

Transcriptomic and genomic heterogeneity in blastic plasmacytoid dendritic cell neoplasms: from ontogeny to oncogenesis

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Key Points

- BPDCNs have varying profiles evoking either pDC or AS-DC signatures, and phenotype can sometimes be confusing, notably with T-cell ALL.
- Genetics is heterogeneous: a lymphoid-like profile (*IKZF1*) is highly prevalent, whereas immune defects can mimic AS-DC characteristics.

Oncogenesis and ontogeny of blastic plasmacytoid dendritic cell neoplasm (BPDCN) remain uncertain, between canonical plasmacytoid dendritic cells (pDCs) and AXL⁺ SIGLEC6⁺ DCs (AS-DCs). We compared 12 BPDCN to 164 acute leukemia by Affymetrix HG-U133 Plus 2.0 arrays: BPDCN were closer to B-cell acute lymphoblastic leukemia (ALL), with enrichment in pDC, B-cell signatures, vesicular transport, deubiquitination pathways, and AS-DC signatures, but only in some cases. Importantly, 1 T-cell ALL clustered with BPDCN, with compatible morphology, immunophenotype (cCD3⁺ sCD3⁻ CD123⁺ cTCL1⁺ CD304⁺), and genetics. Many oncogenetic pathways are deregulated in BPDCN compared with normal pDC, such as cell-cycle kinases, and importantly, the transcription factor *SOX4*, involved in B ontogeny, pDC ontogeny, and cancer cell invasion. High-throughput sequencing (HaloPlex) showed myeloid mutations (*TET2*, 62%; *ASXL1*, 46%; *ZRSR2*, 31%) associated with lymphoid mutations (*IKZF1*), whereas single-nucleotide polymorphism (SNP) array (Affymetrix SNP array 6.0) revealed frequent losses (mean: 9 per patient) involving key hematological oncogenes (*RB1*, *IKZF1/2/3*, *ETV6*, *NR3C1*, *CDKN2A/B*, *TP53*) and immune response genes (*IFNGR*, *TGFB*, *CLEC4C*, *IFNA* cluster). Various markers suggest an AS-DC origin, but not in all patients, and some of these abnormalities are related to the leukemogenesis process, such as the 9p deletion, leading to decreased expression of genes encoding type I interferons. In addition, the AS-DC profile is only found in a subgroup of patients. Overall, the cellular ontogenic origin of BPDCN remains to be characterized, and these results highlight the heterogeneity of BPDCN, with a risk of a diagnostic trap.

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The data reported in this article have been deposited in the Gene Expression Omnibus (GEO) database, including GSM705329 to GSM705333, and GSE89565. Only transcriptomic data (Affymetrix HG-U133 Plus 2.0 arrays) have been partially presented. In our previous study¹⁹: 12 BPDCN out of 13, 65 AML out of 79, and 35

T-ALL out of 61 are available in the GEO database (GSE89565). The 5 primary cDCs and the 5 primary pDCs are also available on the GEO database (GSE28490, GSM705321-25 and 29-33, respectively).

The full-text version of this article contains a data supplement.

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Introduction

Dendritic cells (DCs) consist of conventional or myeloid dendritic cells (cDCs) and plasmacytoid dendritic cells (pDCs). pDCs are lineage[−] CD4⁺ CD123^{high} HLA-DR^{high} and produce large amounts of interferon (IFN) type I in response to viral infections.¹ They develop from myeloid and/or lymphoid progenitors. The myeloid branch includes common myeloid progenitors (CMPs) and common DC progenitors (CDPs) with both cDC and pDC potential,^{2,3} while the lymphoid branch involves common lymphoid progenitors.^{4–6} Recently, AXL⁺ SIGLEC6⁺ CD11c^{−/low} DCs (AS-DCs) have been described among the CD123^{high} HLA-DR^{high} DCs.^{4,7} Single-cell RNA sequencing shows that this population shares pDC- and cDC-like enriched signatures. Characterized by low IFN secretion and the ability to stimulate T-cell proliferation, they have properties closer to cDC and might be a transition state from pDC to cDC, explaining conflicting descriptions of pDCs.^{7,8} They express cDC markers, such as CD2, CD33, CD5, CD86, but also pDC markers, such as CD123^{high} and CD303,⁷ and present some B-cell features: IGLL1⁺ SIGLEC1⁺ CD22⁺ LYZ⁺ compared with canonical pDCs.^{4,7,9–12}

Blastic plasmacytoid dendritic cell neoplasm (BPDCN) is a rare and aggressive leukemia derived from pDCs, which is characterized by skin involvement, and affects mainly elderly men.^{13–18} Our team previously described a BPDCN-specific transcriptomic profile leading to dysregulation of *LXR* target genes involved in cholesterol homeostasis.¹⁹ The mutational landscape of BPDCN is close to myeloid malignancies, impacting epigenetics (*TET2*, *ASXL1*, *EZH2*, *ATRX*, *IDH1*, *IDH2*, *DNMT3A*), the RAS pathway (*NRAS*, *KRAS*), splicing (*SF3B1*, *SRSF2*, *ZRSR2*), kinase signaling (*FLT3*, *KIT*), and tumor removal (*TP53*, *RB1*, *ATM*).^{20–27} Moreover, myeloid neoplasms can be associated with or followed by BPDCN,²⁸ especially chronic myelomonocytic leukemia (CMML),^{29,30} with shared clonal mutations in 2 studies.^{31,32} Conversely, the rare case reports of BPDCN associated with lymphoid neoplasms never show common genetic events, suggesting cooccurrence instead of a common clonal origin.^{33–35} Conversely, deletions of *CDKN2A/CDKN2B* and *IKZF1* are particularly prevalent in BPDCN, similarly to lymphoid neoplasms, as well as *MYC* rearrangements, underpinning similarities with high-grade B-cell lymphoma.^{25,36–38} Moreover, the CAL-1 cell line, derived from a BPDCN patient, is *MYC*-rearranged and exhibits an AS-DC profile with the following phenotype: AXL⁺ SIGLEC1⁺ IGLL1⁺ BCL11A⁺ ID2⁺ SPIB⁺ SPI1⁺ PAX5[−] TCF3[−] ID3[−].^{4,37} Variability of lineage marker expression is also frequent in BPDCN, with expression of AS-DC markers, such as CD22, CD2, or CD33.¹⁷ However, the exhaustive AS-DC phenotype has never been investigated in BPDCN patients. In addition, some other lymphoid or myeloid lineage markers can be expressed, such as CD7, cCD79a, CD117, and/or CD15, which do not correspond to the AS-DC phenotype.^{20,18,39–41}

Data obtained on BPDCN in recent years have not clarified whether BPDCN derive from canonical pDCs or from AS-DCs, or whether BPDCN can be divided into 2 groups: one derived from canonical pDCs and the other from AS-DCs. In this study, we clarify the transcriptomics signature of BPDCN in comparison with other acute leukemias (ALs) and correlate it with copy number variations (CNV) and mutations to investigate pathways involved in BPDCN oncogenesis and ontogeny.

Methods

Patients and materials

Bone marrow (BM) aspirates or peripheral blood (PB) samples were obtained for BPDCN diagnostic purposes from 12 patients (French BPDCN network, authorization number DC-2008-713) and compared with a control cohort of 164 patients with AL, including acute myeloid leukemia (AML; *n* = 79), B-cell acute lymphoblastic leukemia (B-ALL; *n* = 24), and T-cell acute lymphoblastic leukemia (T-ALL; *n* = 61) (supplemental Methods). All BPDCN patients had cutaneous involvement with diagnostic confirmation on skin biopsies for 10 of them (Table 1). This study was approved by the local ethics committee (CPP EST II, Besançon, France; 31 January 2017).

Molecular annotations

T-cell receptor (TCR) δ and γ rearrangements and direct sequencing of *TCR* and *NOTCH1/FBXW7* genes were performed as previously described.⁴² Internal tandem duplications and mutations of the tyrosine kinase domain of *FLT3* (*FLT3-ITD* and *FLT3-TKD*) as well as *CEBP α* mutations were centrally assessed on ABI 2720 Genetic Analyzer (Thermo Fisher Scientific, Wilmington, NC) with Sequencing Analysis v2.5 software.

NGS and bioinformatics analysis

Next-generation sequencing (NGS) was performed from a HaloPlex^{HS} Target Enrichment System designed for Illumina Sequencing (Agilent) targeting 68 genes (supplemental Table 1) in paired-end, 2 × 150 cycles on a MiSeq platform (Illumina Inc, San Diego, CA). Raw data were analyzed using an in-house bioinformatics pipeline (supplemental Methods).⁴³ Sequencing by the Sanger method was performed on an ABI PRISM 3500 DNA Genetic Analyzer (Thermo Fisher Scientific) with a BigDye Terminator v3.1 Cycle Sequencing kit (Thermo Fisher Scientific).

Single-nucleotide polymorphism array analysis

Genome-wide detection of CNV and copy-neutral loss of heterozygosity were performed using the Genome-Wide Human single-nucleotide polymorphism (SNP) Array 6.0 (Affymetrix, Santa Clara, CA). CNV was identified and depicted with tools listed in the supplemental Methods.

Gene expression profiling and data analysis

Gene expression profiling was obtained using Human Genome U133 Plus 2.0 arrays (Affymetrix) from 176 leukemia samples (12 BPDCN, 164 AL) and compared with 5 normal cell-sorted cDCs and 5 pDCs from the GEO database, GSE28490: GSM705321-25 and 29-33, respectively (see supplemental Methods for analysis and supplemental Table 2 for published signatures).

Statistics

Statistical analyses were performed using Prism software v6 (GraphPad Software, San Diego, CA), using nonparametric tests for quantitative variables with non-Gaussian distribution, Spearman correlation, and the Kruskal-Wallis test, as appropriate. All statistical tests were 2-sided, with 5% significance.

Table 1. Clinical and biological features of the cohort

Patient number	Age, y	Sex	Sample	Infiltration, %	Hematological sites	Extrahematological sites	Prior history (time before diagnosis)	Karyotype	Markers associated to pDC lineage	BPCDN score	Immature lineage markers*	Myeloid lineage markers*	B lineage markers*	T lineage markers*	Anatomopathological confirmation
P1	38	M	PB	65	PB, BM	Skin	None	44,X,-Y,del(9)(q11;q31),-13(12)/44,ide,add(4q7)/add(5q7)(3)/46,XY[14]	CD4 ⁺ CD56 ⁺ HLA-DR ⁺ CD123 ⁺ CD303 ⁺ CD304 ⁺ TCL1 ⁺	5/5	Tdt ⁺	CD33 ⁺	None	CD2 ⁺	Yes
P2	69	M	BM	90	BM, LN	Skin	Colon cancer	46,XY[20]	CD4 ⁺ CD56 ⁺ HLA-DR ⁺ CD123 ⁺ CD303 ⁺ CD304 ⁺ TCL1 ⁺	3/5	None	None	None	CD7 ⁺	No
P3	70	M	PB	70	PB, BM	Skin	CMMML	44,X,-Y,del(6)(q23q25-27)/2,add(9)(q24),inv(9)(p11;q13)ct(12;15)(p10;p10),-13(18)/46,XY,inv(9)(p11;q13)c[2]	CD4 ⁺ CD56 ⁺ HLA-DR ⁺ CD123 ⁺ CD303 ⁺ CD304 ⁺ TCL1 ⁺	5/5	None	CD33 ⁺ CD117 ⁺	None	Neg	Yes
P4	51	M	PB	71	PB, BM, LN, spleen	Skin, gums	None	46,XY,del(9)(q11q22),del(9)(q11;q13),del(10)(q12;q13)[1]/46,XY,ide,;(7)(q10)[18]/46,XY[11]	CD4 ⁺ CD56 ⁺ CD123 ⁺ CD303 ⁺ CD304 ⁺ TCL1 ⁺	5/5	None	None	None	CD2 ⁺ CD5 ⁺ low (18%)	Yes
P5	15	M	BM	100	PB, BM, LN, spleen	Skin, liver	None	43,XY,(3;6),der(7)(7;19),-9,der(12)(5;12),-13,-19[7]/86,ide,x2[3]	CD4 ⁺ CD56 ⁺ HLA-DR ⁺ CD123 ⁺ CD303 ⁺ CD304 ⁺ TCL1 ⁺	5/5	None	None	None	Neg (CD7 and CD2 NA)	Yes
P6	66	M	BM	74	PB, BM, LN	Skin	None	46,XY[20]	CD4 ⁺ CD56 ⁺ HLA-DR ⁺ CD123 ⁺ CD303 ⁺ CD304 ⁺ TCL1 ⁺	5/5	None	CD33 ⁺	CD22 ⁺	CD7 ⁺	Yes
P8	60	M	PB	70	PB, BM, LN	Skin	None	46XY[18]/44,X,-Y,(4;12),-9,-13,+mar[9]	CD4 ⁺ CD56 ⁺ HLA-DR ⁺ CD123 ⁺ CD303 ⁺ CD304 ⁺ TCL1 ⁺	5/5	None	CD33 ⁺	None	CD7 ⁺ CD2 ⁺	Yes
P9	74	M	PB	75	PB, BM	Skin	None	46,XY[20]	CD4 ⁺ CD56 ⁺ HLA-DR ⁺ CD123 ⁺ CD303 ⁺ CD304 ⁺ TCL1 ⁺	5/5	None	None	None	CD7 ⁺ CD2 ⁺	Yes
P11	59	M	BM	95	PB, BM, LN	Skin	None	46,XY[20]	CD4 ⁺ CD56 ⁺ HLA-DR ⁺ CD123 ⁺ CD303 ⁺ CD304 ⁺ TCL1 ⁺	5/5	None	None	None	Neg	Yes
P12	61	M	BM	82	PB, BM	Skin	None	46,XY[20]	CD4 ⁺ CD56 ⁺ HLA-DR ⁺ CD123 ⁺ CD303 ⁺ CD304 ⁺	3/5	None	None	None	CD2 ⁺	Yes
P18	75	F	PB	90	PB, BM, spleen	Skin	None	45,XX,del(5)(q13q34),7inv(11)(p11;q21),der(15)(15qter-15q10::?;-18q10-18qter),-18[15]/44,ide,;22,dmin [2]/88,ide,x2,del(6)(q16)[2]/46,XX[11]	CD4 ⁺ CD56 ⁺ HLA-DR ⁺ CD123 ⁺ CD303 ⁺ CD304 ⁺	5/5	None	None	None	CD7 ⁺	Yes
P89	82	M	PB	80	PB, BM, LN	Skin, tonsil	None	46,XY[20]	CD4 ⁺ CD56 ⁺ HLA-DR ⁺ CD123 ⁺ CD303 ⁺ CD304 ⁺ TCL1 ⁺	5/5	None	CD33 ⁺	None	Neg	No
P13	46	F	BM	NA	PB, BM	Absent	None	45,XX,del(1)(p21),-5,-7,del(11)(q13),del(13)(q13q22),-15,-mar[13]/46,XX[17]	CD4 ⁺ CD56 ⁺ CD123 ⁺ CD304 ⁺ CD303 ⁺ TCL1 ⁺	2/5	Tdt ⁺	CD117 ⁺	None	cCD3 ⁺ sCD3 ⁺ CD2 ⁺ CD5 ⁺ low (20%)	No

DOD, dead of disease; F, female; LN, lymph node; M, male; NA, not available; Neg, negative; None, no positive marker.

*Only positive markers are indicated. Immature lineage marker: CD34, Tdt; myeloid lineage marker: CD33, CD117, CD13, CD15, CD65, CD14, CD64, CD11c, MPO; B lineage marker: CD19, CD20, CD22, CD79a, T lineage marker: cCD3 and sCD3 (cytoplasmic and surface), CD2, CD7, CD4, CD8, TCR αβ, TCR gd; BPCDN score, scoring system for BPCDN diagnostic.⁴⁰

Results

Patients

Eleven men and 1 woman diagnosed with BPDCN (median age 63.5 years old, sex ratio: M/F = 11/1) had cutaneous lesions at diagnosis, and a characteristic phenotype of BPDCN on PB or BM (BPDCN score >3)³⁹; with high expression of CD123, HLA-DR and cTCL1; expression of CD4, CD56, CD304, and/or CD303, without expression of CD34, or strong lineage commitment markers, such as MPO, cCD3, cCD79a, CD14, and CD11c. The blastic population also expressed T-cell markers in 7 cases (CD2, CD5, and/or CD7), myeloid markers in 6 patients (CD33 and/or CD117), and 1 case expressed the B-cell marker CD22 (Table 1).

A T-ALL reclassified as BPDCN

By unsupervised analysis, the 12 BPDCN samples all clustered together, close to normal pDCs and cDCs, and apart from other AL. Patient P13, initially diagnosed with T-ALL, also clustered with BPDCN patients (Figure 1A). The diagnosis was thus reconsidered for this 46-year-old woman exhibiting cCD3⁺ blasts (75%, medium intensity), without expression of surface CD3. According to TCR classification of T-ALL, this case was initially designated as immature IM0 T-ALL, with cTCR β [−] and germline TCR δ , γ , β . Tumor cells expressed CD4 and CD2, low levels of TdT and CD10, and no CD5 or CD7 expression. Considering our transcriptomic results, pDC markers were evaluated in a second step: despite the absence of expression of CD303 and CD56, CD304 was partially expressed (34%) and CD123 and cTCL1 were expressed at high levels (Table 1; Figure 1B). Morphologically, medullar blasts were characterized by regular nuclei and small nucleoli, no cytoplasmic granulation, small vacuoles, and pseudopodia in some cells (Figure 1C). The BPDCN score was only 2, because of cCD3 positivity, but blasts expressed high levels of CD123, and one of the most specific BPDCN markers, cTCL1. In addition, genetic and cytogenetic aberrations found in this patient were similar to the 12 other BPDCN cases, as described below. Taken together, these results led us to retrospectively classify patient P13 as BPDCN for further analyses.

Transcriptomic signature of BPDCN

A signature of 824 probe sets differentiating BPDCN from other AL was established (supplemental Table 3). Genes associated with pDC lineage were markedly overexpressed in the 13 BPDCN cases, such as *LAMP5* (BADLAMP), *HES6*, *LILRB4* (ILT3), *LILRA4* (ILT7), *IL3RA* (CD123), and *CLEC4C* (CD303), *GLUL*, *IRF7*, *TLR7*, and HLA class II genes, compared with AML, T-ALL, or B-ALL (supplemental Table 3). Some pDC and B-cell markers, such as *TCL1A*, *TCF4*, *IGLL1*, *LRMP*, and *NPC1*, displayed expression levels similar to B-ALL in BPDCN, arguing for a B-lymphoid origin (supplemental Figure 1). Furthermore, some AS-DC markers, such as *SIGLEC6*, were highly expressed in 5 cases, including the CD22⁺ patient P4 (Figure 2A). Interestingly, another group of 5 cases was isolated (supplemental Figure 2A) using signatures of AS-DCs,⁷ as well as other signatures: CD5⁺ CD81⁺ pDCs,¹¹ pDC commitment,⁶ activated vs resting pDCs, but also myeloid vs lymphoid lineage²⁶ and hematopoietic progenitors with B-lymphoid potential (hematopoietic stem cells, multilymphoid progenitors and Pro-B).⁴⁴ This group was not highlighted using signatures defining

CMP, granulocyte-monocyte progenitors, megakaryocytic-erythroid progenitors, and earliest thymic progenitor.⁴⁴ Collectively, gene set enrichment analysis (GSEA) also confirmed that BPDCN were enriched in gene sets upregulated in canonical pDCs as well as AS-DC signatures compared with other leukemias, but also in gene sets involved in protein misfolding, protein transport from the endoplasmic reticulum to the membrane, and inflammatory response to antigenic stimuli (supplemental Table 4).

Next, we compared BPDCN to each type of AL, beginning with AML. We applied the signature of Dijkman et al,²¹ which clearly validated the same distinction between BPDCN and AML in our cohort (supplemental Figure 2B). Immune and inflammatory responses and cytokine-mediated signaling pathway appeared upregulated in AML compared with BPDCN, as well as cell adhesion and proliferation (Figure 2B). BPDCN always appeared enriched in pDC gene sets and endoplasmic reticulum genes, but also in the deubiquitination process by GSEA (supplemental Table 5; Figure 2C). When BPDCN were compared with ALL by GSEA, many more pathways were found to be regulated; notably vesicle trafficking pathways and B-cell genes were upregulated in BPDCN, consistent with the B-cell gene enrichment mentioned above (supplemental Table 4; Figure 2D). Interestingly, BPDCN were also enriched in the NF- κ B pathway and RUNX targets (Figure 2E-F), corticosteroid pathways, and *KMT2A* rearrangements by GSEA. Pathways involved in inflammatory or adaptive immune response and in cell organization or signal transduction were downregulated in T-ALL compared with BPDCN (Figure 2B). Finally, when compared with B-ALL, hypergeometric tests did not reveal any significantly different pathway, and GSEA was also less informative (Figure 2B; supplemental Table 4).

The transcriptional signature of BPDCN is clearly different from the pDC signature

As expected, BPDCN transcriptomes were enriched in pDC genes, but differential gene expression analysis revealed a much larger number of microarray probes (1228) that were regulated when we compared the 13 BPDCN with the 5 normal pDC (supplemental Table 3). BPDCN were clearly different from pDCs when applying published signatures (Figure 2G-H).^{7,26} Genes differentially expressed between normal pDC and BPDCN made it possible to identify oncogenesis pathways in BPDCN. As expected, cell proliferation and division were upregulated in BPDCN, whereas regulation of cell shape and signal transduction was downregulated (Figure 2B).

The top upregulated genes in BPDCN compared with normal pDC included *IGLL1*, *GLUL*, *CLEC11A*, *UBE2T*, *BCL6*, *TLR2*, *UBE2C*, *BCL2*, *LRMP*, *UBE2S*, and *SOX4* (Figure 2I; supplemental Table 3). In contrast, the most relevant downregulated genes in BPDCN compared with normal pDC were *GZMB*, *CLEC4C*, *NPC1*, *BCL11A*, *NPC2*, genes regulating the immune system (*IL10RA*, *CXCR3*), and ubiquitination (*UBE2W*, *UBE3C*) (Figure 2I).

BPDCN exhibit many mutations and chromosomal imbalances

The NGS panel was informative for all cases, with many detected mutations ranging from 1 to 7 (median 3). Twenty genes were found

to be mutated among the 68 of the panel (Figure 3A; supplemental Table 5). Epigenetic modifiers were particularly mutated, affecting DNA methylation with *TET2* (8/13, 62%), *IDH2* (1/13, 8%), or chromatin remodeling with *ASXL1* (6/13, 46% including 3 c.1934dupG confirmed by Sanger sequencing), *ARID1A* (1/13, 8%), and *BCOR* (1/13, 8%). Other mutations concerned splicing factors *ZRSR2* (4/13, 31%), *SRSF2* (2/13, 15%); tumor suppressor genes *TP53* (2/13, 15%), *ATM* (2/13, 15%); transcription factors *ETV6* (1/13, 8%), *IKZF1* (2/13, 15%), *IKZF3* (1/13, 8%), *ZEB2* (1/13, 8%); RAS pathway *NRAS* (1/13, 8%), *KRAS* (1/13, 8%), or cytokine signaling pathway *JAK2* (2/13, 15%), *STAT3* (1/13, 8%), *CXCR4* (1/13, 8%), *NOTCH2* (1/13, 8%), and *MET* (1/13, 8%) (Figure 3A; supplemental Table 5).

BPDCN patients also carried many abnormalities in SNP array analysis, with a total of 117 chromosomal imbalances for the 13 BPDCN patients and a median of 9 aberrations per patient (range 2 to 19) (Figure 3B; supplemental Table 6). Deletions ($n = 97$) were more frequent than gains ($n = 20$). Patient P13 carried 10 chromosomal losses and 2 chromosomal gains, in agreement with this patient's requalification as BPDCN.

Notably, the minimal common deletion region (MCDR) on the 7p arm found in 8 patients (61%) included the *IKZF1* locus. Moreover, no del(7p) were identified in patients P9 and P89, but stop-gain mutations of *IKZF1* (p.E29X and p.Q1429X, respectively) were detected by NGS (Figure 3A; supplemental Table 5). The MCDR 12p13.1-2 included *ETV6*, *CDKN1B*, and *LRP6* genes (62% of patients). A ninth patient was mutated for *ETV6* with a stop-gain p.Q267X (P4), leading to a total of 69% of patients with *ETV6* dysregulation. The MCDR 13q14.1-13q14.3, including *RB1* and *INTS6*, was lost in 10 patients (78%), whereas a 9p21.3 MCDR was found in 5 patients (38%), with loss of *CDKN2A/CDKN2B* and *IFN* genes. The 9q21.32-21.33 region was also deleted in 6 patients (P1 P2, P4, P5, P8, P18) (46%), notably including the neurotrophic receptor tyrosine kinase *NTRK2*. The sensitivity of SNP array was assessed by 5q31.3 deletions, including *NR3C1*, previously investigated for the 12 initially diagnosed BPDCN⁴⁵ and confirmed here by SNP array. The 15q24.1-15q25.3 MCDR was detected in 4 patients, including *NTRK3* (31%). Three patients (P1, P3, P4) had a 6q23.3 deletion (23%), located next to *MYB*, prompting us to suspect a rearrangement of this gene, known to be involved in recurrent fusion transcript in BPDCN.²³ Similarly, a del(8q24) downstream to *MYC* was detected in patient P2. Expression data were also in favor of an *MYC* rearrangement, with overexpression of *MYC* in 2 cases (P2 and P6) compared with other patients (Figure 3C). Two del(17p) were detected, in patients P8 and P11, involving the *TP53* locus 17p13.1. One of them (P8) was also *TP53*-mutated, leading to a allelic inactivation of the gene (supplemental Table 5). Patient P5 was also mutated for *TP53*, with a variant allele frequency (VAF) of 98.25%, evoking a loss of heterozygosity, as no deletion was shown. Three patients presented homozygous deletions: in 9p21.3 for patient P1, and in 12p13.2 to p12.3 for patients P2 and P3. A total of 70 genes are included in these deleted regions (supplemental Table 7), including genes of IFN (*IFNA1*, 2, 4 to 8, 10, 14, 16, 17, 21, *IFNB1*) and proteins of cholesterol metabolism, such as low-density Lipoprotein Receptor-Related Protein 6 (*LRP6*).

Impact of chromosomal imbalances on transcriptional expression

When selecting the 354 most relevant genes whose expression is potentially impacted by CNV (supplemental Table 8), ingenuity pathways analysis revealed that the most frequently implicated pathways were infectious disease (24 impaired molecules: 5 gains and 19 losses), cell cycle (41 molecules: 33 loss and 8 gains), developmental disorders (23 molecules: 19 losses and 4 gains), and hematological disease (20 molecules: 14 losses and 6 gains) (supplemental Figure 3; supplemental Tables 8-9). Cellular assembly, organization, and function development were particularly impacted, notably molecular transport, cell-to-cell signaling and cell cycle, as well as genes implicated in immune response, with deletions and downregulation of *IFNGR1* (IFN γ receptor 1), *TGFBRI*, *TNFRSF1A* (TNF receptor superfamily member 1A), *TNFAIP3* (TNF- α -induced protein 3), *IFI6* (IFN, α -inducible protein 6), members of the C-type lectin domain family *CLEC2B*, *CLEC4E*, and *CLEC4C*, which were lost in some cases. Cluster A of the HOX family of genes (7p15) and genes of ubiquitination (*USP42*, *UBQLN1*, *USPL1*, *CUL4A*, *USP12*) were also recurrently deleted and downregulated, whereas *UBE2H* was upregulated and gained in 3 patients. Among upregulated genes, *GLUL*, *FCER1A*, and *APP* were also gained in 2 or 3 patients. Moreover, *ETV6* was invalidated in most of the patients (9/13), leading to lower expression than in AML and T-ALL. Interestingly, a part of B-ALL expressed *ETV6* at the same level as BPDCN, and these B-ALL also highly expressed *TCL1A*, similarly to BPDCN (Figure 3D). Thus, *ETV6* and *TCL1A* expression was inversely correlated (Spearman, $r = -0.2197[-0.3600; -0.0698]$, $P = .0034$), and *TCL1A* is only overexpressed in cases with low *ETV6* expression (Kruskal-Wallis, $P < .0001$).

Discussion

Oncogenesis of BPDCN is still debated, although a growing body of data has accumulated in the last few years.^{19,23,26,37,45-47} Our study highlights the fact that many oncogenetic processes are activated in BPDCN compared with normal pDCs: cell proliferation and division are upregulated, notably G1/S transition of the mitotic cell cycle. Cell-cycle kinases, such as *CDKN1B*, *CDKN2A*, and *CDKN2B*, are frequently deleted (69%), whereas *GLUL*, involved in cell proliferation, apoptosis inhibition, and response to glucocorticoids in ALL,⁴⁸ is upregulated in our cohort of 13 cases compared with pDCs and gained in 3 patients by SNP array. Interestingly, *SOX4* is upregulated in BPDCN compared with pDCs. *SOX4* is a transcription factor critical for lymphoid and pDC differentiation⁴⁹ because it upregulates *TCF4*,⁵⁰ an E-box transcription factor known to promote pDC lineage commitment,^{47,51,52} whereas its deletion induces transdifferentiation into cDC cells.⁵³ *SOX4* is also expressed in a wide variety of human cancers, promoting migration, invasion, and resistance to apoptosis.⁵⁴ In hepatocellular carcinoma, cell migration particularly depends of the *SOX4* target *NRP1*, also known as CD304, which is expressed by BPDCN,⁵⁴ and *SOX4* plays a key role in AML leukemogenesis, constituting an independent poor prognostic factor.^{49,55} Because *SOX4* targets *TCF4* and *NRP1* appear crucial for BPDCN, its upregulation may represent a major element of BPDCN oncogenesis.

Furthermore, 3 cancer-related genes are particularly deleted or mutated: *ETV6* (69%), *RB1* (69%), and *IKZF1* (62%), each of them

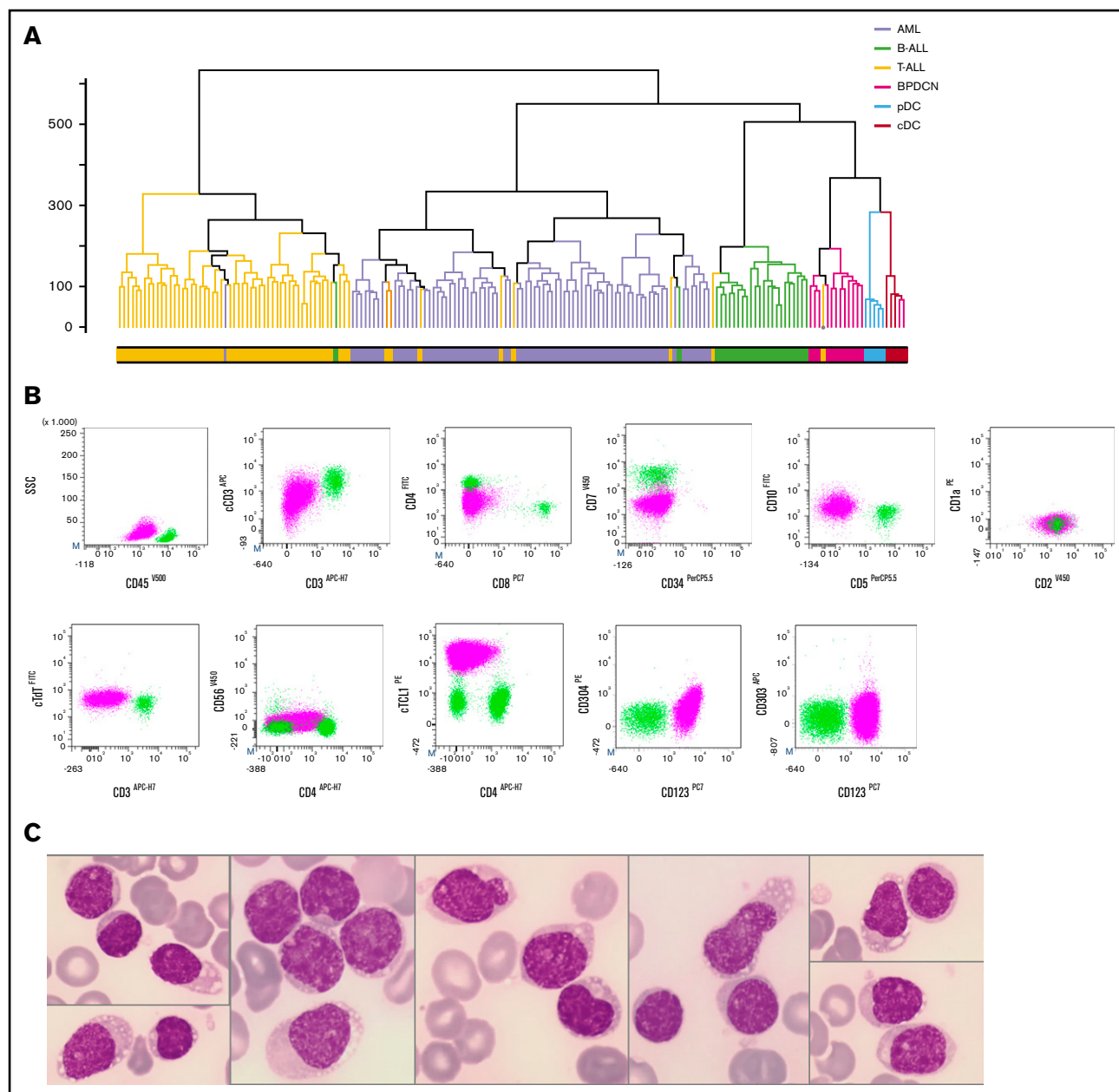


Figure 1. Reclassifying a T-ALL as BPDCN by gene expression profiling. (A) Unsupervised hierarchical clustering of 176 AL patients and 10 normal DC transcripts: B-ALL (green), T-ALL (yellow), AML (lavender), BPDCN (magenta), reclassified patient P13 (yellow with gray dot), normal cDC (red), and normal pDC (light blue). (B) Flow cytometry analysis of patient P13 BM: blastic population (magenta) and CD45⁺ lymphocytes (light green). The blastic population expresses CD45^{low} cCD3^{low} and CD4⁺, CD2⁺, CD10⁺ but no CD7 and surface CD3. The blastic population expresses the pDC markers CD123, TCL1^{high} and a low level of CD304. CD303 and CD56 were not expressed. (C) BM examination of patient P13 showed blasts, with regular nuclei and small nucleoli, no cytoplasmic granulation, and rare small vacuoles and/or pseudopodia (May-Grünwald-Giemsa stain, magnification $\times 1000$).

being crucial for BPDCN.^{25,31,38,56} In 2 patients, *ETV6* deletions were biallelic, evoking an early event, as already suggested,⁵⁶ and leading to lower expression of *ETV6* than in AML and T-ALL. We found that *ETV6* and *TCL1A* expression were inversely correlated and *TCL1A* is only overexpressed in cases with low *ETV6* expression, corresponding to our BPDCN cases and a subgroup of B-ALL, suggesting a link between *ETV6* downregulation and *TCL1A* upregulation. Interestingly, Fears et al⁵⁷ showed a reverse relationship in B-ALL, with high *ETV6* levels associated

with decreased endogenous *TCL1A* expression. Our results highlight an original mechanism of *TCL1A* deregulation in BPDCN, different from aggressive B-cell lymphoma^{58,59} and from T-cell prolymphocytic leukemia with chromosomal translocation that makes *TCL1A* (14q32.13) dependent on *TCR* enhancer elements.

In addition, *NTRK* genes were deleted in 9 out of 13 patients (69%) (1 *NTRK1*, 4 *NTRK2*, 3 *NTRK3*, and 1 *NTRK2* + *NTRK3*). Their

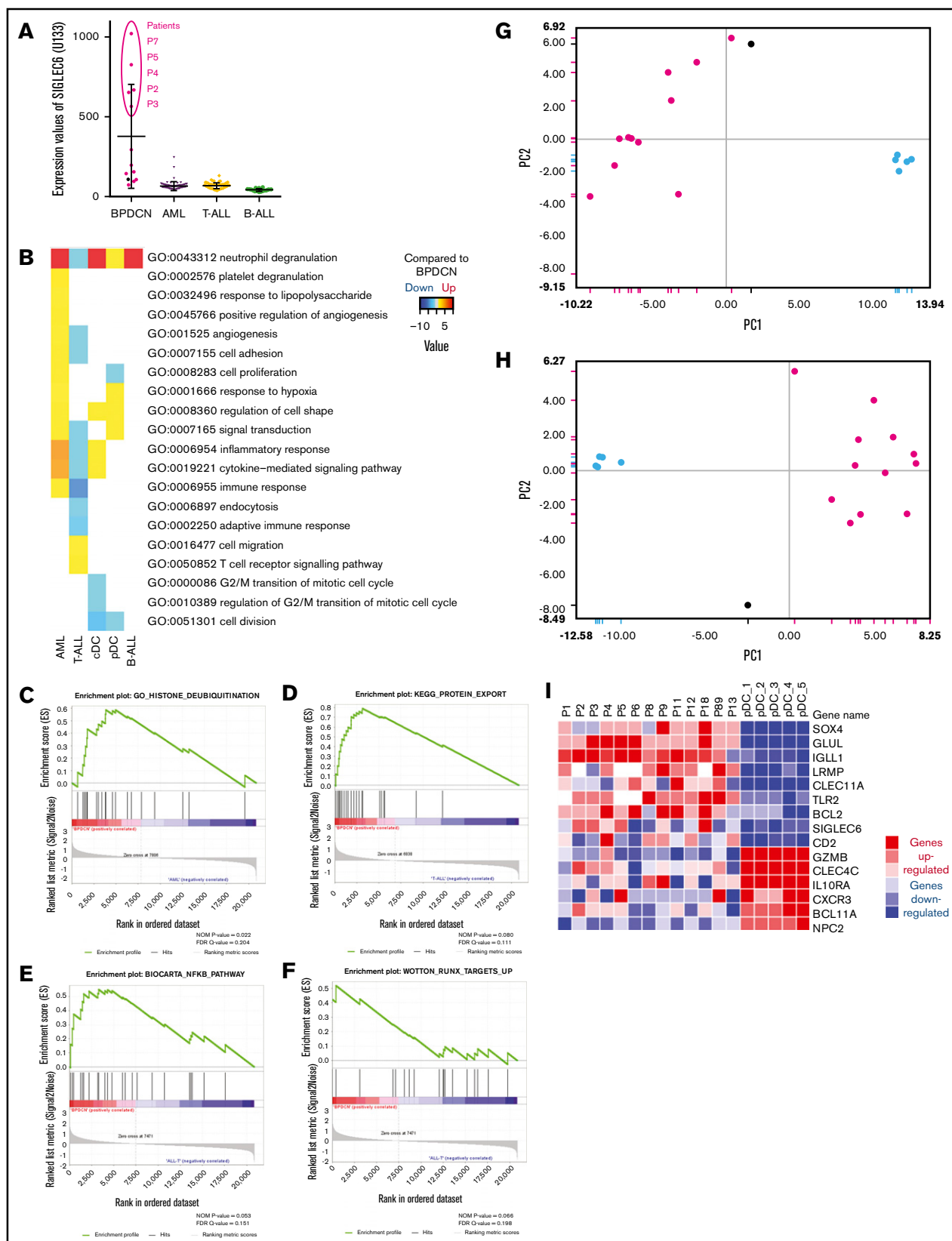


Figure 2. Transcriptomic signature of BPDCN. (A) Expression levels of SIGLEC6 probes on microarray. (B) Main gene ontology pathways deregulated for each type compared with BPDCN, using hypergeometric probability score with 20 HM and $P = .05$. Downregulated pathways are light blue, and upregulated pathways are yellow to bright red. (C-F) GSEA examples of gene sets enriched in BPDCN compared with AML (C) or T-ALL (D-F). (G-H) Principal component analysis based on published signatures

overexpression and activating mutations are widely described in solid tumors, as well as fusion transcripts, including *ETV6-NTRK3* in rare cases of ALL or AML.⁶⁰ Conversely, loss-of-function mutations are rarer, without a clear role in tumorigenesis.⁶⁰ Similarly, the impact of such deletions is unknown in BPDCN.

Regulation of cell shape, adhesion, and migration is also impacted in BPDCN. In particular, the *HOXA* cluster is recurrently deleted, with *HOXA9* having an important regulatory role in cytokine induction of endothelium adhesion molecules, ELAM and VCAM-1.⁶¹ In addition to the role of the *SOX4-NRP1* axis in migration, this deletion could also contribute to BM-to-skin or skin-to-BM cell spreading, as they have protumoral effects, promoting the metastatic process of solid tumors.⁶²⁻⁶⁴ *HOXA9* and *SOX4* deregulation may occur in a second stage, after an early BM oncogenic event, such as the *ETV6* deletions, as previously suggested.⁵⁶

Our study clearly shows high expression of pDC-related genes, consistent with their pDC origin, and confirms activation of the NF- κ B and corticosteroid pathways.^{19,21,26,45} The activation of the canonical NF- κ B pathway is particularly interesting, because it would explain the remarkable efficiency of bortezomib in BPDCN, as already demonstrated.^{26,65} *RUNX* targets are also enriched in BPDCN, consistent with *RUNX2* overexpression, previously described in BPDCN.²¹ Interestingly, *RUNX2* is involved in survival, differentiation, and release of pDCs from BM.⁶⁶ In BPDCN, its overexpression is considered an oncogenic event, dysregulating pDC differentiation, in some cases through t(6;8)(p21;q24) involving *MYC* and *RUNX2*.⁶⁷

Another key point is the role of pDCs in the immune system, impacted in BPDCN, notably IFN regulation (*IFNGR1*, *IFI6*) and pathogen recognition with the CLEC family, including the specific pDC marker *CLEC4C* (CD303). The latter is known to be negative or low in 25% to 30% of BPDCN cases.^{17,40}

As discussed above, the contribution of the myeloid and lymphoid branch of pDC genesis is still under debate. *SIGLEC6*, suggestive of the AS-DC cell subtype, appeared upregulated here in 5 cases, as already shown in previous reports in the literature,^{4,7} and could support an AS-DC origin of these cases, as hypothesized for the CAL-1 cell line.⁴ However, these results appear sample dependent, as already described, with some primary neoplastic cells able to secrete IFN type I and others not, evoking an AS-DC cell of origin.¹⁴ This heterogeneity may suggest that BPDCN could have heterogeneous ontogeny, with some of them being derived from canonical pDCs (7 to 8 cases in our cohort, depending on the signatures used) and others from AS-DCs (5 to 6 cases). As a consequence of the myeloid or lymphoid origin of BPDCN, this heterogeneity could induce variable expression of CD33, CD22, CD2, or CD7 expression, as found in our 13 cases and in our larger cohorts of BPDCN.^{18,38} However, some AS-DC characteristics could also be explained by the oncogenic process, such as low IFN secretion in BPDCN compared with normal pDCs, which can be attributed to recurrent deletions of IFN genes, or CD2 expression upregulated by

SOX4.⁶⁸ Regrettably, as our cohort would be divided into potential subgroups of <10 cases, we are unable to determine the ontogeny of BPDCN.

The mutational landscape of BPDCN is considered to be myeloid-like (*TET2*, *ASXL1*, *ZRSR2*, *SRSF2*, *BCOR*, *JAK2*), and case reports of CMML evolving to BPDCN³⁰⁻³² suggest that at least part of BPDCN may originate from a CMP. Nevertheless, the myeloid-like profile of BPDCN cannot be definitively ascertained because genetic data are still limited to small cohorts,²²⁻²⁷ because of the rarity of cases. A lymphoid-like origin of BPDCN is supported by the overexpression of *IGLL1*, *LRMP*, *BCL11A*, *BCL2* and genes involved in lymphoid proliferation, such as *BCL6*, *IKZF1*, *ETV6*, and *MYC*. Of note, *IKZF1* abnormalities are highly prevalent in BPDCN, but absent in myeloid neoplasms, except for rare cases in the very particular context of pediatric AML.⁶⁹ In fact, the myeloid-like profile of BPDCN mainly concerns preleukemic mutations and not transforming events, similarly to AML and MDS arising from clonal hematopoiesis of indeterminate potential (CHIP).^{70,71} In elderly patients with CHIP, epigenetic abnormalities (*TET2*, *DNMT3A*, *ASXL1* mutations) typically appear at low VAF (usually <20%), constituting a strong risk factor for subsequent hematological neoplasm (hazard ratio >10).^{70,71} At the diagnosis of neoplasm, VAF reached higher levels, because of clonal evolution. Similarly, patients are elderly in BPDCN and frequently exhibit *TET2* or *ASXL1* mutations (85% of our cases). This early epigenetic event could be followed by nonmyeloid oncogenic events, including canonical lymphoid abnormalities (69% of cases with *IKZF1/2/3* or *CDKN2A/B* inactivation, plus *ATM*, *MYC*, *STAT3*) and pan-cancer mutations (*NRAS*, *KRAS*, *TP53*) in the pDC lineage or in cells where the pDC/AS-DC commitment would be promoted by TCF4 upregulation via *SOX4* deregulation.

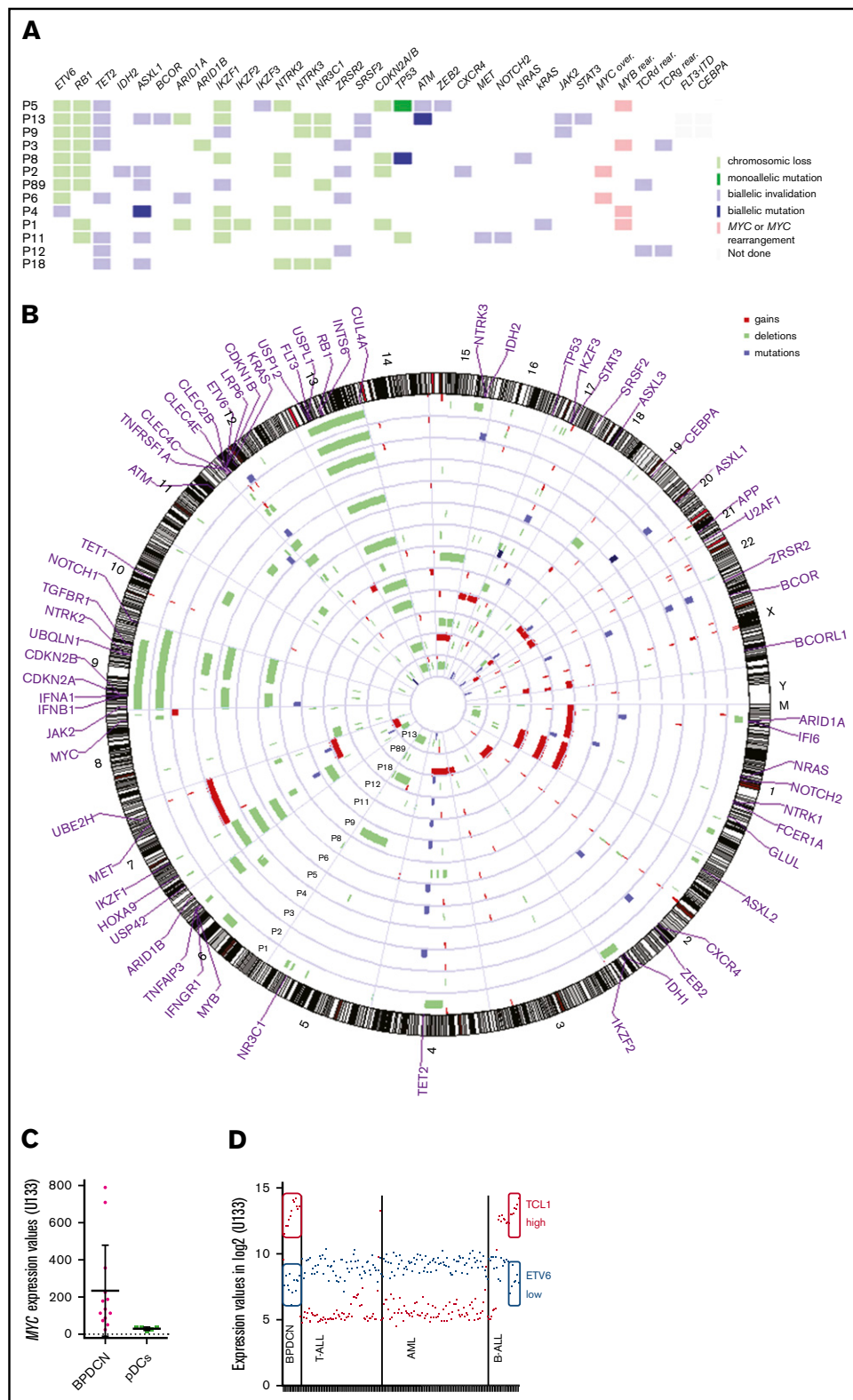
BPDCN displays immunophenotype heterogeneity and misclassification risks. We illustrate this challenge with patient P13. Despite the absence of CD56 and CD303 expression, and cytoplasmic expression of CD3, conceptually negative in BPDCN (but already described to be positive intracellularly in BPDCN⁷²), a strong commitment to the pDC lineage was shown with gene expression profile, chromosomal imbalances, and positivity of the following markers: CD4, CD123, CD304, and cTCL1. This patient prompts us to perform a BPDCN differential diagnosis in the case of immature AL of uncertain lineage (cCD3⁺ sCD3⁻ CD7⁻, without TCR γ and TCR δ rearrangement) when only 1 marker points to T-ALL or AML diagnosis and a fortiori if skin manifestations are detected. Specific markers for differential diagnosis should be used in such circumstances by flow cytometry or immunohistochemistry, such as TCL1, CD123, CD303, *RUNX2*, *HES6*, *SPIB*, and TCF4.^{21,51,73}

In conclusion, the more we learn about pDC ontogeny, the more BPDCN oncogenesis seems complex, with a wide variety of oncogenic mechanisms involved. Developing advanced strategies for diagnosis remains crucial, with mandatory specific

Figure 2. (continued) differentiating BPDCN from pDCs (panel G: Sapienza et al²⁶; panel H: Villani et al⁷). (I) Heatmap depicting the expression levels of selected upregulated (red) or downregulated (blue) genes in BPDCN compared with normal pDCs. In the dot plot, the 12 BPDCN are depicted in magenta, patient P13 in gray, AML in purple, B-ALL in green, T-ALL in yellow, and normal pDC in light blue.

Figure 3. Genetic abnormalities in BPDN.

(A) Mutational profile and key deletions or rearrangements in BPDN. (B) Circle plot in BPDN. Blue: biallelic mutation; lavender: monoallelic mutation; lime green: chromosomal loss; green: biallelic invalidation by both chromosome loss and mutation of the other allele; light red: MYC or MYC rearrangement; gray: not done. In the karyogram, deletions are depicted by green bars on the left of the chromosome, gains by red bars on the right of the chromosome, and mutations by blue bars. (C) Expression levels of MYC in BPDN and pDCs. (D) Expression levels of ETV6 in blue and TCL1A in red on microarrays. High expression of TCL1A and low expression of ETV6 are circled in full line.



markers. We also need to evaluate the clinical and prognostic impact of this heterogeneity in larger cohorts, and the specific deregulated pathways mentioned above should encourage the development of specific targeted therapies in BPDN. To

further investigate potential subgroups with different ontogeny, a validation cohort is required and will be explored by RNA sequencing in an additional 30 cases, coherent with the scarcity of BPDN.

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Authorship

Contribution: F.G.-O., C.R., E.M., and V.A. conceptualized the project; F.G.-O., E.M., and C.R. supervised the research; C.R., A.R., F.R., L.S., S. Blichle, F.A.-D., S.G., and C.F. performed experiments; F.R., M. Cheok, C.R., A.G., P.-J.V., F.J. performed bioinformatic analyses; C.R., F.G.-O., E.M., V.A., C.P. were responsible for patient samples; V.H., S. Bouyer, V.S., P. Saussoy, J.F., and P.F. contributed to the collection of samples, and clinical and biological data for the French

BPDCN network; T.P. provided help with immunohistochemistry and anatomopathology analysis; F.R., A.R., F.G.-O., and C.R. wrote the manuscript; C.P., E.M., V.A., P. Saas, C.F., E. Deconinck, E. Daguidau, O.A., M. Callanan, F.J., and J.C. commented on the manuscript; and all authors provided input, edited, and approved the final version of the manuscript.

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References

1. Gilliet M, Cao W, Liu Y-J. Plasmacytoid dendritic cells: sensing nucleic acids in viral infection and autoimmune diseases. *Nat Rev Immunol*. 2008;8(8):594-606.
2. Naik SH, Sathe P, Park H-Y, et al. Development of plasmacytoid and conventional dendritic cell subtypes from single precursor cells derived in vitro and in vivo. *Nat Immunol*. 2007;8(11):1217-1226.
3. Onai N, Obata-Onai A, Schmid MA, Ohteki T, Jarrossay D, Manz MG. Identification of clonogenic common Flt3+M-CSFR+ plasmacytoid and conventional dendritic cell progenitors in mouse bone marrow. *Nat Immunol*. 2007;8(11):1207-1216.
4. Dekker JD, Rhee C, Hu Z, et al. Lymphoid origin of a lineage of intrinsically activated plasmacytoid dendritic cell in mice and humans. *bioRxiv*. Published online 2018:310680. doi:
5. Dress RJ, Dutertre C-A, Giladi A, et al. Plasmacytoid dendritic cells develop from Ly6D⁺ lymphoid progenitors distinct from the myeloid lineage. *Nat Immunol*. 2019;20(7):852-864.
6. Rodrigues PF, Alberti-Servera L, Eremin A, Grajales-Reyes GE, Ivanek R, Tussiwand R. Distinct progenitor lineages contribute to the heterogeneity of plasmacytoid dendritic cells. *Nat Immunol*. 2018;19(7):711-722.
7. Villani A-C, Satija R, Reynolds G, et al. Single-cell RNA-seq reveals new types of human blood dendritic cells, monocytes, and progenitors. *Science*. 2017;356(6335):eaah4573.
8. See P, Dutertre C-A, Chen J, et al. Mapping the human DC lineage through the integration of high-dimensional techniques. *Science*. 2017;356(6342):eaag3009.
9. Matsui T, Connolly JE, Michnevit M, et al. CD2 distinguishes two subsets of human plasmacytoid dendritic cells with distinct phenotype and functions. *J Immunol*. 2009;182(11):6815-6823.
10. Alcántara-Hernández M, Leyle R, Wagar LE, et al. High-dimensional phenotypic mapping of human dendritic cells reveals interindividual variation and tissue specialization. *Immunity*. 2017;47(6):1037-1050.e6.
11. Zhang H, Gregorio JD, Iwahori T, et al. A distinct subset of plasmacytoid dendritic cells induces activation and differentiation of B and T lymphocytes. *Proc Natl Acad Sci USA*. 2017;114(8):1988-1993.
12. Ye Y, Gaugler B, Mohty M, Malard F. Plasmacytoid dendritic cell biology and its role in immune-mediated diseases. *Clin Transl Immunology*. 2020;9(5):e1139.
13. Swerdlow SH, Campo E, Harris NL, et al. *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues*. Revised 4th ed. Lyon, France: International Agency for Research on Cancer; 2017.
14. Chaperot L, Bendriss N, Manches O, et al. Identification of a leukemic counterpart of the plasmacytoid dendritic cells. *Blood*. 2001;97(10):3210-3217.
15. Petrella T, Comeau MR, Maynadié M, et al. "Agranular CD4⁺ CD56⁺ hematodermic neoplasm" (blastic NK-cell lymphoma) originates from a population of CD56⁺ precursor cells related to plasmacytoid monocytes. *Am J Surg Pathol*. 2002;26(7):852-862.
16. Julia F, Petrella T, Beylot-Barry M, et al. Blastic plasmacytoid dendritic cell neoplasm: clinical features in 90 patients. *Br J Dermatol*. 2013;169(3):579-586.

17. Garnache-Ottou F, Vidal C, Biichlé S, et al. How should we diagnose and treat blastic plasmacytoid dendritic cell neoplasm patients? *Blood Adv*. 2019; 3(24):4238-4251.
18. Lúcio P, Parreira A, Orfao A. CD123hi dendritic cell lymphoma: an unusual case of non-Hodgkin lymphoma. *Ann Intern Med*. 1999;131(7):549-550.
19. Ceroi A, Masson D, Roggy A, et al. LXR agonist treatment of blastic plasmacytoid dendritic cell neoplasm restores cholesterol efflux and triggers apoptosis. *Blood*. 2016;128(23):2694-2707.
20. Alayed K, Patel KP, Konoplev S, et al. TET2 mutations, myelodysplastic features, and a distinct immunoprofile characterize blastic plasmacytoid dendritic cell neoplasm in the bone marrow. *Am J Hematol*. 2013;88(12):1055-1061.
21. Dijkman R, van Doorn R, Suzhai K, Willemze R, Vermeer MH, Tensen CP. Gene-expression profiling and array-based CGH classify CD4+CD56+ hematodermic neoplasm and cutaneous myelomonocytic leukemia as distinct disease entities. *Blood*. 2007;109(4):1720-1727.
22. Menezes J, Acquadro F, Wiseman M, et al. Exome sequencing reveals novel and recurrent mutations with clinical impact in blastic plasmacytoid dendritic cell neoplasm. *Leukemia*. 2014;28(4):823-829.
23. Suzuki K, Suzuki Y, Hama A, et al. Recurrent MYB rearrangement in blastic plasmacytoid dendritic cell neoplasm. *Leukemia*. 2017;31(7):1629-1633.
24. Jardin F, Callanan M, Penther D, et al. Recurrent genomic aberrations combined with deletions of various tumour suppressor genes may deregulate the G1/S transition in CD4+CD56+ haematodermic neoplasms and contribute to the aggressiveness of the disease [published correction appears in *Leukemia*. 2009;23(4):825-826]. *Leukemia*. 2009;23(4):698-707.
25. Lucioni M, Novara F, Fiandrino G, et al. Twenty-one cases of blastic plasmacytoid dendritic cell neoplasm: focus on biallelic locus 9p21.3 deletion. *Blood*. 2011;118(17):4591-4594.
26. Sapienza MR, Fuligni F, Agostinelli C, et al; AIRC 5xMille consortium 'Genetics-driven targeted management of lymphoid malignancies and the Italian Registry on Blastic Plasmacytoid Dendritic Cell Neoplasm. Molecular profiling of blastic plasmacytoid dendritic cell neoplasm reveals a unique pattern and suggests selective sensitivity to NF-κB pathway inhibition. *Leukemia*. 2014;28(8):1606-1616.
27. Stenzinger A, Endris V, Pfarr N, et al. Targeted ultra-deep sequencing reveals recurrent and mutually exclusive mutations of cancer genes in blastic plasmacytoid dendritic cell neoplasm. *Oncotarget*. 2014;5(15):6404-6413.
28. Ramachandran V, Park KE, Loya A, Duvic M. Second primary malignancies in blastic plasmacytoid dendritic cell neoplasm: a national database study. *J Am Acad Dermatol*. 2020;83(6):1786-1789.
29. Hu Z, Sun T. Blastic plasmacytoid dendritic cell neoplasm associated with chronic myelomonocytic leukemia. *Blood*. 2016;128(12):1664.
30. Lebecque B, Vial J-P, Pigneux A, Lechevalier N. Chronic myelomonocytic leukaemia followed by blastic plasmacytoid dendritic cell neoplasm. *Br J Haematol*. 2019;185(3):398.
31. Patnaik MM, Lasho T, Howard M, et al. Biallelic inactivation of the retinoblastoma gene results in transformation of chronic myelomonocytic leukemia to a blastic plasmacytoid dendritic cell neoplasm: shared clonal origins of two aggressive neoplasms. *Blood Cancer J*. 2018;8(9):1-5.
32. Brunetti L, Di Battista V, Venanzi A, et al. Blastic plasmacytoid dendritic cell neoplasm and chronic myelomonocytic leukemia: a shared clonal origin. *Leukemia*. 2017;31(5):1238-1240.
33. Milley S, Thomas L, Balme B, Dalle S. Blastic plasmacytoid dendritic cell neoplasm following Waldenström macroglobulinemia. *J Eur Acad Dermatol Venereol*. 2016;30(5):904-905.
34. Khan AM, Munir A, Raval M, Mehdi S. Blastic plasmacytoid dendritic cell neoplasm in the background of myeloproliferative disorder and chronic lymphocytic leukaemia. *BMJ Case Rep*. 2019;12(7):e230332.
35. Harvell JD, Fulton R, Jones CD, Terris DJ, Warnke RA. Composite dendritic cell neoplasm (NOS) and small lymphocytic lymphoma. *Appl Immunohistochem Mol Morphol*. 2000;8(4):322-328.
36. Zachariadis V, Schoumans J, Barbany G, et al. Homozygous deletions of CDKN2A are present in all dic(9;20)(p13.2;q11.2)-positive B-cell precursor acute lymphoblastic leukaemias and may be important for leukaemic transformation. *Br J Haematol*. 2012;159(4):488-491.
37. Sakamoto K, Katayama R, Asaka R, et al. Recurrent 8q24 rearrangement in blastic plasmacytoid dendritic cell neoplasm: association with immunoblastoid cytomorphology, MYC expression, and drug response. *Leukemia*. 2018;32(12):2590-2603.
38. Bastidas Torres AN, Cats D, Mei H, et al. Whole-genome analysis uncovers recurrent IKZF1 inactivation and aberrant cell adhesion in blastic plasmacytoid dendritic cell neoplasm. *Genes Chromosomes Cancer*. 2020;59(5):295-308.
39. Garnache-Ottou F, Chaperot L, Biichle S, et al. Expression of the myeloid-associated marker CD33 is not an exclusive factor for leukemic plasmacytoid dendritic cells. *Blood*. 2005;105(3):1256-1264.
40. Garnache-Ottou F, Feuillard J, Ferrand C, et al; GOELAMS and GEIL study. Extended diagnostic criteria for plasmacytoid dendritic cell leukaemia. *Br J Haematol*. 2009;145(5):624-636.
41. Poret E, Angelot-Delettre F, Biichle S, et al. Biological Description of 109 Cases of Blastic Plasmacytoid Dendritic Cell Neoplasm (BPDCN) from the French Network of BPDCN. Presented at the: 57th Annual Meeting Exposition of American Society of Hematology; December 7, 2015; Orlando.
42. Asnafi V, Beldjord K, Boulanger E, et al. Analysis of TCR, pT alpha, and RAG-1 in T-acute lymphoblastic leukemias improves understanding of early human T-lymphoid lineage commitment. *Blood*. 2003;101(7):2693-2703.
43. Vially PJ, Mareschal S, Bertrand P, et al. GenerateReports: an IonTorrent plugin summarizing a whole NGS experiment for clinical interpretation. 23rd Annual International Conference on Intelligent Systems for Molecular Biology (ISMB) and 14th European Conference on Computational Biology (ECCB) Dublin, Ireland. July 12, 2015.
44. Laurenti E, Doulatov S, Zandi S, et al. The transcriptional architecture of early human hematopoiesis identifies multilevel control of lymphoid commitment. *Nat Immunol*. 2013;14(7):756-763.

45. Emadali A, Hoghoughi N, Duley S, et al. Haploinsufficiency for NR3C1, the gene encoding the glucocorticoid receptor, in blastic plasmacytoid dendritic cell neoplasms. *Blood*. 2016;127(24):3040-3053.
46. Sapienza MR, Abate F, Melle F, et al. Blastic plasmacytoid dendritic cell neoplasm: genomics mark epigenetic dysregulation as a primary therapeutic target. *Haematologica*. 2019;104(4):729-737.
47. Ceribelli M, Hou ZE, Kelly PN, et al. A Druggable TCF4- and BRD4-dependent transcriptional network sustains malignancy in blastic plasmacytoid dendritic cell neoplasm. *Cancer Cell*. 2016;30(5):764-778.
48. Dyczynski M, Vesterlund M, Björklund A-C, et al. Metabolic reprogramming of acute lymphoblastic leukemia cells in response to glucocorticoid treatment. *Cell Death Dis*. 2018;9(9):3-13.
49. Aue G, Du Y, Cleveland SM, et al. Sox4 cooperates with PU.1 haploinsufficiency in murine myeloid leukemia. *Blood*. 2011;118(17):4674-4681.
50. Saegusa M, Hashimura M, Kuwata T. Sox4 functions as a positive regulator of β -catenin signaling through upregulation of TCF4 during morular differentiation of endometrial carcinomas. *Lab Invest*. 2012;92(4):511-521.
51. Sukswai N, Aung PP, Yin CC, et al. Dual expression of TCF4 and CD123 is highly sensitive and specific for blastic plasmacytoid dendritic cell neoplasm. *Am J Surg Pathol*. 2019;43(10):1429-1437.
52. Cisse B, Caton ML, Lehner M, et al. Transcription factor E2-2 is an essential and specific regulator of plasmacytoid dendritic cell development. *Cell*. 2008;135(1):37-48.
53. Ghosh HS, Cisse B, Bunin A, Lewis KL, Reizis B. Continuous expression of the transcription factor e2-2 maintains the cell fate of mature plasmacytoid dendritic cells. *Immunity*. 2010;33(6):905-916.
54. Liao Y-L, Sun Y-M, Chau G-Y, et al. Identification of SOX4 target genes using phylogenetic footprinting-based prediction from expression microarrays suggests that overexpression of SOX4 potentiates metastasis in hepatocellular carcinoma. *Oncogene*. 2008;27(42):5578-5589.
55. Lu J-W, Hsieh M-S, Hou H-A, Chen C-Y, Tien H-F, Lin L-I. Overexpression of SOX4 correlates with poor prognosis of acute myeloid leukemia and is leukemogenic in zebrafish. *Blood Cancer J*. 2017;7(8):e593.
56. Tang Z, Li Y, Wang W, et al. Genomic aberrations involving 12p/ETV6 are highly prevalent in blastic plasmacytoid dendritic cell neoplasms and might represent early clonal events. *Leuk Res*. 2018;73:86-94.
57. Fears S, Chakrabarti SR, Nucifora G, Rowley JD. Differential expression of TCL1 during pre-B-cell acute lymphoblastic leukemia progression. *Cancer Genet Cytogenet*. 2002;135(2):110-119.
58. Aggarwal M, Villuendas R, Gomez G, et al. TCL1A expression delineates biological and clinical variability in B-cell lymphoma. *Mod Pathol*. 2009;22(2):206-215.
59. Ramuz O, Bouabdallah R, Devillard E, et al. Identification of TCL1A as an immunohistochemical marker of adverse outcome in diffuse large B-cell lymphomas. *Int J Oncol*. 2005;26(1):151-157.
60. Cocco E, Scaltriti M, Drilon A. NTRK fusion-positive cancers and TRK inhibitor therapy. *Nat Rev Clin Oncol*. 2018;15(12):731-747.
61. Bandyopadhyay S, Harris DP, Adams GN, et al. HOXA9 methylation by PRMT5 is essential for endothelial cell expression of leukocyte adhesion molecules. *Mol Cell Biol*. 2012;32(7):1202-1213.
62. Schlesinger M, Bendas G. Vascular cell adhesion molecule-1 (VCAM-1)—an increasing insight into its role in tumorigenicity and metastasis. *Int J Cancer*. 2015;136(11):2504-2514.
63. Sharma R, Sharma R, Khaket TP, Dutta C, Chakraborty B, Mukherjee TK. Breast cancer metastasis: putative therapeutic role of vascular cell adhesion molecule-1. *Cell Oncol (Dordr)*. 2017;40(3):199-208.
64. Schadendorf D, Heidel J, Gawlik C, Suter L, Czarnetzki BM. Association with clinical outcome of expression of VLA-4 in primary cutaneous malignant melanoma as well as P-selectin and E-selectin on intratumoral vessels. *J Natl Cancer Inst*. 1995;87(5):366-371.
65. Philippe L, Ceroi A, Bôle-Richard E, et al. Bortezomib as a new therapeutic approach for blastic plasmacytoid dendritic cell neoplasm. *Haematologica*. 2017;102(11):1861-1868.
66. Chopin M, Preston SP, Lun ATL, et al. RUNX2 mediates plasmacytoid dendritic cell egress from the bone marrow and controls viral immunity. *Cell Rep*. 2016;15(4):866-878.
67. Kubota S, Tokunaga K, Umezaki T, et al. Lineage-specific RUNX2 super-enhancer activates MYC and promotes the development of blastic plasmacytoid dendritic cell neoplasm [published correction appears in Nat Commun. 2019;10(1):3943]. *Nat Commun*. 2019;10(1):1-16.
68. Wotton D, Lake RA, Farr CJ, Owen MJ. The high mobility group transcription factor, SOX4, transactivates the human CD2 enhancer. *J Biol Chem*. 1995;270(13):7515-7522.
69. de Rooij JDE, Beuling E, van den Heuvel-Eibrink MM, et al. Recurrent deletions of IKZF1 in pediatric acute myeloid leukemia. *Haematologica*. 2015;100(9):1151-1159.
70. Jaiswal S, Fontanillas P, Flannick J, et al. Age-related clonal hematopoiesis associated with adverse outcomes. *N Engl J Med*. 2014;371(26):2488-2498.
71. Genovese G, Kähler AK, Handsaker RE, et al. Clonal hematopoiesis and blood-cancer risk inferred from blood DNA sequence. *N Engl J Med*. 2014;371(26):2477-2487.
72. Khoury JD, Medeiros LJ, Manning JT, Sulak LE, Bueso-Ramos C, Jones D. CD56(+) Td(+) blastic natural killer cell tumor of the skin: a primitive systemic malignancy related to myelomonocytic leukemia. *Cancer*. 2002;94(9):2401-2408.
73. Montes-Moreno S, Ramos-Medina R, Martínez-López A, et al. SPIB, a novel immunohistochemical marker for human blastic plasmacytoid dendritic cell neoplasms: characterization of its expression in major hematolymphoid neoplasms. *Blood*. 2013;121(4):643-647.