

TO THE EDITOR:

Distinct patterns of clonal evolution in patients with concurrent myelo- and lymphoproliferative neoplasms

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BCR-ABL-negative myeloproliferative neoplasms (MPNs) are a group of clonal stem cell disorders characterized by the overproduction of mature myeloid cells. However, MPN patients also have a 2.8- to 3.4-fold increased risk of developing a lymphoproliferative disorder (LPD) compared with the general population.^{1,2} A range of lymphoid neoplasms have been documented in MPN patients, most commonly chronic lymphocytic leukemia (CLL), which can be diagnosed prior to, simultaneous with, or following the myeloid neoplasm.³

The pathogenic mechanism underlying the increased risk of LPD in MPN patients is unknown, but it has been postulated that these disorders could share a common clonal origin.⁴ Genetic studies have demonstrated that certain genes, such as *SF3B1*, *TET2*, *TP53*, and *BCOR*, are mutated in both myelo- and lymphoproliferative diseases, as well as in healthy individuals with age-related clonal hematopoiesis (ARCH), a known precursor to overt malignancy.⁵⁻¹⁰ Moreover, emerging evidence suggests that CLL and hairy cell leukemia, mature B-cell neoplasms traditionally thought to arise in lineage-committed cells, may originate from multipotent hematopoietic stem and progenitor cells (HSPCs), as disease-defining mutations have been identified in nonlymphoid progenitors and differentiated myeloid cells in some patients.¹¹⁻¹³ It follows that ARCH, driven by pathogenic mutations in multipotent HSPCs, could serve as a substrate for the development of concurrent MPN/LPD.

To address this hypothesis, detailed genetic characterization of patients with concurrent MPN/LPD is required; prior reports have exclusively focused on *JAK2* or *CALR* MPN driver mutations, with the majority showing no contribution to lymphoid disease.^{1,4,14-22} Here, we analyzed 3 patients diagnosed with both myelofibrosis (MF) and a mature B-cell LPD; their clinical features are summarized in Table 1 and supplemental Figure 1 (available on the *Blood* Web site). All biological samples used in this study were collected in accordance with the Research Ethics Board of the University Health Network (REB#01-0573 and REB#02-0763). Patient-specific mutations were identified by sequencing DNA from whole peripheral blood (PB) using the Illumina TruSight Myeloid Panel, which covers regions of 54 genes relevant to ARCH and hematologic malignancy (Table 1; supplemental Tables 1 and 2; see supplemental Methods for

details). Subsequently, to determine the contribution of these variants to normal and neoplastic hematopoiesis, digital droplet PCR (ddPCR) was performed on DNA from 5 subpopulations sorted from cryopreserved PB mononuclear cells (MNCs): monocytes, T cells, non-B-cell progenitors, normal B cells, and malignant B cells (supplemental Figure 2).

Targeted sequencing of PB from patient A, a male diagnosed with CLL and primary MF, identified mutations in *CALR*, *BCOR*, *FBXW7*, and *NOTCH1* (Table 1). ddPCR detected all 4 mutations in unsorted MNCs (Figure 1A); however, compared with VAFs generated by sequencing whole PB, these values were lower for MPN-associated genes and higher for CLL-associated genes, a consequence of Ficoll-Hypaque separation, which depletes neutrophils during the isolation of MNCs. Analysis of sorted PB subfractions demonstrated that the *BCOR*, *FBXW7*, and *NOTCH1* mutations were detected only in CLL cells; the *CALR* deletion was found only in progenitors and monocytes; and the T- and normal B-cell fractions were devoid of all variants (Figure 1A). Thus, mutation distribution enabled clear delineation of MPN, CLL, and normal hematopoietic cells within this patient's blood. Notably, the MPN clone included not only mature myeloid cells, but also dominated PB progenitors, a consequence of the circulating clonal CD34⁺ cells characteristic of MF.²³

Patient B, a female with a low-grade CD5⁺CD20⁺ LPD and PET-MF, carried a *JAK2* V617F mutation as well as *DNMT3a* and *TP53* variants (Table 1). All 3 mutations were detected in the monocytes and progenitors but were absent from LPD cells (Figure 1A). Notably, in the normal B- and T-cell fractions, the *DNMT3a* mutation was detected in the absence of *JAK2* and *TP53* alterations, indicating the presence of *DNMT3a*-driven ARCH ancestral to the development of this patient's MPN. However, given that this variant was absent from CLL cells, this ARCH clone did not serve as the origin of her LPD.

A similar mutational distribution was noted in patient C, a male with CLL and PET-MF. Sequencing identified pathogenic variants in *MPL*, *SRSF2*, and *TET2*, all of which were present in monocytes and progenitors, but not CLL cells (Figure 1A). In T cells, the *MPL* and *TET2* mutations were detected at low frequency (<2%), without the *SRSF2* variant. Although the

Table 1. Clinical and molecular characteristics of study patients at the time of sample collection

ID	Age/sex	Hematologic diagnoses	Prior treatments	FISH	Karyotype	PB counts			Mutation data		
						WBC ($\times 10^9/L$)	Lymphocytes ($\times 10^9/L$)	Blasts ($\times 10^9/L$)	Gene	Protein change	VAF (%)
A	84/M	1a. CLL* 1b. PMF*	1. Chlorambucil 2. Ibrutinib	+12	nd	20.6	6.74	0.21	NOTCH1	p.Pro2514Argfs*4	5.2
									FBXW7	p.Arg465Leu	15.1
									BCOR	p.Gln1528*	25.2
									CALR	p.Leu367Thrfs*46	38.7
B	76/F	1. PET-MF with dysplastic acceleration 2. CD5+ LPD, favor marginal zone derivation	Hydroxyurea	Negative for t(11;14)	46,XX,add(7)(q11.2),+8,-11,add(14)(q32),-17,+mar[10]	16.0	10.14	0.16	DNMT3A	p.Asn516Thrfs*135	20.0
									JAK2	p.Val617Phe	18.4
									TP53	p.His193Pro	21.1
C	56/M	1. PET-MF 2. CLL	Hydroxyurea	nd†	46, XY [20]	43.5	28.84	0	MPL	p.Trp515Leu	15.8
									SRSF2	p.Pro95His	8.8
									TET2	p.Leu446Phefs*9	7.5
									TET2	p.His1219Arg	10.3

F, female; FISH, fluorescence in situ hybridization; M, male; nd, not done; PET-MF, post-essential thrombocythemia myelofibrosis; PMF, primary myelofibrosis; VAF, variant allele frequency; WBC, white blood cell count.

*Simultaneous diagnoses.

†Single nucleotide polymorphism array analysis showed biallelic loss of a 1.17 Mb region at chromosome 13q14 and loss of heterozygosity of chromosome 1p.

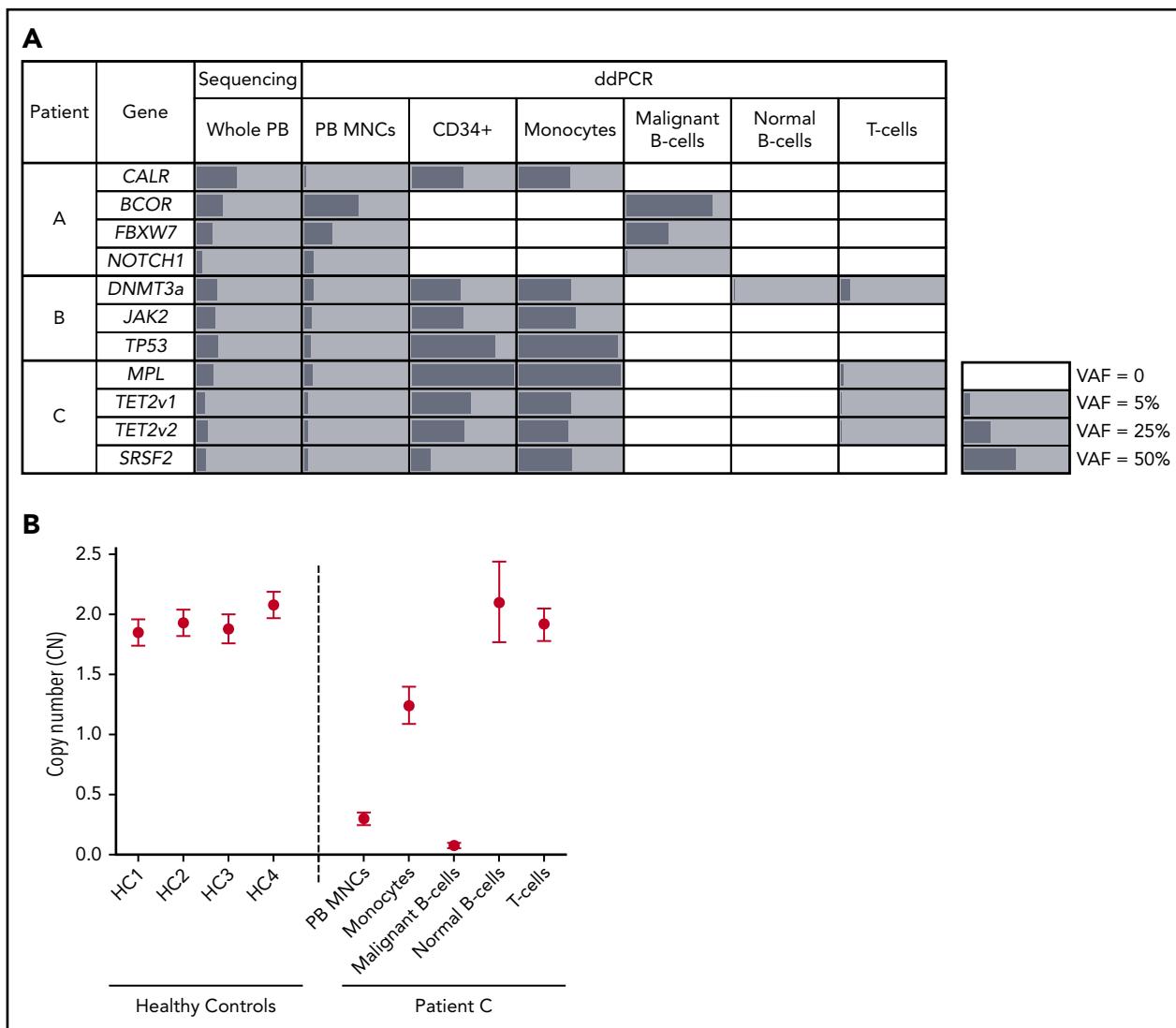


Figure 1. Mutational and copy number (CN) analysis of sorted PB subpopulations in patients with concurrent MPN/LPD. (A) Allele frequencies (%) of patient-specific variants in whole PB, unsorted PB MNCs, and PB subpopulations as determined by targeted sequencing (whole PB) or ddPCR (unsorted MNCs and sorted fractions). Mutation-positive fractions are shaded in gray, with the length of the bars proportional to the VAF. White squares indicate populations where variants were not detected. *TET2 v1* (*TET2* variant 1) refers to the *TET2* L446fs variant; *TET2 v2* (*TET2* variant 2) refers to the *TET2* H1219R variant. In patient C, the *MPL* W515L VAF exceeded 99% in the CD34⁺/monocyte fractions, consistent with the known loss of heterozygosity of chromosome 1p in this patient. (B) CN at the chromosome 13q14 locus as determined by ddPCR in unsorted PB MNCs and PB subpopulations from patient C as well as PB MNCs from 4 healthy controls (HC). CN measurements are depicted by a circle, with error bars indicating the 95% confidence interval.

presence of 3 mutations in this fraction could suggest contamination by cells from the MPN clone, this is unlikely given the purity of the sorted fraction (supplemental Figure 3), and the notable absence of one of the MPN-associated variants. Instead, this pattern is suggestive of underlying ARCH, skewed toward T- vs B-lymphopoiesis, which, like patient B, was ancestral only to the myeloid neoplasm.

These results demonstrate that, at this level of genetic interrogation, mutational patterns segregate the MPN and LPD disease clones in patients with concurrent diagnoses. Importantly, ARCH was detected in 2 of 3 patients in this study, and in both instances, exclusively involved mutations that contributed to myeloid disease. Thus, ARCH with known driver did not serve as the shared origin of concurrent MPN/LPD in these individuals. Although our sequencing panel captures the canonical ARCH

drivers, rare somatic driver, nondriver, or CN alterations shared between the 2 disease processes cannot be ruled out.

To address this possibility, we took 2 experimental approaches. First, X-inactivation patterns were assessed in the monocyte and malignant B cells of patient B, our sole female subject. The active X-chromosome, as determined by the human androgen receptor (HUMARA) assay, differed between MPN and LPD cells (supplemental Figure 4), establishing the distinct clonal origins of the 2 diseases. Second, given the recent finding that loss of chromosome 13q14 in the PB of healthy individuals is associated with an increased risk of CLL,²⁴ we interrogated 13q14 CN status in patient C, who was known to carry this lesion. As expected, CLL cells showed evidence of biallelic 13q14 deletions, whereas the CN of normal B- and T-cell populations at this locus was comparable to diploid controls (Figure 1B). Surprisingly, monocytes

showed evidence of monoallelic deletion of 13q14, suggesting that loss of 1 copy of this locus was a shared ancestral event for this patient's MPN and LPD. Consistent with a role for this lesion in MPN pathogenesis, 13q loss is a frequently observed karyotypic abnormality in MF.²⁵

Thus, our study adds clarity to the apparent contradictory results from previous studies of patients with concurrent MPN/LPD: distinct clonal origins were suggested in a study of X-inactivation pattern in 3 females with MPN and CLL,¹⁸ whereas other reports have found that *JAK2* mutations can be detected in LPD cells in a subset of patients.^{1,19-21} Our findings suggest that the pathogenic processes underlying concurrent MPN/LPD can vary between patients. In some cases (as observed in patient B), the 2 disease clones originate independently and environmental factors or a germ-line predisposition state likely drive their parallel development. Alternatively, as seen in patient C, the 2 disease processes can have a common clonal origin, with an early shared genetic lesion prior to divergence. Notably, mutations in canonical ARCH drivers were not found to be the unifying origin of MPN and LPD disease clones in this study. Instead, we observed that a CN alteration could serve as an early shared genetic lesion. This finding highlights the key role played by noncanonical ARCH in the pathogenesis of hematologic malignancies and underscores a requirement for genome-wide mutational and CN analyses in future studies of concurrent MPN/LPD.

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Authorship

Contribution: J.A.K. designed the study and performed research, collected clinical information, analyzed data, and wrote the manuscript; J.J.F.M. performed research, analyzed data, and wrote the manuscript; S.M.D. performed research and analyzed data; A.A. collected patient samples; A.T. provided clinical flow cytometric data; M.D.M. provided patient samples; M.A.S., T.S., and S.K.-R. performed and analyzed sequencing data; J.E.D. and V.G. designed research and wrote the manuscript; and all authors reviewed the manuscript and approved it for publication.

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Footnote

The online version of this article contains a data supplement.

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TO THE EDITOR:

Increased risk of colon cancer and osteogenic sarcoma in Diamond-Blackfan anemia

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Inherited bone marrow failure syndromes (IBMFSs), characterized by bone marrow failure and developmental anomalies, are among a group of rare cancer predisposition syndromes. As illustrated by Fanconi anemia (FA),¹ the unique biology of this IBMFS has provided important insights into drivers of malignancy. Diamond-Blackfan anemia (DBA), one of the IBMFSs, is associated with red cell aplasia and congenital anomalies. The majority of cases with DBA result from dominant, loss-of-function mutations/deletions in ~20 genes encoding either a small or large subunit-associated ribosomal protein (RP) (reviewed in Vlachos et al²). We previously described and quantified the cancer risk in DBA with a cumulative incidence of solid tumors (STs) and acute myeloid leukemia (AML) of ~20% by 46 years of age³ using the Diamond Blackfan Anemia Registry (DBAR) of North America.^{4,5} As of July 2016, the DBAR consisted of 702 patients, accrued through physician or self-referral. We now report longitudinal data describing a further increase in the incidence of colon cancer and osteogenic sarcoma.

The DBAR of North America (United States, Canada, and Mexico), established in 1991 and registered at www.clinicaltrials.gov (#NCT00106015), is the largest, international DBA patient cohort with prospective follow-up.^{4,5} Written informed consent as per the Declaration of Helsinki was obtained from all study participants. The current analysis includes an additional 6 years with 94 additional patients and 2918 patient-years than in the previous report. We classify cancer type and age of onset from 702 patients enrolled from November 1991 through July 2016. A competing risks approach was used to estimate cause-specific hazard functions and cumulative incidences for the first adverse event (hematopoietic stem cell transplantation [HSCT], death from nonmalignant causes, AML, or ST) as previously described.³ Results were compared with the expected number of malignancies (observed/expected [O/E] ratio) derived from the Surveillance, Epidemiology and End Results Program (SEER 9)

(<https://seer.cancer.gov/data/>), with adjustment for age, sex, and birth cohort. Pathology reports and medical records were obtained to verify the neoplastic diagnoses in 30% of cases. Tumors not included in SEER were excluded from this analysis and myelodysplastic syndrome (MDS) was analyzed separately. The DBAR is voluntary and is not actively searching for those patients with cancer specifically but enrolls all comers at any age who want to be enrolled. Thus, there may be biased enrollment of older individuals, some with cancer prior to the time of enrollment, as well as a bias toward concern about a perceived increased cancer risk by a parent or patient. In total, there were 8 patients with cancer and one with MDS/AML diagnosed prior to their enrollment (described in Table 1). Of note, a certain bias would be created if enrollment was restricted to only those patients without a cancer diagnosis prior to enrollment in the DBAR.

The total of 702 enrolled patients contributed 12 376 person-years of follow-up. The male to female ratio was 1:1.02. There were 39 cancers; 5 in post-HSCT patients (Table 1). Fourteen of the 34 cancers in patients without HSCT were verified by pathology/physician reports. The median age at the first cancer (AML and ST, excluding nonmelanoma skin cancers) was 35 years (range, 11-70 years). Three evaluable AML cases and 25 STs were reported in 26 patients without a prior history of HSCT (Table 1).

Some malignancies were excluded from our analyses. Three basal cell carcinomas and 3 squamous cell carcinomas of the skin (median age, 54 years for each, younger than population-incidence) were excluded because basal cell carcinomas and squamous cell carcinomas are not included in the SEER database, leading to 28 statistically evaluable cancers in 26 patients. One hundred patients had received an HSCT. Five post-HSCT malignancies were also excluded from analysis.