually early clonal events.[103] *BCOR* mutations likely cooperate with *MYC* translocations in promoting B-PLL. *BCOR* mutations were detected in 9% of mantle cell lymphoma patients[104] and may cooperate with *KDM5C* mutations by increasing H3K4 and try-methylation in the late stages of mantle cell lymphoma.[105]

Clonal *BCOR* mutations or losses occurred in 24% of splenic diffuse red-pulp small B-cell lymphoma,[106] whereas they were absent in hairy cell leukemia and hairy cell leukemia variant and were only rarely found in splenic marginal zone lymphoma. Other B-cell lymphomas mutated for *BCOR* are listed in supplemental Table 1.

BCOR mutations in T-cell malignancies

BCOR mutations were detected in 2% to 3% of pediatric T-cell acute lymphoblastic leukemia with high TAL1 expression, being mutually exclusive with TLX1 and TLX3 expression (TLX-related).[107]

T-cell prolymphocytic leukemia is an aggressive disease carrying the inv(14) (q11q32)/t(14;14) (q11;q32) or t(X;14)(q28;q11). However, other genetic alterations may contribute to promote T-cell prolymphocytic leukemia, such as *BCOR* mutations that occur in 8% to 9% of cases.[108,109] *BCOR* deletion, at Xp11.4, is also revealed by Comparative Genomic Hybridization (CGH) array.[108]

Extranodal natural killer/T-cell lymphoma nasal type (ENKTL) frequently carries mutations in the JAK-STAT pathway.[110] *BCOR* mutations occur in 20.6% to 32% patients,[110,111] suggesting they may play a pathogenetic role in ENKTL.[112] However, the BCOR K607E mutation is not restricted to natural killer/T-cell lymphomas (31.9%), being also observed in angioimmunoblastic T-cell lymphomas (11.1%) and peripheral T-cell lymphomas not otherwise specified (33.3%).[113] Because Epstein-Barr virus infection promotes ENKTL through epigenetic mechanisms,[114] *BCOR* mutations could cooperate with Epstein-Barr virus by amplifying epigenetic deregulation.

BCOR and *BCORL1* mutations in nonmalignant hematologic diseases

Acquired aplastic anemia

Aplastic anemia (AA) shows a high frequency of clonal hematopoiesis.[115,116] Mutations of *BCOR, BCORL1*, *DNMT3A*, *ASXL1*, and *PIGA* have been detected in AA.[117-120] Frequency of *BCOR/BCORL1* mutations in AA ranged between 0% and 10.9%.[118-122] This suggests that, when such mutations occur without a proper ancestral hit, they are possibly unable to drive an efficient clonal expansion and are thus overridden once normal polyclonal hematopoietic stem cells recover.

AA patients carry a disproportionate number of *BCOR* and *BCORL1* mutations compared with their expected frequency in an age-matched population. Thus, these mutations are more likely to be selected by the AA bone marrow milieu rather than representing an age-related outgrowth.[120,122] In AA, the autoimmune attack of T lymphocytes against HSCs can result into selective growth advantage of cells that, by acquiring somatic mutations, become less immunogenic.[117] Unlike *DNMT3A* and *ASXL1* mutations, those involving *PIGA*, *BCOR*, and *BCORL1* tended to disappear or showed stable clone size. Moreover, AA patients carrying *BCOR, BCORL1*, and *PIGA* mutations responded better to immunosuppressive therapy than patients with other mutations and showed a good OS and progression-free survival.[120] Conversely, *DNMT3A* and *ASXL1* mutations tended to increase their clone size and were associated with worse outcome.[120] Similar findings have been reported for *BCOR* and *BCORL1* mutations in pure red cell aplasia.[123]

MDS and AML usually develop in 15% to 26% of AA patients over a period of 10 years.[115] Unlike high-risk *ASXL1* and *RUNX1* mutations that promote evolution of AA to MDS/AML, *BCOR* and *BCORL1* mutations impart a low risk of transformation into MDS/AML.[115,124,125]

Erythrocytosis

Erythrocytosis defined by the strict 2008 World Health Organization classification criteria (hemoglobin > 18.5 g/dL or Hematocrit (Hct) ≥ 52% in males; hemoglobin > 16.5 g/dL or Hct ≥ 48% in females), associates with cardiovascular morbidity/mortality and all-cause mortality,[126] independently of conventional risk factors. Moreover, cardiovascular morbidity is strongly associated with clonal hematopoiesis, mostly because of *BCOR/BCORL1* mutations (16%).[126] Similar to AA, *BCOR/BCORL1* mutations in erythrocytosis associate with a low risk of transformation into MDS/AML.[126]

Role of *BCOR* in the pathogenesis of hematologic malignancies

Given the disruptive nature of *BCOR* mutations, the role of *BCOR* in promoting hematologic malignancies was mainly investigated in animal models whose endogenous gene had been inactivated. Initial *Bcor* loss-of-function studies in zebrafish and *Xenopus* recapitulated the phenotype of OFCD syndrome.[127] *Bcor* knockout mouse models are discussed below.

Myeloid malignancies

A conditional loss-of-function model targeting exons 9 and 10 of the *Bcor* allele allowing their removal via expression of either a

retrovirus-expressing Cre ex vivo or a Vav-iCre recombinase in vivo was generated.[37] Excision led to a premature stop codon with deletion of carboxy-terminal *Bcor* domain required for proper formation of PRC1.1 complex.[24] *Bcor* mutant cells cultured under myeloid stem/progenitor conditions showed higher proliferation rates than control cells. *Bcor* knockout mice exhibited a marked increase of peripheral blood neutrophils without significant changes in red blood cells, platelet and lymphocyte levels. Conversely, other investigators failed to demonstrate peripheral blood counts alterations, using the same conditional model crossed with *HSC-SCL-Cre-ER^T* mice to facilitate tamoxifen-inducible *Bcor* deletion specifically in HSCs.[128] Nevertheless, mutant mice showed expanded BM cKit$^+$Sca1$^-$Lin$^-$ myeloid progenitors with enhanced repopulating capacity in vivo.[128] Both mouse models exhibited overexpression of Hox genes. Specifically, *Bcor* loss reduced the levels of RING1B in the complex, leading to a reduced monoubiquitylation of H2A at position 119 of HoxA promoters with consequent upregulated Hox transcription.

More recently, we developed a conditional *Bcor* knockout mutant[87] targeting exons 8 to 10 resulting in a premature stop codon in exon 11 and tested the effects of *Bcor* loss in hematopoiesis using *Mx1-Cre* mice.[87] Mice displayed leukopenia, mainly because of B-cell lymphopenia, red blood cell reduction with increased mean corpuscle volume, and progressive increase of platelet counts. Thrombocytosis was caused by accumulation of megakaryocytic-erythroid and megakaryocytic progenitors due to apoptosis resistance. Thus, *Bcor* loss of function induces derepression of Hox and *Cebp* family genes,[37] myeloid differentiation, and thrombocytosis.[87] However, it is insufficient to promote myeloid malignancies alone, clearly pointing to the need of additional cooperative events, as depicted in Figure 5.

Because *BCOR* and *DNMT3A* are frequently comutated in AML,[9] we generated *Bcor/Dnmt3a* double knockout mice[87] that rapidly developed a lethal leukemic phenotype characterized by immature erythroid cells expansion[87] (Figure 6). The aberrant erythroid skewing was induced by an altered molecular program affecting major cell cycle regulators (Mdm2, Tp53) and erythroid-specific transcriptional factors (Gata1-2)[87] (Figure 6). Another mouse model of acute erythroid leukemia involving loss of *Bcor* and *Dnmt3a* (in addition to *Trp53*) and characterized by deregulation of aberrantly methylated driver genes has been recently reported[129] (Figure 6).

BCOR and *RAS* mutations cooccur in both AML and MDS. *Bcor*-deficient mice crossed with *Kras* mutant animals[128] developed a lethal disease characterized by leucocytosis, splenomegaly, and increased leukemic blasts through Hoxa 9 upregulation (Figure 6). *BCOR* and *TET2* are also frequently comutated in MDS patients.[51,76,130] Accordingly, *Bcor* and *Tet2* disruption in mice induced a lethal MDS phenotype with differentiation block, apoptosis, and activation of myeloid regulator genes of the *Cebp* and Hoxa family through reduction of H2AK119ub levels[31] (Figure 6).

One of the BCOR functional domains (Figure 1) directly binds to the common MLL fusion partner AF9 (MLLT3), contributing to promote MLL rearranged leukemia.[131] Mutagenesis studies identified point mutations selectively disrupting the capability of BCOR to bind MLLT3. Expression of these *BCOR* point mutations in BM stem/progenitor cells caused partial differentiation and abrogated the leukemogenic potential in a mouse model[131] through downregulation of EYA1 phosphatase and c-MYC protein expression.[131]

We recently found *BCOR* mutations in the AML cell lines MUTZ-2, KG-1, and HL60-R (E. Tiachi and B. Falini, unpublished data). *BCOR* reconstitution in HL60-R cells inhibited cell growth and increased vitamin D_3–induced differentiation (E. Tiachi and B. Falini, unpublished data). *BCORL1* mutations were detected in the AML-193, SKM1, and OCI-AML5 cell lines.[10] The MUTZ-2 AML cell line carries both *BCOR* and *BCORL1* mutations.[10] All these cell lines may serve for functional studies and drug testing in vitro and in vivo.

Lymphoid malignancies

About 10% of *NUP98-PHF2* (*NP23*) transgenic mice develop an aggressive pro-B1 ALL that carries spontaneous *Bcor* indel mutations, leading to premature stop codons, usually within a 9-bp hotspot in exon 8.[132,133] Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-Cas9 insertion of *Bcor* frameshift mutation into *NP23* hematopoietic stem/progenitor cells and their transplantation into recipient irradiated mice led to pro-B1 ALL development,[134] suggesting a cooperation between mutated *Bcor* and *NP23* fusion. The human counterpart of mouse B-1 cells remains elusive, and *Bcor* mutations are very rare in human B-cell ALL. However, mouse *NP23/Bcor* pro-B1 ALL tends to acquire *Jak* mutations and may serve as a model for human B-progenitor ALL with *Jak* mutation and rearrangements causing overexpression of CRLF2,[132] a receptor for thymic stromal lymphopoietin critical for B-1 cell development.

Emu-Myc mice spontaneously develop a B-cell leukemia/lymphoma–like malignancy with 100% penetrance. Destructive *Bcor* mutations and loss of *Cdkn2a* cooperate with overexpressed Myc to promote this disease.[135]

Mice expressing Bcor lacking the BCL6-binding domain[136] showed impaired B lymphopoiesis, and 50% of animals developed lethal T-ALL with late latency. However, the concomitant *p53* loss accelerated T-ALL development (Figure 6). Thymic leukemic blasts displayed activated Notch1 and upregulation of its target genes *Myc* (via *Bcor* loss of function[135]) and *Hes1*. These findings suggest a tumor suppressor role for *Bcor* in T-ALL, antagonizing the transcriptional activation of T-ALL related oncogenes by *Notch1*.[136] *Bcor* loss of function may induce leukemia abrogating the PRC1.1 complex formation because mice without the ZF-CxxC DNA-binding domain of Kdm2b develop T-ALL.[50]

BCOR carrying the K607E mutation (located near the BCL6 binding site) binds to BCL6, PCGF1, and RING1B proteins with lower affinity than BCOR wild type.[113] Ectopic expression of *BCOR*-K607E mutant drives the constitutive activation of T cells (ie, enhanced cell proliferation, increased phosphorylation of AKT, and production of interleukin-2). Similar effects were mimicked by silencing *BCOR* in T cells.[113] Similarly to AML, the BCOR mutant led to upregulation of HOX genes.[113]

Conclusions and perspectives

BCOR is involved in the regulation of embryogenesis, mesenchymal stem cell function, hematopoiesis, and lymphoid development. *BCOR* and *BCORL1* disruptive mutations contribute to

the origin of various hematologic malignancies and are similar to those found in OFCD and Shukla-Vernon syndromes.

Therapeutic targeting of BCOR-containing PRC1.1 complex functions (eg, H2AK119 ubiquitylation and H3K36 demethylation) should be explored.[137] AML cells with *BCOR* mutations alone are sensitive to the tankyrase/WNT inhibitor XAV-939 and the multikinase inhibitor crizotinib.[138] *BCOR/RUNX1*-comutated AML cells are sensitive to JAK kinase inhibitors,[138] whereas acute erythroid leukemia driven by *Bcor* and *Dnmt3a* loss is susceptible to CDK7/CDK9 inhibitors.[129] Better understanding of the role played by BCOR and BCORL1 in leukemogenesis and screening for synthetically lethal partners of these mutations may help unraveling new therapeutic opportunities.

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Footnotes

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