Review

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Epigenomics

BCOR involvement in cancer

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BCL-6 corepressor (*BCOR*) is a gene that encodes for an epigenetic regulator involved in the specification of cell differentiation and body structure development and takes part in the noncanonical polycomb repressive complex 1. This review provides a comprehensive summary of BCOR's involvement in oncology, illustrating that various *BCOR* aberrations, such as the internal tandem duplications of the PCGF Ub-like fold discriminator domain and different gene fusions (mainly *BCOR-CCNB3, BCOR-MAML3 and ZC3H7B-BCOR*), represent driver elements of various sarcomas such as clear cell sarcoma of the kidney, primitive mesenchymal myxoid tumor of infancy, small round–blue-cell sarcoma, endometrial stromal sarcoma and histologically heterogeneous CNS neoplasms group with similar genomic methylation patterns known as CNS-HGNET-BCOR. Furthermore, other *BCOR* alterations (often loss of function mutations) recur in a large variety of mesenchymal, epithelial, neural and hematological tumors, suggesting a central role in cancer evolution.

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BCL-6 corepressor is an epigenetic regulator

It is known that tumorigenesis depends on the sequential acquisition of proto-oncogene-activating mutations and/or oncosuppressor loss of function. In this oversimplified perspective, epigenetic alterations are less easily framed, even if the resulting inappropriate expression of oncogenes and oncosuppressors can play an equally crucial role in cancer development and progression. Therefore, epigenetic regulators are potential proto-oncogenes or oncosuppressors, depending on the function of their target genes. A gene implicated in transcriptional regulation, *BCOR* – BCL-6 corepressor, is increasingly reported as mutated in different human cancers, with a key role in neoplastic transformation or in tumor progression.

BCOR is located on chromosome X, in the Xp11.4 locus, and derives its name from its function as an interacting corepressor of BCL-6 that enhances BCL-6-mediated transcriptional repression [1]. The gene has 16 alternative exons coding several protein isoforms, with the principal isoform, encoded by 14 exons, that gives rise to a protein of 1755 amino acids. *BCOR* shows virtually ubiquitous expression, but only a few isoforms retain known protein interactions, depending on the domains preserved by alternative splicing.

The function of BCOR is primarily mediated by two domains: the BCL-6-binding domain that interacts with the transcriptional repressor BCL-6 and the PCGF Ub-like fold discriminator (PUFD), a common domain recurrent in different proteins implicated in histone regulation that binds the RING finger and WD40-associated ubiquitin-like (RAWUL) domain of PCGF proteins (polycomb group RING finger homologs; Figure 1).

BCOR takes part into one specific type of polycomb repressive complex that mediates transcriptional repression through epigenetic modifications of histones. Polycomb repressive complexes (PRCs) are molecular complexes involved in histone modification processes that are usually classified into two types: PRC1, which adds an ubiquitin moiety to histone H2A at Lys119 (H2AK119ub1), and PRC2, which catalyzes the addition of one to three methyl groups to histone H3 at Lys27, leading to H3K27me1, H3K27me2 or H3K27me3 [2]. PRCs silent a wide range of genes, including homeobox group genes (*HOX*), the first identified PRC targets [2,3]. The core protein subunits of PRC2 complex are Suz12 (with a Zinc finger domain), Eed (with a WD repeat domain that recognizes trimethylated peptides) and Ezh2 (the SET-domain containing catalytic subunit) [4]. These core

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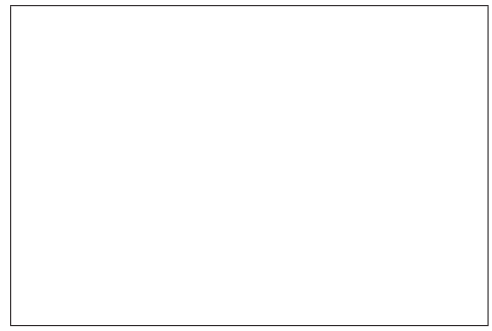

Figure 1. Structure and functional domains of BCOR, including BCL-6- and MLLT3-binding domains, ankyrin repeats and the PCGF Ub-like fold discriminator domain. A schematic representation of the exon structure is also shown below the protein domains.

Table 1. Polycomb repressive complex 1.1 complex components.

PRC1.1 subunits	Principal interactions	Functional notes
BCOR	PCGF1, KDM2B, BCL6, MLLT3, IRF8	PRC1.1 core protein. It seems able to bind AF9, a protein associated with SEC complex, but the relevance of this interaction has not been determined
BCORL1	PCGF1, KDM2B, CTBP1, HDACs	PRC1.1 core protein, alternatively to BCoR. It does not bind BCL-6, but the transcriptional corepressor CTBP1. Interacts with different Class II HDACs
PCGF1	BCOR, KDM2B, RYBP, YAF2	PRC1.1 core protein
KDM2B	BCOR, PCGF1, SKP1, unmethylated CpG islands	PRC1.1 core protein. It demethylates H3K36me3/me2. It can participate in the formation of the SCF complex
RING1a/RING1b	PCGF1, RYBP, YAF2, CBX8	Canonical and noncanonical PRC1s core protein. It ubiquitinates H2AK119
UBP7	BCOR, PCGF1, CBX8, RING1a/b, PCGF2/MEL18, PCGF4/BMI1, PTEN, MDM2, P53, DMNT	De-ubiquitinating action. It can be associated with other noncanonical PRC1 as well as the classic PRC1.
SKP1	KDM2B, other SCF proteins	Ubiquitinating action. It binds KDM2B. It participates in the formation of the SCF complex
RYBP/YAF2	PGGF1, RING1b, YY1	They may link PRCs to YY1
Associated proteins	**Principal interactions**	**Functional notes**
YY1	RYBP, YAF2, DNA, INO80 complex, P53, HDACs	Transcription factor. The real relevance in the *in vivo* PRCs recruitment is not yet determined
CBX8	RING1a/b, H3K27me3, PCGF1/2/3/5/6, PHC1/2, MLLT1	It recruits the PRCs that contain it at H3K27me3 histones, establishing a functional cross-link with the PRC2s. Not present in PRC1.1
BCL6	BCOR, NCOR1, SMRT, HDACs, NuRD, MET3, DNA	Transcription factor. It recruits PRC1.1 by link with BCOR. It also has PRC independent activity: it is able to bind other proteins involved in gene silencing

PRC: Polycomb repressive complex

components associate with different proteins that work to regulate PRC2 enzymatic activity or its recruitment to specific genomic loci.

Conversely, all PRC1 complexes contain a core consisting of a RING1 protein (either RING1A or RING1B), which is an E3-ubiquitin ligase that adds an ubiquitin group to histone H2A at Lysine 119, and a PCGF protein (PCGF 1–6). Different PRC1 complexes are defined by the different PCGF protein that associates to the complex and directs interaction with distinct auxiliary proteins, giving rise to PRC1 complexes with differential target specificity and chromatin recruitment patterns [5]. Moreover, based on the cofactor that binds to the RING1 subunit, PRC1 complexes are often divided into 'canonical,' 'noncanonical' or 'variant.' Canonical PRC1 complexes (cPRC1) are assembled around PCGF2/4 and contain one chromobox protein (CBX2, 4, 6–8) that is able to recognize and bind the H3K27me3 histone mark implemented by the PRC2 complex [6]. Conversely, noncanonical PRC1 complexes (ncPRC1) bind to the RYBP or its paralog YAF2 and associate with PCGF1, PCGF3/5 or PCGF6 to give rise to PRC1.1, PRC1.3/1.5 or PRC1.6, respectively [4,6]. RYBP and YAF2 play a fundamental role in stimulating E3 ligase activity of PRC1 and enhancing H2AK119ub deposition [7]. Importantly, RYBP and YAF2 lack the capacity to bind to H3K27me3 and can be recruited to chromatin in cells lacking functional PRC2.

BCOR participates in the constitution of one of the six currently described noncanonical variants of PRC1, the PRC1.1 (Table 1) [8–17]. PRC1.1's core components include RING1A/B and PCGF1 that heterodimerize via their

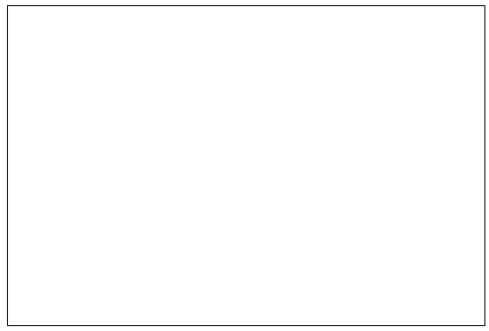

Figure 2. Schematic representation of the polycomb repressive complex 1.1 model. The core complex is composed of the catalytic enzyme RING1A/B that forms a dimer with PCGF1 through the RING finger domains, and that deposits an ubiquitin moiety to histone H2A at Lys119 (H2AK119ub). BCOR binds to PCGF1 by means of its PUFD domain, while RYBP is bound to the RAWUL domain of RING1A/B. Recruitment to chromatin is due to KDM2B that recognizes nonmethylated CpG islands by its CXXC-binding domain. Other members of the complex are SKP1, that associates with KDM2B, and USP7, acting as a deubiquitinating enzyme.
PUFD: PCGF Ub-like fold discriminator.

N-terminal RING domains (Figure 2). The RING1/PCGF1 heterodimer forms a scaffold for PRC1 assembly. PRC1.1 contains RYBP or its homolog YAF2, altogether with BCOR and other proteins (Figure 2). BCOR associates with the complex by binding to PCGF1 through its PUFD domain located at the C-terminus. Since RYBP and YAF2 are not able to bind H3K27me3, recruitment to chromatin depends on the activity of KDM2B, an H3K36 me2 histone demethylase (Figure 2) that harbors a Zinc finger CxxC-binding domain involved in DNA binding that recognizes and drives targeting of PRC1.1 to nonmethylated CpG islands as observed in murine embryonic stem (ES) cells [5,18].

In mammals, PRCs are recruited to target sites by multiple mechanisms, including the binding of hypomethylated CpG islands, the specific interaction with transcription factors and long ncRNAs, and the recognition of chromatin marks deposited by other histone-modifying complexes [6]. This last mechanism is at least partly responsible for the coordinated activity of PRC1 and PRC2, since it is known that the PRC2-dependent H3K27me3 mark increases affinity and recruits CBX-containing cPRC1 [19], while KDM2B-mediated recruitment to nonmethylated CpG islands of ncPRC1 drives H2AK119ub that conversely promotes binding of PRC2 [5]. However, it is also known that in some contexts PRC1.1 can act independently from PRC2; for example, in leukemic stem cells PRC1.1 was found to bind a unique set of genes independently of PRC2 and of the H3K27me3 histone mark [8].

Not surprisingly, the genes encoding for polycomb proteins (PcG) were initially identified, along with trithorax (TrxG), as important elements for homeostasis of body development in *Drosophila Melanogaster*. Indeed, even in mammals and humans, PcG gene germinal mutations often cause severe morphological defects and complex syndromes [20–22]. Specifically, a number of *BCOR* germinal loss of function mutations (>40 were previously identified) [23] induce oculo-facio-cardio-dental syndrome (OFCD) [24], inherited in an X-linked dominant mode and lethal in males [25]. The typical phenotype of OFCD syndrome is characterized by ocular defects, such as congenital cataracts and microphthalmia, craniofacial dysmorphisms, such as bifid nose tip and palatoschisis, cardiac abnormalities, such as atrial and ventricular septal defects, as well as dental abnormalities, such as radiculomegaly and oligodontia [26]. An attenuated variant of OFCD syndrome, known as Lenz's microphthalmia, has also been described, holding a classical X-linked recessive inheritance and therefore asymptomatic in females [24].

Nevertheless, the normal evolution of body structure during embryo development is not the only biological process that depends on proper activity of the PcG group proteins. These are also involved in the maintenance of cell identity and are necessary for correct cell differentiation [27]. Studies performed on murine ES showed that BCOR is required for the formation of primitive erythrocytes, plays a role in B- and T-cell development and is necessary for the timely expression of genes regulating ES cell pluripotency and ectodermal and mesodermal development [28]. Moreover, in ES cells, BCOR is highly expressed and required for the maintenance of the pluripotent state, since its depletion induces a robust differentiative phenotype, further supporting the idea that BCOR is a core regulator of the primed pluripotent state [29].

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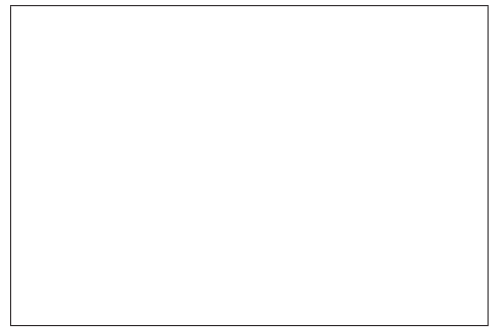

Figure 3. Schematic representation of different *BCOR* alterations, including internal tandem duplications and chimeric fusion transcripts. The numbers inside the boxes represent the exons, while open boxes indicate UTR regions. **(A)** ITD-BCOR, **(B)**BCOR-CCNB3, **(C)** BCOR-MAML3, **(D)** ZC3H7B-BCOR and **(E)** BCOR-RARA.
ITD: internal tandem duplication.

Due to its central role in pluripotency maintenance, differentiation induction and cell fate determination, it is not surprising that mutations in *BCOR* play a central role in cancer development. This review provides an in-depth description of the different tumor types for which the pathogenetic role of *BCOR* seems to be particularly relevant.

Sarcomas

Clear cell sarcoma of the kidney

Studies of clear cell sarcoma of the kidney (CCSK) have revealed a lot about the oncogenic role of *BCOR* somatic alteration on the diagnosis and biological understanding of this disease. CCSK is the second most common renal malignancy in childhood, representing 4–5% of all renal tumors [30] and accounting for approximately 20 new cases per year in the USA [31]. Although CCSK is recognized as an independent entity from Wilms' tumor, there is still much to learn about this sarcoma.

Microscopically, the classical pattern of CCSK presentation (91% of all cases) [31] is characterized by an architecture consisting of nests or cords of round cells with clear cytoplasm, separated by regular fibrovascular septa [32]. The extracellular matrix, rich in mucopolysaccharides, contributes to confer the 'clear' appearance of the tumor. However, classical architecture always accompanies at least one other pattern among the many described, albeit to a lesser extent [31,33].

From a molecular point of view, CCSK appears as a genetically stable tumor, and over the years, the genetic alterations found were few and not recurring. A decisive step forward was made between 2015 and 2016, when five independent studies described and confirmed the recurrence of different internal tandem duplications (ITD) in the last exon of the *BCOR* gene, involving the PUFD domain (Figure 3A) [34–38]. These events were found in less than 75% of analyzed cases (Table 2), and many different types of ITD were identified in CCSK, all residing in exon 15 of the PUFD domain, and all preserving the protein frame. In particular, the different ITDs involved the region encompassing amino acids 1700–1755, with a minimal identified duplication of 22 amino acids up to a maximum of 38, and a minimal overlapping duplicated region covering protein positions 1725–1737 [38]. The involved region is almost always duplicated, while only in very rare cases was a partial triplication found [38]. These achievements, confirmed by subsequent works [39,40], laid the basis for the investigation of CCSK pathobiology. In fact, the features of duplication, that is strictly in-frame and associated with overexpression of the protein encoded by *BCOR*, suggested important speculations about the origins of CCSK.

There have been several attempts to attribute biological significance in terms of gain or loss of function to the ITD-BCOR; however, until now, no predicted structural model of the BCOR protein carrying the ITD has been analyzed and released, even though protein–protein interaction studies suggested that ITD-BCOR might represent an hypomorphic allele with altered PCGF1-binding affinity [13]. Currently, we are still far from having conclusive results on the question, and even though there is evidence supporting a loss-of-function effect of ITD-BCOR, (overexpression of Cyclin D1 [41,42], of PRC2 targets and of several classes of HOX genes [36]), the lack of any frameshift or nonsense mutation in this setting is instead in favor of a gain-of-function event.

Table 2. BCOR mutations in different tumor hystotypes.

Tumor family	Tumor hystotype	Examined subgroup	BCOR		Molecular methods†	Ref.
			Genetic alteration %	Genetic alteration type		
Sarcomas	Clear cell sarcoma of the kidney	/	91% (10/11)	ITD-exon 15	RNA-seq/Targeted RT-PCR	[35]
			85% (23/27)	ITD-exon 15	WES/RNA-seq	[32]
			9.1% (1/11)	BCOR-CCNB3 fusion	RNA-seq/Targeted RT-PCR	[35]
			83% (132/159)	ITD-exon 15	PCR/Targeted RT-PCR	[34]
			91% (20/22)	ITD-exon 15	RNA-seq	[33]
			100% (8/8)	ITD-exon 15	RNA-seq	[31]
			75% (3/4)	ITD-exon 15	RNA-seq/Targeted RT-PCR/FISH	[36]
			Two case reports	BCOR-CCNB3 fusion	FISH	[84]
			Five case reports	ITD-exon 15	Targeted PCR	[100]
			100% (20/20)	ITD-exon 15	Targeted PCR	[30]
	Primitive myxoid mesenchymal tumor of infancy	/	100% (5/5)	ITD-exon 15	Targeted PCR	[42]
			86% (6/7)	ITD-exon 15	RNA-seq/Targeted RT-PCR/FISH	[36]
			One case report	ITD-exon 15	Targeted PCR	[100]
			One case report	ITD-exon 15	Targeted PCR	[41]
	Undifferentiated/unclassified sarcomas: various hystologies	/	1.5% (11/753)	BCOR-CCNB3 fusion	Targeted RT-PCR	[47]
			14% (6/43)	BCOR-CCNB3 fusion	RNA-seq	[48]
	Undifferentiated/unclassified sarcomas: (Ewing-like) small blue–round-cell or spindle cell sarcoma/undifferentiated round-cell sarcoma	/	4% (24/594)	BCOR-CCNB3 fusion	RNA-seq	[44]
			6.5% (12/184)	Five BCOR-CCNB3 fusions, one BCOR-MAML3 fusion, one ZC3H7B-BCOR fusion, five BCOR-ITD	RNA-seq	[56]
			13% (11/87)	BCOR-CCNB3 fusion	RNA-seq/Targeted RT-PCR/FISH	[58]
			12% (2/17)	BCOR-CCNB3 fusion	Targeted RT-PCR/FISH	[57]
			2.5% (5/200)	BCOR-CCNB3 fusion	Targeted RT-PCR	[59]
			Ten cases	BCOR-CCNB3 fusion	Targeted RT-PCR	[46]
			5% (2/41)	BCOR-CCNB3 fusion	Targeted RT-PCR/FISH	[53]
			4.3% (7/164)	BCOR-CCNB3 fusion	Targeted RT-PCR/FISH	[49]
			One case report	KMT2D-BCOR fusion	RNA-seq/Targeted RT-PCR	[55]
		Soft tissue URCSs	Four case reports	BCOR-CCNB3 fusion	Targeted RT-PCR	[50]
			41% (9/22)	ITD-exon 15	RNA-seq/Targeted RT-PCR/FISH	[36]
		SBRCSs lacking *EWSR1, FUS, SYT* and *CIC* gene rearrangements	22% (19/86)	11 BCOR-CCNB3 fusion, two BCOR-MAML3 fusions, two ZC3H7B-BCOR fusions, four other BCOR rearrangments	FISH	[51]
		BCOR-CCNB3 fusion Positive sarcomas	36 case reports	BCOR-CCNB3 fusion	RNA-seq/Targeted RT-PCR	[55]
	Various hystologies	Round cell sarcoma of bone	4% (24/594)	BCOR-CCNB3 fusion	n.a.	[52]

†Only the techniques used in the individual studies to establish BCOR mutational status have been reported.
5′-RACE PCR: 5′-Rapid amplification of cDNA ends PCR; AML: Acute myeloid leukemia; array CGH: Array comparative genome hybridization; CMML: Chronic myelo-monocytic leukemia; DIPG: Diffuse intrinsic pontine glioma; FISH: Fluorescent *in situ* hybridization; ITD: internal tandem duplication; MDS: Myelodysplastic syndrome; n.a.: Not available; NGS: Next-generation sequencing; pGBM: Pediatric glioblastoma; RNA-seq: RNA sequencing; RT-PCR: Real-time PCR; WES: Whole exome sequencing; WGS: Whole genome sequencing; WT: Wild type

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Table 2. BCOR mutations in different tumor hystotypes (cont.).

Tumor family	Tumor hystotype	Examined subgroup	BCOR		Molecular methods†	Ref.
			Genetic alteration %	Genetic alteration type		
		Soft tissue tumors	1.5% (2/133)	BCOR-CCNB3 fusion	RNA-microarray	[54]
	Endometrial stromal sarcoma	/	29% (9/31)	Five ZC3H7B-BCOR fusion, three BCOR-ZC3H7B fusion, one ITD exon 15	Various	[81]
			9% (3/27)	Two ZC3H7B-BCOR fusion, one ZC3H7B-BCOR fusion + BCOR-ZC3H7B fusion	Various	[69]
			Two case reports	One ZC3H7B-BCOR fusion, one ZC3H7B-BCOR fusion + BCOR-ZC3H7B fusion	RNA-seq/Targeted RT-PCR	[78]
			Three case reports	ZC3H7B-BCOR fusions	Targeted RT-PCR/FISH	[77]
			17 case reports	ZC3H7B-BCOR fusions	Targeted RNA NGS/FISH	[79]
			Three case reports	ITD-exon 15	Targeted PCR	[80]
	Rhabdomyosarcoma	/	8.3% (5/60)	Frameshift insert/deletion, nonsense	WES/RNA-seq	[67]
			7% (10/147)	Seven Frameshift insert/deletion, one nonsense, two focal homozygous deletion	WGS/WES/RNA-seq	[65]
		Anaplastic RMS	One case report	Frameshift insertion	Targeted PCR	[66]
		PAX-fusion negative RMS	9.5% (9/94)	Six frameshift insert/deletion, one nonsense, two focal homozygous deletion	WGS/WES/RNA-seq	[65]
		PAX-fusion positive RMS	1.9% (1/53)	Frameshift insert/deletion	WGS/WES/RNA-seq	[65]
	Ossifying fibromyxoid tumor	/	2.5% (1/39)	ZC3H7B-BCOR fusion	RNA-seq	[85]
CNS neoplasm	CNS neoplasms	'CNS-HG-NET-BCOR altereted' cluster of methylation tumor	79% (15/19)	ITD-exon 15	DNA and RNA NGS/targeted PCR	[83]
			Five case reports	ITD-exon 15	Targeted PCR	[100]
			One case report	ITD-exon 15	Targeted PCR	[102]
	Medulloblastoma	/	2% (4/189)	One nonsense, three frameshift insert/deletion	Various	[93]
			2.2% (1/46)	Nonsense	WGS/WES	[94]
			1.6% (2/125)	Frameshift insert/deletion	WGS/WES	[95]
			3% (3/92)	Nonsense, frameshift insert/deletion	WES	[96]
		Sonic-Hedgehog-driven MB	7% (4/58)	One nonsense, three frameshift insert/deletion	Various	[93]
			5.2% (7/133)	Missense, nonsense, frameshift insert/deletion	WGS/WES	[97]
		Sonic-Hedgehog-driven MB in infants (age <3)	8% (4/50)	n.a.	WGS/WES	[97]
		Sonic-Hedgehog-driven MB in children (age 3 ≤ × <18)	3% (1/33)	n.a.	WGS/WES	[97]
		Sonic-Hedgehog-driven MB in adults (age ≥18)	4% (2/50)	n.a.	WGS/WES	[97]

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Tumor family	Tumor hystotype	Examined subgroup	BCOR		Molecular methods†	Ref.
			Genetic alteration %	Genetic alteration type		
	Retinoblastoma	/	10% (7/71)	One missense, two nonsense, two frameshift insert/deletions, two large deletions	WES	[87]
			13% (6/46)	Frameshift insert/deletion, large deletion	WGS/SNP array	[88]
			4.2% (4/94)	Focal deletion	WGS/SNP array	[89]
	Gliomas, various hystologies	Recurrent high-grade astroblastoma	25% (1/4)	Frameshift insert/deletion	Targeted DNA NGS/array CGH	[92]
		pGBM and DIPG	4,3% (14/326)	Nonsense, frameshift insert/deletion	WGS/WES/RNA-seq	[91]
		Diffuse glioma	14% (1/7)	Missense	WES	[90]
Hemolymphopoietic system tumors	Myeloid neoplasm	MDS	5.3% (6/114)	Nonsense, frameshift insert/deletion, splice site	WES	[112]
			2.8% (2/71)	Deletion (Xp11.4)	Targeted DNA NGS/array CGH	[108]
			4.2% (40/944)	Various	Targeted DNA NGS/array CGH	[109]
			7% (2/29)	Nonsense, frameshift insert/deletion	WES	[110]
			4.2% (15/354)	Eight frameshift insert/deletion, five nonsense and two splice site	Targeted PCR	[111]
		MDS with multilineage dysplasia	8% (2/25)	Frameshift insertion, splice site	WES	[112]
		MDS with excess blasts	5.4% (2/37)	Frameshift deletion, nonsense	WES	[112]
		AML with chromosome 11 trisomy	4% (1/23)	n.a.	Targeted DNA NGS	[123]
		AML with chromosome 13 trisomy	25% of 34	n.a.	WES/targeted DNA NGS	[124]
		Cohesin-altered myeloid neoplasm	11.4% (14/123)	n.a.	WES/targeted DNA NGS	[122]
		Cohesin-WT myeloid neoplasm	5% (47/937)	n.a.	WES/targeted DNA NGS	[122]
		Unselected AML	8% (72/494 adult, vs 3/179 in children)	n.a.	DNA and RNA NGS	[113]
			5% of 143	n.a.	WES/targeted DNA NGS	[114]
			8.7% (58/664)	n.a.	Targeted DNA NGS	[115]
		Primary AML in adults	3% (6/197)	n.a.	Targeted DNA NGS	[116]
			1.6% (4/247)	n.a.	WES / Targeted DNA NGS	[114]
			5% (83/1603)	n.a.	Targeted DNA NGS	[125]
			5% (19/377)	Missense, nonsense, frameshift insert/deletion, splice site	Targeted DNA NGS	[121]
		Aged ≤65 with intermediate cytogenetic prognosis	15.2% (7/46)	n.a.	Targeted DNA NGS	[121]
		AML with balanced chromosomic rearrangements	8% (18/224, but 38% in cases with inv(3)(q21q26.2/t(3;3)(q21q26.2))	n.a.	Targeted DNA NGS	[125]

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Table 2. BCOR mutations in different tumor hystotypes (cont.).

Tumor family	Tumor hystotype	Examined subgroup	BCOR		Molecular methods†	Ref.
			Genetic alteration %	Genetic alteration type		
		AML with unbalanced chromosomic rearrangements	9% (31/349)	n.a.	Targeted DNA NGS	[125]
		Secondary AML	7.2% (16/221)	n.a.	WES/targeted DNA NGS	[114]
		RUNX1-mutated	18% (29/163)	n.a.	Targeted DNA NGS	[128]
			10.8% (13/1381)	n.a.	Targeted DNA NGS	[129]
		Normal karyotype AML	2.5% (1/40)	Focal deletion	Targeted DNA NGS	[126]
			4.2% (10/262)	n.a.	Targeted PCR	[127]
			4% (27/716)	n.a.	Targeted DNA NGS	[125]
		Normal karyotype	17.1% (14/82)	Frameshift insert/deletion, nonsense, splice site	Targeted PCR	[127]
		Pediatric normal karyotype AML	6.3% (3/48)	Missense	Targeted PCR	[120]
		Pediatric AML	1.1% (2/182)	One frameshift insert/deletion, one splice site	WES/targeted DNA NGS	[117]
			1.1% (4/369)	n.a.	Targeted PCR	[118]
			5% (2/40)	Frameshift insert/deletion, nonsense	Targeted DNA NGS/SNP array	[119]
			4.8% (4/83)	Missense	Targeted PCR	[120]
		Acute promyelocytic leukemia	One case report	BCOR-RARα	5′-RACE-PCR	[132]
			One case report	BCOR-RARα	Targeted PCR	[133]
		CMML	10% (15/150)	n.a.	WES/targeted DNA NGS	[130]
			7.4% (3/54)	One frameshift insert/deletion, three nonsense	Targeted PCR	[111]
			7.7% (2/26)	Frameshift insertion, splice site	WES	[112]
	Lymphoid neoplasm	Extranodal NK/T-cell lymphoma nasal type	17% ([5/30] or 20.6% [7/34] including cell lines)	Two missense, two frameshift insertion/deletion, three nonsense	WES/targeted DNA NGS/RNA-seq	[146]
			32% (8/25)	Frameshift insert/deletion, large deletion, nonsense, splice site	Targeted DNA NGS	[147]
			21% (24/113 including cell lines)	n.a.	Targeted DNA NGS	[147]
		T-cell prolymphocytic leukemia	9% (2/23)	Missense	Targeted DNA NGS	[139]
			8% (4/51)	Deletion (Xp11.4, 50%)	Targeted DNA NGS/array CGH	[140]
		Chronic lymphocytic leukemia	3% (4/149)	Three frameshift insert/deletion, one nonsense	WES/SNP array	[141]
			1.6% 10/643	n.a.	Targeted DNA NGS	[144]
			6.3% (3/48)	Missense, frameshift insert/deletion	Targeted DNA NGS	[142]
			1.2% (5/428)	Nonsense, frameshift insert/deletion	WGS/WES/RNA-seq/array CGH	[143]

†Only the techniques used in the individual studies to establish BCOR mutational status have been reported.
5′-RACE PCR: 5′-Rapid amplification of cDNA ends PCR; AML: Acute myeloid leukemia; array CGH: Array comparative genome hybridization; CMML: Chronic myelo-monocytic leukemia; DIPG: Diffuse intrinsic pontine glioma; FISH: Fluorescent *in situ* hybridization; ITD: internal tandem duplication; MDS: Myelodysplastic syndrome; n.a.: Not available; NGS: Next-generation sequencing; pGBM: Pediatric glioblastoma; RNA-seq: RNA sequencing; RT-PCR: Real-time PCR; WES: Whole exome sequencing; WGS: Whole genome sequencing; WT: Wild type

Table 2. BCOR mutations in different tumor hystotypes (cont.).

Tumor family	Tumor hystotype	Examined subgroup	BCOR		Molecular methods†	Ref.
			Genetic alteration %	Genetic alteration type		
		Splenic diffuse red pulp lymphoma	24% (10/42)	Three frameshift insert/deletion, two nonsense, one splice site, four large deletions	WES/targeted DNA NGS/array CGH	[145]
	Other	Mixed-phenotype acute leukemia	8.3% (1/12)	n.a.	Targeted DNA NGS	[131]
Carcinomas	Salivary glands cancer, various hystologies	Recurrent and metastatic SGC	8% (4/50)	Missense	Targeted DNA NGS	[149]
	Adenoid cystic carcinoma	Metastatic ACCs	60% (3/5)	Frameshift insertion, nonsense	WGS/RNA-seq	[151]
	Endometrial carcinoma	POLE-negative nonultramutated Endometrioid endometrial carcinoma	13% (10/76)	Missense, splice site	WES/targeted DNA NGS	[152]
	Gynecologic carcinosarcoma		n.a.	n.a (Missense N1459S 2 time recurrent)	WES	[153]
	Thymoma and thymic carcinoma	B3 thymoma	50% (3/6)	Missense, frameshift insert/deletion	WES	[148]
			One case report	Frameshift insert/deletion	WGS/RNA-seq/array CGH	[150]

† Only the techniques used in the individual studies to establish BCOR mutational status have been reported.
5′-RACE PCR: 5′-Rapid amplification of cDNA ends PCR; AML: Acute myeloid leukemia; array CGH: Array comparative genome hybridization; CMML: Chronic myelo-monocytic leukemia; DIPG: Diffuse intrinsic pontine glioma; FISH: Fluorescent *in situ* hybridization; ITD: internal tandem duplication; MDS: Myelodysplastic syndrome; n.a.: Not available; NGS: Next-generation sequencing; pGBM: Pediatric glioblastoma; RNA-seq: RNA sequencing; RT-PCR: Real-time PCR; WES: Whole exome sequencing; WGS: Whole genome sequencing; WT: Wild type

Primitive mesenchymal myxoid tumor of infancy

Primitive mesenchymal myxoid tumor of infancy (PMMTI) is a newly introduced myofibroblastic pediatric tumor [43]. It is an intermediate grade mesenchymal neoplasm with a high local recurrence rate, but with low metastatic potential [44]. Histologically, it is composed of cells that are fused in a myxoid matrix. Morphologically, differential diagnosis is warranted with respect to other stromal tumors, such as congenital infant fibrosarcoma, fibromyxoid sarcoma, myofibrosarcoma and dermatofibrosarcoma protuberans [44], from which it clearly diverges from the immunophenotypical pattern and specific molecular features. Actually, few studies that investigated PMMTI at a molecular level have found the presence of ITD-BCOR (Figure 3A) associated with the overexpression of BCOR in almost all the cases analyzed [40,45,46], similarly to CCSK. Recently, Kao *et al.* demonstrated that these two tumors also show a very similar transcriptional signature that, together with epidemiological, morphological and genetic overlap, suggests the possibility that PMMTI is the equivalent of CCSK arising in soft tissue [40].

Small round–blue-cell sarcomas

The typical pediatric tumors of Ewing sarcoma family can be molecularly distinguished by the presence of translocations involving the *EWSR1* gene and one of *ETS*/*FLI* family genes. These tumors are morphologically distinct per their microscopic appearance consisting of a bed of round monomorphic cells with regular nuclei and dispersed chromatin, with or without the presence of rosettes [47]. However, progressive diffusion of molecular profiling and high-throughput sequencing technologies has allowed the identification of an increasing number of molecular alterations in undifferentiated sarcomas that are morphologically similar or indistinguishable from Ewing's sarcoma, but lacking the aforementioned molecular features. The need to set up a new classification scheme for these tumors led to the use of the term 'Ewing-like small round-blue-cell sarcoma' (SRBCS), although the specific molecular entities belonging to this subgroup often present atypical, undifferentiated or with nonspecific histology (undifferentiated round cell sarcomas [URCS]). Among the soft tissue URCS, Kao *et al.* have shown that nearly half of them carry ITD-BCOR [40], whereas Pierron *et al.* identified a group characterized by the presence of the *BCOR-CCNB3* fusion gene (Figure 3B) and overexpression of the resulting chimeric protein [48]. These tumors showed a transcriptional signature different from pediatric tumors of Ewing sarcoma family (they were negative for BCOR immunostaining [49]) and a broader morphological spectrum [48,50], which is why the classification of Ewing-like sarcomas appears reductive. Other works have frequently found cases of *BCOR-CCNB3* positive sarcomas, in variable percentages depending on the group of tumors analyzed, but in any case definitely consistent

(Table 2) [40,50–63]. *CCNB3* encodes for cyclin B3, a protein with promeiotic function in which its expression is normally limited to the testis. CCNB3 activity in the chimeric protein (evaluated through cellular models of engineered fibroblasts) suggested a possible oncogenic driver role due to the observed ectopic expression of CCNB3 in this type of tumors, although further evaluations are needed to assess the role of BCOR in the chimeric protein, as well as any potential new activity acquired from the fusion of the two proteins [48].

Regarding 'Ewing-like' SRBCS, Specht *et al.* [55] described, in addition to the *BCOR-CCNB3* fusion gene, the presence of distinct rearrangements of *BCOR* in eight cases: two cases carrying BCOR-MAML3 fusion (Figure 3C), two carrying ZC3H7B-BCOR (Figure 3D) and four with internal rearrangements of *BCOR*. In the same study, the increased expression of *HOX* genes was reported in one of the two cases with BCOR-MAML3 fusion, suggestive of a loss of function of BCOR and PRC activity. *MAML3* encodes a member of the mastermind-like (MAML) family of transcriptional coactivators that constitutes a component of the Notch signaling pathway, with roles in biological processes such as cell proliferation, differentiation and survival. The BCOR-MAML3 fusion transcript contains exons 2-5 of MAML3, thus retaining the transactivation domain, but losing the Notch-binding site of MAML3. *ZC3H7B* encodes a nuclear protein containing a domain able to interact with the rotavirus NSP3 36 protein [64], as well as several other domains involved in multiprotein and nucleic acid/protein interactions. Little can be said at present about the ways in which these two genes exert their oncogenic roles in SRBCS, since their activity in the context of chimeric proteins containing BCOR requires further study. However, BCOR is involved in its entirety in the BCOR-MAML3 fusion and in its terminal 3′ portion only in the ZC3H7B-BCOR chimeric transcript. The recurrence of these two fusion genes in SRBCS was also confirmed in the most recent study by Watson *et al.* [60], which highlighted the existence of SRBCS characterized by the presence of ITD-BCOR (Table 2). An extremely interesting result of this last study is that all SRBCS-carrying *BCOR* alterations generate a common cluster at the transcriptional level, thus reinforcing a view in which *BCOR* alterations, either ITD or rearrangements, exert a common pathogenic pathway leading to abnormal activity of the PRC1.1 complex. In fact, as in CCSK, a significant enrichment of gene sets regulating morphogenesis, differentiation of neurons and tyrosine kinase receptors was observed, and *HOX* genes are overexpressed. In the same study, the authors noticed an overexpression of SMARCA2, a component of the chromatin modifier SWI/SNF complex, and its activity was opposite to that of the PRC1 and PRC2 complexes [65], with observed strict association and functional linkage [66]. This insight may suggest that PRCs loss-of-function caused by *BCOR* alterations can lead to increased SWI/SNF complex activity. This hypothesis is reinforced via *in vitro* work in which the codeletion of *EZH2* reverts the oncogenic phenotype caused by the loss of function of the *SMARCB1* gene [67], similarly to *SMARCA2*, which encodes for a subunit of the SWI/SNF complex. Recently, Kao *et al.* [59] also identified a single case of URCS harboring the *KMT2D-BCOR* fusion gene.

Rhabdomyosarcoma

BCOR's involvement appears to also be relevant in rhabdomyosarcoma (RMS), another typical blue–round-cell malignant childhood tumor. Histologically, the two major RMS subtypes are alveolar RMS and embryonal RMS, both of which have distinct molecular and clinical hallmarks [68]. Alveolar RMS results in poor prognosis and is genetically defined by the presence of a fusion involving *PAX3* or *PAX7* genes, two transcription factors. The embryonal RMS subtype typically affects younger children and is generally comprised of PAX-negative tumors. A rare subtype of RMS is anaplastic, where a single case was recently reported to carry a *BCOR* alteration, together with mutation in *ARID1A*, a member of the SWI/SNF family, and of *SETD2*, a histone methyl-transferase [69]. Two studies [68,70] confirmed that *BCOR* mutations in RMS recur in about 7–8% of total cases, with an apparent major involvement in PAX-negative RMS, for which the percentage nears 10% of cases (Table 2).

Endometrial stromal sarcoma

Endometrial stromal sarcoma (ESS) is a gynecological sarcoma composed of cells that resemble those of the endometrial stroma in the proliferative phase. The WHO classification recognizes four distinct entities [71,72]: benign stromal endometrial nodules; low-grade ESS genetically characterized by various translocations among which the most frequent involves *JAZF1* and/or *PHF1*; high-grade ESS generally carrying *YWHAE-NUTM2* fusion gene [73]; and undifferentiated endometrial sarcoma with a complex karyotype.

In particular, the involvement of the *YWHAE-NUTM2* fusion gene is noteworthy since this was the first genetic alteration associated with CCSK [74], as well as the second in order of frequency (with a percentage between 0 and 12% of cases in various studies) [34–38,75–77]. *YWHAE* genes (tyrosine 3-monooxygenase/tryptophan 5-

monooxygenase activation protein) encode different variants of the 14-3-3 protein family, which includes seven highly conserved and ubiquitously expressed proteins that seem to play a role in modulation of cytoskeletal organization, metabolism, differentiation and proliferation [78]. *NUTM2* genes of unknown function belong instead to the FAM22 family, which was more recently renamed NUTM2 because of sequence homology to the *NUT* gene locus that is involved in the oncogenesis of malignant tumor of midline [79].

Several patients with *ZC3H7B-BCOR* and/or *BCOR-ZC3H7B* [72,80–82] fusion genes were also described and initially included among low-grade ESS; however, it was recently proposed to identify ZC3H7B-BCOR ESS as an independent subgroup of high-grade ESS (Table 2) due to new prognostic evidence and myxoid leiomyosarcoma-like histologic appearance [80]. Recently, on subsets of 31 various grade ESS, Mariño-Enriquez *et al.* identified three cases carrying ITD-BCOR, one defined as high-grade ESS and two as undifferentiated ESS, all three cases holding typical immunophenotypic features of ITD-BCOR-positive tumors, such as the overexpression of cyclin D1 and BCOR [83].

This description limited to the mesenchymal tumor histotypes carrying BCOR-related abnormalities clearly underlined that the evident morphological and molecular overlaps between CCSK and PMMTI, as well as the common recurrence of ITD-BCOR, are not the only similarities found among these tumor histotypes. In fact, the *YWHAE-NUTM2* fusion gene, typical of some CCSKs, seems to be the driver element of high-grade ESS subgroup. Conversely, low-grade ESS is often characterized by the involvement of *BCOR* as a fusion gene with *ZC3H7B*. The absence of substantial histological differences between ITD-BCOR-positive CCSK and those carrying YWHAE-NUTM2, together with the simultaneous presence of BCOR molecular anomalies and YWHAE-NUTM2 fusion in the ESS, as well as the evidence that various *BCOR* aberrations (such as ITD, ZC3H7B-BCOR and BCOR-CCNB3) lead to the overexpression of BCOR and of cyclin D1 [34–36,40–42,46,62,83–86] are all elements that reinforce the hypothesis that these alterations activate a common pathogenetic pathway, or at least a partially shared one.

It is also interesting that the link between CCSK and SRBCS due to the common involvement of BCOR was also strengthened by the findings of YWHAE-NUTM2B/E and ITD-BCOR in a group of URCS of soft tissue, the last previously identified also in SRBCS [60], as well as by the description of three cases of CCSK carrying the *BCOR-CCNB3* fusion gene [87].

Another example of biological intersection between these tumors is the finding of ZC3H7B-BCOR fusion typical of low-grade ESS in some cases of SRBCS [55] and in one case of ossifying fibromyxoid tumor [88], a rare soft tissue tumor.

CNS neoplasms

BCOR is hypothesized to have an active role in neuronal development. In fact, BCOR expression patterns in several tissues during the various phases of embryonic development, conducted by Wamstad and Bardwell on mouse models, revealed intense BCOR expression in the neural tube and retina during development [89].

Therefore, it is not surprising that several CNS tumors carry *BCOR* alterations (Table 2). Mutations with supposed loss of function (e.g., nonsense, frameshift, splice sites and deletions) have been identified in up to 13% of retinoblastomas [90–92]; between 1 and 14% of various glial tumors, particularly in high-grade tumors [93–95] and also in pediatric patients [86,94], with a 25% peak found in recurrent high-grade astroblastoma [95]. Moreover, *BCOR* genetic alterations also appear in 2–8% of medulloblastomas [96–100], and in particular, such aberrations seem to be more represented in SHH-driven cases, especially in infant patients [100].

CNS-HGNET-BCOR

Primary CNS neuroectodermal tumors (CNS-PNET) are highly malignant neoplasms that predominantly affect children but may also occur in adolescents and adults. Histologically, CNS-PNETs are characterized by poorly differentiated embryonic cells, with a propensity both for glial and neuronal differentiation [101]. The WHO classification of 2016 [102] has removed CNS-PNET as an independent group of tumors, grouping them in a class of 'embryonic tumors' with medulloblastoma, as an example. In the attempt to establish new classification criteria to better define the molecular nature of CNS-PNET, Sturm *et al.* [86] proposed to evaluate a large series of CNS tumors via genomic methylation patterns. From this, new molecular entities emerged, clustering in well-defined methylation subgroups, but often lacking histological homogeneity. One of these new histologically nonhomogeneous entities was shown to carry in the vast majority of cases ITD-BCOR alteration. This group of tumors, defined CNS-HGNET-BCOR (high-grade primitive neuroectodermal tumors of the CNS with alterations of BCOR), showed a remarkable overexpression of BCOR similar to what is found in other tumors-carrying ITD-

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BCOR (Table 2). Curiously, as reported by Santiago *et al.* [46], the triad 'CCSK/PMMTI/CNS-HGNET-BCOR,' after the group 'rhabdoid tumor of the kidney/extra-renal rhabdoid tumor of soft tissues/atypical teratoid-rhabdoid tumor of the CNS,' would constitute the second trio of tumors joined by molecular anomalies and kidney, soft tissues, and brain districts. It is however necessary to highlight that Yoshida *et al.* recently reported important immunophenotypical differences between CNS-HGNET-BCOR and CSSK/PMMTI [103].

In addition to the recurrence of ITD-BCOR, CNS tumors and mesenchymal tumors with BCOR abnormalities also share common characteristics regarding transcriptional regulation. In fact, WNT and in particular SHH pathways, wherein upregulation had already been reported as a typical feature of CCSK [104], were also found upregulated in a CNS-HGNET-BCOR patient [105], as well as in many cases of retinoblastoma [106] and even represented the driver element of two subgroups of medulloblastoma [107]. The recurrence of the deregulation of these signaling pathways in at least two entities carrying ITD-BCOR supports the hypothesis that such deregulation may actually affect all ITD-BCOR-positive tumors, and it can be envisaged as a potential therapeutic target [105].

It is also interesting to note that, like mesenchymal tumors, also among CNS tumors with BCOR involvement, there are numerous cases arising in the pediatric setting. This evidence can indicate that, coherently with the functions of BCOR at the embryonic level, mutations in this gene lead to a premature disruption of the differentiation pathway in progenitor stem cells. However, further studies are necessary to support this hypothesis.

Hemolymphopoietic system neoplasms

There is evidence regarding the importance of BCOR for physiological hematopoiesis. Mutations of this gene in knockout organisms result in hematological abnormalities, consisting in functional deficiency of primitive erythrocytes and lymphocyte depletion, and confirming the relevance of BCOR on the activity of BCL-6, a known mitogenic agent for lymphoid cells [28]. Conversely, the loss-of-function of BCOR in murine bone marrow cells produces significantly enhanced proliferation and myeloid differentiation rates with upregulated expression of *HOX* genes [108]. Even if the introduction of next-generation sequencing (NGS) techniques for the molecular screening of large cohorts of patients affected by hemolymphopoietic system neoplasms highlighted the clear involvement of BCOR alterations in these diseases, at present it is still challenging to clarify the biological role of BCOR in myeloid and lymphoid precursors and to translate this molecular knowledge for clinical applications. Actually, there are still few reports aimed at clarifying the role of BCOR in lymphoid and myeloid oncogenesis [109,110]. Tanaka *et al.* generated transgenic mice harboring exon 4 deletion leading to loss of the BCL-6-binding domain, which developed lethal acute T-cell lymphoblastic leukemia in a Notch1-dependent manner and demonstrated myc upregulation [109]. Another report, by Lefebure *et al.* [110], showed that BCOR is a tumor suppressor in the Eμ-myc lymphoma murine model and its loss-of-function mutations act as myc-cooperating events in this setting.

Myeloid neoplasms

The involvement of BCOR alterations in clonal disorders of myeloid lineage (Table 2) has been assessed primarily for myelodysplasias [111–115] and acute myeloid leukemia (AML) [116–119], with cases ranging from less than 1–10% in unselected cohorts. By analyzing these and other cohort studies, it was possible to identify clinical contexts where the presence of BCOR alterations is most frequent. Particularly in AML, it is clear that the frequency of *BCOR* mutations is significantly higher in older patients than in pediatric ones [116,120–122], with pediatric AMLs with normal karyotype as the exception [123] and higher in secondary AML with respect to primary AML [117]. NGS techniques allow for the identification of new molecular subgroups potentially related to specific clinical characteristics, in addition to those already identifiable by cytogenetic studies. In the context of these specific entities, association with *BCOR* comutations (Table 2) is particularly relevant in the following instances: intermediate cytogenetic prognosis and FLT3-ITD-negative AML in adult patients under 65 [124], nonselected myeloid neoplasms with mutations in cohesin-coding genes [125], AML with changes associated with myelodysplasia [114], AML with trisomy 11 or 13 [126,127], AML with balanced or unbalanced chromosomal rearrangements [128], AML with normal karyotype [128–130] (in particular, pediatric AML) [123] or if co-occurring mutations in *NPM1, CEBPA, FLT3-ITD, MLL-PTD* or *RUNX1* are present [130–132].

The high percentage of BCOR involvement in patients with chronic myelo-monocytic leukemia [114,115,133] or with acute leukemia with mixed phenotype is also noteworthy [134]. Lastly, two cases of a variant of acute promyelocytic leukemia have been described for which *BCOR* represented the partner of *RARα* (the transcription factor typically involved in APL) in the chimeric gene (Figure 3E) [135,136].

BCOR alterations found in myeloid clonal diseases are apparently clustered around exon 4 and for the most part are mutations with presumed loss-of-function. This consideration derived both from the analysis of the type of alterations found and the evaluated expression of *HOX* genes, the target of PRC activity when these genes are strongly overexpressed. The prognostic significance of the presence of BCOR mutations in myeloid clonal diseases was also evaluated. Damm *et al.* identified the presence of mutated *BCOR* as an independent negative prognostic factor in MDS [114]. Regarding adult AML, Terada *et al.* identified *BCOR* mutations as unfavorable prognostic factors in 5-year overall survival (8.7% BCOR mutated vs 34.0% BCOR WT) [124].

Finally, *BCOR* alterations have been identified and/or described in several studies conducted on acquired aplastic anemia patients, in percentages similar to those described for nonselected MDS and AML [137–141]. This suggested the possibility that these mutations may contribute to clonal selection during the onset of myeloid malignancies on the aplastic anemia background.

Lymphoid neoplasms

In the lymphoid line, there is evidence of a significant presence of *BCOR* genetic alterations, seemingly all causing loss-of-function of the corresponding protein. *BCOR* alterations have been identified in up to 9% of prolymphocytic T leukemias [142,143], chronic lymphocytic leukemias [144–147] and, respectively, in 24% and up to 32% of the evaluated cases of diffuse splenic lymphoma of the red pulp [148] and of extra-nodal NK/T-cells lymphoma, nasal type [149,150]. The latter one is an aggressive lymphoma, strongly associated with EBV infection, which arises primarily, but not exclusively, in nasal and paranasal areas. Considering the high recurrence of mutation and inferences deriving from murine models, it is clear that a wider evaluation of BCOR involvement in these tumors is warranted.

Carcinomas

The functional studies conducted on BCOR showed that the activity of this gene primarily affects cells committed to the mesenchymal and neuroectodermal lineages. In fact, knockout organisms have shown alterations, especially in tissues involved in OFCD syndrome, as well as important hematological abnormalities [28]. Analysis of BCOR expression in various tissues during murine embryogenesis also confirmed strong expression in the eye, limb buds and branchial arches, neural tube, adnexal and nervous system tissues, and craniofacial structures [89]. These studies are partly strengthened by the analysis of tumor histotypes associated with *BCOR* mutations. The most represented tumors are stromal tumors, CNS neoplasms and hemolymphopoietic system tumors, sometimes concentrated in the head and neck or upper chest districts in organs such as the thymus [151] and salivary glands [152]. In the context of thymic tumors, it is particularly interesting that *BCOR* mutations recur in 50% of cases of aggressive B3-thymoma [153]. Considering the high percentage of recurrence, an active role of BCOR in the pathogenesis of this thymoma subtype, as well as the potential use of this gene as a classifying marker, is hypothesized. As for salivary glands tumors, the occurrence of *BCOR* mutations seems to characterize more aggressive diseases, regardless of the histological type. This is particularly true of adenoid cystic carcinoma, which is a rare type of cancer that most frequently occurs in salivary glands, but which can also be found in breast, lacrimal gland, lung, Bartholin's gland, trachea and paranasal sinuses [154]. While definitive publications on the recurrence and role of *BCOR* mutations in various epithelial malignancies are still lacking, it is evident from comprehensive sequencing efforts (https://portal.gdc.cancer.gov; http://www.cbioportal.org) that *BCOR* abnormalities are recurrent in uterine corpus endometrial carcinoma, colon and stomach adenocarcinoma, and lung tumors regardless of histology. In the context of The Cancer Genome Atlas project, in the EBV + gastric carcinomas subgroup, the percentage of mutations involving *BCOR* reached 23%. All mutations were nonsense mutations, therefore suggesting a complete loss-of-gene function. The high involvement of *BCOR* mutations in EBV + gastric carcinomas is emphasized by the consideration that BCOR also affects another EBV-related tumor: extra-nodal NK/T-cell lymphoma, nasal type.

A special case is that of the uterus because in this organ, *BCOR* alterations are recurrently involved both in epithelial tumor histotypes (in this case, endometrioid endometrial adenocarcinoma) [155] and in uterine carcinosarcoma (involving BCOR in 23% of cases) [156], in addition to the previously described ESS.

Conclusion

Somatic alterations of BCOR are found in many tumor subtypes, with fusion genes discovered in rare subclasses of sarcomas, Internal Tandem Duplications (ITD) in almost all pediatric Clear Cell Sarcomas of the Kidney, in a

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subgroup of undifferentiated pediatric sarcomas (undifferentiated round cell sarcoma of infancy, primitive myxoid mesenchymal tumor of infancy), in endometrial stromal sarcoma and in the subset of CNS HGNET-BCOR (High-grade neuroepithelial tumor of the central nervous system with BCOR gene alteration). Additionally, loss-of-function mutations were also found in myelodysplasias and acute myeloid leukemias and in selected subtypes of lymphoid neoplasms. A deeper knowledge of the functional role of different BCOR alterations in tumors of the mesenchymal, hematopoietic and neuroectodermal lineages will allow the identification of actionable pathways in these malignancies.

Future perspective

Deeper molecular knowledge of oncology enables better identification of diagnostic or prognostic markers and/or therapeutic targets. The recent introduction of NGS techniques has exponentially amplified the potential to obtain such data and to compare and find similarities among the molecular landscapes of histologically distant tumor types. This review has shown that *BCOR* represents one of the possible genes and a viable factor to focus our efforts. In fact, the recognition of recurrent alterations of this gene in tumors such as CCSK, PMMT1, SRBCS and CNS-HG-NET-BCOR already plays a key role in the diagnosis of these malignancies and there are many examples of new entities wherein BCOR plays a relevant clinical role. Further work will be necessary to dissect the activity of different BCOR mutations in specific cell contexts, and mechanistic and functional studies are warranted to understand the overall effects of these alterations on PRC1.1 function and target recruitment.

We believe that a better knowledge of PRC activity and the epigenetic regulators in which BCOR is an important component can lead to the advancement of new therapies.

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Author's contributions

A Pession conceived the study and revised the paper; M Fiore and A Astolfi drafted the manuscript and collected and interpreted the data; F Melchionda, V Indio and SN Bertuccio participated in planning and revising the manuscript. All the authors read and approved the final manuscript.

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Executive summary

BCOR is an epigenetic regulator

- BCOR is a transcriptional corepressor, wherein the gene is located on the Xp11.4 locus.
- It binds BCL-6 and takes part in the polycomb repressive complex (PRC) 1.1.
- In the PRC1.1 complex, BCOR binds to PCGF1 through its PCGF Ub-like fold discriminator domain.
- PRC1.1 silences genes through ubiquitination of Lys119 in histone H2A (H2AK119).
- BCOR germinal loss-of-function mutations determine oculo-facio-cardio-dental syndrome.
- BCOR is necessary for regulation of embryonic stem cell pluripotency and ectodermal and mesodermal development.

Involvement of BCOR in sarcomas

- BCOR internal tandem duplications (ITD) are present in more than 75% of CCSK, a pediatric renal sarcoma.
- ITD-BCOR are characteristic of almost all primitive mesenchymal myxoid tumor of infancy.
- Soft tissue undifferentiated round cell sarcomas are characterized by ITD-BCOR or BCOR-CCNB3 fusion gene.
- Other fusions found in Ewing-like small blue–round-cell sarcoma involve BCOR-MAML3 or ZC3H7B-BCOR.
- *BCOR* mutations recur in 10% of PAX-negative rhabdomyosarcomas.
- ZC3H7B-BCOR or ITD-BCOR characterize a subgroup of endometrial stromal sarcomas.

BCOR in CNS tumors

- Loss-of-function *BCOR* mutations are found in retinoblastomas, high-grade glial tumors and medulloblastomas.
- A subgroup of CNS-PNET, defined 'High-grade Primitive Neuroectodermal Tumors of the CNS with alterations of BCOR' is characterized by ITD-BCOR.
- Soft tissue undifferentiated round cell sarcomas are characterized by BCOR-ITD or BCOR-CCNB3 fusion gene.
- Other fusions found in Ewing-like small blue–round-cell sarcoma involve BCOR-MAML3 or ZC3H7B-BCOR.

BCOR alterations in hemolymphopoietic system tumors

- Loss-of-function mutations of *BCOR* are present in around 10% of unselected acute myeloid leukemia and Myelodysplastic syndrome.
- BCOR mutations are unfavorable prognostic factors in Myelodysplastic syndrome and adult acute myeloid leukemia.
- Prolymphocytic T leukemias and chronic lymphocytic leukemias carry *BCOR* mutations in less than 10% of cases.
- Up to 20–30% of diffuse splenic lymphoma of the red pulp and extra-nodal NK/T-cells lymphoma, nasal type carry BCOR alterations.

BCOR involvement in carcinomas

- *BCOR* mutations are found in aggressive B3-thymomas, adenoid cystic carcinoma, uterine corpus endometrial carcinoma, EBV + gastric carcinomas, colon and stomach adenocarcinoma, and lung tumors, regardless of histology.

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