blood advances

TO THE EDITOR:

CBL-MZ is not a single biological entity: evidence from genomic analysis and prolonged clinical follow-up

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The term "clonal B cell lymphocytosis of marginal zone origin" (CBL-MZ)[1,2] has recently been suggested for asymptomatic individuals whose routine blood count shows a persistent modest lymphocytosis that is usually accompanied by bone marrow involvement. This immunophenotype is suggestive of marginal zone/postgerminal center derivation, but no other features of a chronic B cell lymphoproliferative disorder are found, other than a low-level paraprotein in some cases. Cases with a clonal lymphocyte count $<5 \times 10^9$/L would fall within the revised World Health Organization (WHO) category of non–chronic lymphocytic leukemia-type monoclonal B-cell lymphocytosis. In 3 previous series of CBL-MZ consisting of 102, 53, and 16 cases with median follow-ups (FUs) of 60, 34, and 44 months, respectively,[1,3,4] the overall incidence of progression was 15.7%, with the majority (11.1%) developing splenomegaly that frequently did not require treatment. However, it remains unclear whether CBL-MZ is the precursor to 1 or several well-defined WHO entities and what factors predict disease progression. To address this, we performed a genomic analysis of a well-characterized cohort of CBL-MZ cases with long FU and provide evidence to show that CBL-MZ is not a single biological entity.

This study includes data from 37 patients with CBL-MZ diagnosed and managed at the Royal Bournemouth Hospital. Clinical, routine laboratory, morphological, immunophenotypic, immunogenetic, and cytogenetic data have been reported on 36 cases using previously described methods.[1] Informed patient consent was obtained according to the Declaration of Helsinki, and the ethical aspect of the study was approved by the Somerset Research and Ethics Committee.

DNA from blood-derived tumor cells (n = 37) at diagnosis was analyzed with a bespoke HaloPlex Target Enrichment System (Agilent Technologies) that enriched 2.39 Mb of genomic DNA for the coding regions of 768 genes, as previously described.[5] From this panel, the following candidate genes were selected for Sanger validation (primers and conditions are listed in supplemental Table 1) based on the high prevalence of somatic mutations in similar mature B-cell malignancies: *KLF2* and *NOTCH2* (splenic marginal zone lymphoma [SMZL]),[5-9] *CCND3* and *BCOR* (splenic diffuse red pulp lymphoma [SDRPL]),[10,11] *MAP2K1* (hairy cell variant [HCL-v]),[12] *MYD88* (lymphoplasmacytic lymphoma [LPL]),[13] *BRAF V600E* (hairy cell leukemia and nodal marginal zone lymphoma [MZL]),[14,15] and *TNFAIP3* and *TP53* (not disease specific). DNA from buccal cells (n = 22) was used to confirm the somatic origin of 14 of 15 variants identified in 7 of these genes.

The study included 20 men and 17 women (1.2:1 ratio). The median age at presentation was 73.2 years (range 47.8-95.5 years). Key clinical and laboratory data and additional demographic and cytogenetic data are provided in Table 1 and supplemental Table 2, respectively. Lymphocyte morphology was heterogeneous in all cases, with a variable percentage of villous and lymphoplasmacytoid cells. No case had the typical morphological features of HCL-v or SDRPL. The immunophenotype was uniform, with expression of moderate SmIg, CD19, and CD49d and lack of CD10, CD38, and CD5, with the exception of 5 cases with weak CD5 positivity.

With a median FU of 9.6 years (range 2.5-22.4), 28 of 37 cases (75.7%) remained stable of whom 11 have died after a median FU of 8.8 years (range 2.5-14.3), and 17 remained stable after a median FU of 9.7 years (range 2.9-14.6 years). Nine of 37 (24.3%) cases showed evidence of progressive disease, and 3 died. The median time to progression was 69 months (range 47-175). Seven patients

Table 1. Patient immunogenetics, cytogenetics, and mutational data

Case	Progressive disease	Diagnosis at progression	Time to progression, mo	Overall survival, mo	Presentation paraprotein, g/L	IGHV gene usage	% IGVH identity	Key cytogenetics abnormalities	Candidate gene mutations
1	Yes	SLLU	147	177.48	No	V5-51	89.7	del(3q)	None
2	Yes	SMZL	65	89.49	No	V3-64*01/*02	92.36	*CK inc i(17)(q10)	*TP53*
3	Yes	SMZL H	66	134	Mk	V3-30*03/*18/V3-30-5*01	95.49	None	*MYD88*
4	Yes	SMZL	119	164.44	No	V4-34*01/*02/*07	96.41	del(14q)	None
5	Yes	SMZL	47	51.09	No	V1-2*04	98.13	del(14q)	*KLF2, N0TCH2*
6	Yes	MZL H	69	118.51	No	V4-34*01/*02	94.14	CK	*TNFAIP3*
7	Yes	SMZL H	57	45.9	No	V5-51*01	96.63	dup(3q)	None
8	Yes	LPL H	150	35.94	GK 1.0	V4-34*01/*02	93.47	CK	*MYD88*
9	Yes	SLLU	175	269	ML 1.7	V3-73*02	96.6	t(2;7)	*MYD88*
10	No	na	na	171.86	No	V3-66*01/*04	87.37	None	None
11	No	na	na	95.57	No	V6-1*01	96.63	+12	None
12	No	na	na	115.68	No	V4-34*01/*02/*12	89.8	+12	None
13	No	na	na	136.51	No	V4-4*02	96.37	None	*CCND3*
14	No	na	na	29.73	GK 9.8	V3-23*01/V3-23D*01	93.4	+12	*MYD88*
15	No	na	na	39.39	No	V4-59*01	95.51	i(17)(q10)	*TP53, CCND3*
16	No	na	na	53.85	No	V3-66*02	100	None	None
17	No	na	na	126.78	No	V4-59*01	97.54	None	None
18	No	na	na	39.66	No	V3-7*02	96.88	del(7q), +12	*TP53, CCND3*
19	No	na	na	107.89	GK 5.2	V3-7*01	95.49	None	*MYD88*
20	No	na	na	19.88	MK 0.5	V1-2*04	100	None	None
21	No	na	na	105	No	V4-38-2*01	92.74	None	None
22	No	na	na	96.16	No	V6-1*01(9 bp ins), V4-59*01	98.32, 91.93	+3,+12, i(17)(q10)	None
23	No	na	na	90.94	No	V4-34*01/*02	95.92	None	None
24	No	na	na	88.41	No	V4-34*01/*02	92.65	None	None
25	No	na	na	40.77	No	V1-69*01/*11/*12/V1-69D*01	89.51	None	None
26	No	na	na	82	ML trace	V4-34*01	100	del(7q)	None
27	No	na	na	149.13	No	V4-30-2*01	91.7	del(14q)	None
28	No	na	na	75.43	No	V3-9*01	93.06	None	*MAP2K1*
29	No	na	na	160.72	GK trace	V3-21*01/*02	94.76	del(7q)	None
30	No	na	na	68.34	No	V1-3*01	93.85	i(17)(q10)	None
31	No	na	na	105.13	No	V4-39*01	96.41	None	None
32	No	na	na	133.55	No	V4-34*01	100	del(7q)	None
33	No	na	na	126.32	MK trace	.	.	i(17)(q10)	None

CK, complex karyotype based on >3 G-banding aberrations; GL, G-lambda; H, histological diagnosis; MK, M-kappa; na, not applicable; SLLU, splenic B-cell lymphoma/leukemia unclassifiable.

Table 1. (continued)

Case	Progressive disease	Diagnosis at progression	Time to progression, mo	Overall survival, mo	Presentation paraprotein, g/L	IGHV gene usage	% IGVH identity	Key cytogenetics abnormalities	Candidate gene mutations
34	No	na	na	146.92	No	V4-39*01	94.07	+12	None
35	No	na	na	130.83	No	V5-51*01	97.98	None	None
36	No	na	na	5192	No	V3-23*01/V3-23D*01	96.18	i(17)(q10)	None
37	No	na	na	153.53	GL trace	V1-69-2*01	96.63	None	None

CK, complex karyotype based on >3 G-banding aberrations; GL, G-lambda; H, histological diagnosis; MK, M-kappa; na, not applicable; SLLU, splenic B-cell lymphoma/leukemia unclassifiable.

(cases 1-5, 7, and 9) developed splenomegaly that was accompanied by progressive lymphocytosis in 4 cases. In 2 patients, splenic histopathology confirmed a diagnosis of SMZL. In 3 patients, lymphocyte morphology and marrow histology at progression, combined with immunogenetic and karyotypic features, were also consistent with SMZL. Two cases, classified as splenic B cell lymphoma/leukemia unclassifiable, were too frail for further investigation; 1 (case 9) had a t(2;7)(p11;q21.2) translocation at diagnosis, which has been associated with MZLs,[16,17] and 1 (case 1) exhibited progressive lymphocytosis with large circulating lymphoid cells. Case 8 developed heavy marrow infiltration with small nonvillous lymphocytes in conjunction with a low-level IgGK paraprotein and cytogenetic analysis showing del(6q) and iso(18q); LPL was considered a likely diagnosis. Case 6 underwent biopsy of orbital and abdominal wall masses, both of which showed histological and immunophenotypic features of MZL.

Fifteen genomic mutations, involving all candidate genes screened, with the exception of *BCOR* and *BRAF V600E*, were identified in 12 cases (Table 1). The most frequent was *MYD88* in 5 (13.5%) cases, involving L265P (n = 3) or S219C (n = 2) and indicating that screening CBL-MZ cases only for the L265P mutation is likely to miss cases with alternate *MYD88* mutations. Three patients had histologically proven SMZL, 1 had a t(2;7)(p11;q21.2) translocation, and 1 had LPL. None of these cases had a mutation of *CXCR4* (data not shown). Three cases (8.1%) had mutations of *TP53*, all accompanied by *TP53* loss, and 3 had PEST domain *CCND3* mutations, although none had the typical features of SDRPL, and all had stable disease. The sole case with a *MAP2K1* mutation (E203K; deleterious and damaging by Mutationtaster and Polyphen2, respectively) was stable with a FU of 66 months. Extended immunophenotypic analysis showed expression of SmIgG, FMC7, CD22, and CD11c (weak) and lack of CD103 and CD25, consistent with HCL-v, splenic B cell lymphoma/leukemia unclassifiable, or SMZL. *NOTCH2* and *KLF2* mutations were present in a single case that used IGHV1-2*04 and progressed to SMZL. The patient with orbital lymphoma had the recently noted association of a *TNFAIP3* mutation and IGHV4-34 usage in this subset of MZL.[18] Three patients had repeat genomic analysis at evolution, and no new mutations were found.

Neither cytogenetic nor immunogenetic data measured at presentation correlated with the natural history of CBL-MZ. In contrast, 5 of 9 patients with progressive disease had ≥1 mutation (3 *MYD88* mutations; single cases with *TP53*, *NOTCH2*, *KLF2*, *TNFAIP3* mutations), of which *TP53* and *NOTCH2* mutations have been associated with disease progression in MZLs, compared with 6 of 28 patients with stable disease ($P = .034$). Five of 6 patients with stable disease who had mutations (3 *CCND3* mutations, 2 mutations in both *MYD88* and *TP53*) died of unrelated causes. Their median FU was considerably shorter (39 months) than that of the other stable cases, raising the question of whether their CBL-MZ would have progressed with extended FU.

In summary, our clinical outcome data indicate that CBL-MZ usually pursues a stable course, but the higher rate of progression in this study compared with previous studies probably reflects the longer FU and reinforces the need for long-term clinical management and patient education on when to seek medical advice. CBL-MZ can evolve into several well-defined WHO disorders, especially those of marginal zone origin. The genomic data are consistent with this observation because, although the genomic abnormalities in CBL-MZ

overlap with those found in any of the well-defined entities into which it could evolve, the incidence of mutations is lower and does not mirror any specific disease. However, important caveats are the relatively small number of cases in the current study and the lack of concordance among genomic studies in other rare disorders, such as HCL-v[19]and SDRPL.[11,20,21] Further larger studies, ideally including immunogenetic, whole genomic sequencing, and epigenetic data, will be required to confirm the relationship between CBL-MZ and established WHO disorders and to identify additional drivers of progressive disease. In the interim, CBL-MZ remains a useful term to define a group of asymptomatic patients with well-defined clinical, morphological, and immunophenotypic features requiring long-term FU.

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

Acknowledgments: The authors gratefully acknowledge all of the patients who contributed material and clinical information to this study.

This work was funded by Bloodwise (11052, 12036), the Kay Kendall Leukaemia Fund (873, 1104), Cancer Research UK (C34999/A18087, ECMC C24563/A15581), Wessex Medical Research, and the Bournemouth Leukaemia Fund.

Contribution: H.P., N.R.M.-B., M.J.J.R.-Z., M.P., and A.X. performed the experiments; Z.A.D. performed the molecular diagnostic assays; M.J.J.R.-Z. and J.G. conducted the statistical and bioinformatics analyses; R.W. and D.G.O. contributed patient samples and data; J.C.S. and D.G.O. initiated and designed the study; D.G.O., H.P., and J.C.S. wrote the manuscript; and all authors critically reviewed the final manuscript.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

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DOI 10.1182/bloodadvances.2018019760