Clonal hematopoiesis in diffuse large B-cell lymphoma: clinical impact and genetic relatedness to lymphoma and therapy-related myeloid neoplasm

Clonal hematopoiesis (CH) is an age-related phenomenon characterized by the overrepresentation of blood cells derived from a single clone, conferring an increased risk of myeloid neoplasms (MN), cardiovascular disease, and death from non-hematological malignancies.[1,2] Recent work demonstrated a high prevalence of CH that divergently evolves to lymphoma and MN in patients with angioimmunoblastic T-cell lymphoma (AITL).[3] Several studies have shown CH occurs in 10-30% of patients with B-cell lymphoma (BCL).[4,5] Whether CH impacts the outcome of BCL remains controversial.[4,5] Moreover, whether CH clones divergently evolve to BCL and MN is unclear. Herein, we evaluated a cohort of patients with diffuse large B-cell lymphoma (DLBCL) and high-grade B-cell lymphoma (HGBCL) who were analyzed for mutations using a targeted next-generation sequencing (NGS) panel covering 400 genes (MSK-IMPACT).[6] We aimed to investigate: i) the prevalence of CH and its impact on outcome; ii) the risk of t-MN in patients harboring CH mutations; iii) the possibility of divergent clonal evolution from CH to BCL and MN.

NGS data on diagnostic tissue between January 2015 and September 2021 were available for 362 (94%) DLBCL and 23 (6%) HGBCL patients. Among DLBCL, 187 (48.5%) were of germinal center B-cell (GCB) subtype and 173 (45%) of non-GCB subtype and two (0.5%) without an available immunophenotype. Median age was 64 years (range, 19-95) at the time of lymphoma diagnosis and 65 years (range, 20-95) at the time of CH detection. Time points for CH detection were variable due to the retrospective nature of the study: 206 and 179 patients were tested after and before chemotherapy, respectively. One hundred and twenty-seven patients had paired MSK-IMPACT performed on uninvolved bone marrow (BM) or peripheral blood (PB) (*Online Supplementary Figure S1*). For cases with paired analysis, a 0.01 variant allele frequency (VAF) cutoff in BM/PB was used for CH calling.[5,7,8] In this subgroup, CH was present in 37 of 127 (29%) patients and absent in 90 of 127 (71%) (referred to as CH+ and CH- cohorts, respectively). For the 258 cases without paired analysis, CH calling was performed using NGS data from lymphoma tissue and/or saliva samples based on the following criteria suggesting tissue infiltration by blood: i) variants were reported as common in CH genes in the literature;[9] and ii) variants had a VAF <1/10 of the highest VAF of any DLBCL-associated mutation in lymphoma tissue. As a result, 17 (7%) and 241 (93%) patients were classified as CH+ and CH-, respectively. Overall, the CH prevalence was 14% (54/385).

The most common CH mutations were *DNMT3A* (20/54; 37%), *TET2* (13/54; 24%), *TP53* (12/54; 22%), *PPM1D* (7/54; 13%) and *ASXL1* (7/54; 13%). The VAF of the CH mutations ranged from 0.01 to 0.28 (*Online Supplementary Figure S2*). No significant association between the highest VAF and age at CH detection was seen (P=0.9). Twenty-seven patients had VAF ≥0.05 in any CH and 11 had >2 CH. Twenty-one patients harbored CH in DNA-repair pathway genes (*TP53, PPM1D, CHEK2* and *ATM*).

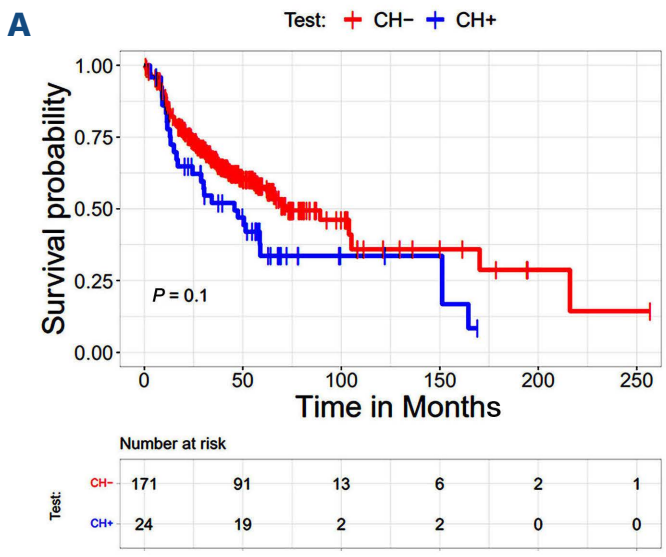
CH+ patients were significantly older at the time points of DLBCL diagnosis (median age 70 years *vs*. 63 years; P<0.01) and CH detection (71 years *vs*. 64 years; P<0.01) as compared to CH- patients. There were no significant differences in CBC, B symptoms, lactate dehydrogenase (LDH) levels, age-adjusted International Prognostic Index (IPI), disease stage, Ki-67, DLBCL subtype, treatment regimen, tolerance to chemotherapy (absolute neutrophil count [ANC] or BM reserve post chemotherapy) between CH+ and CH- patients (Table1).

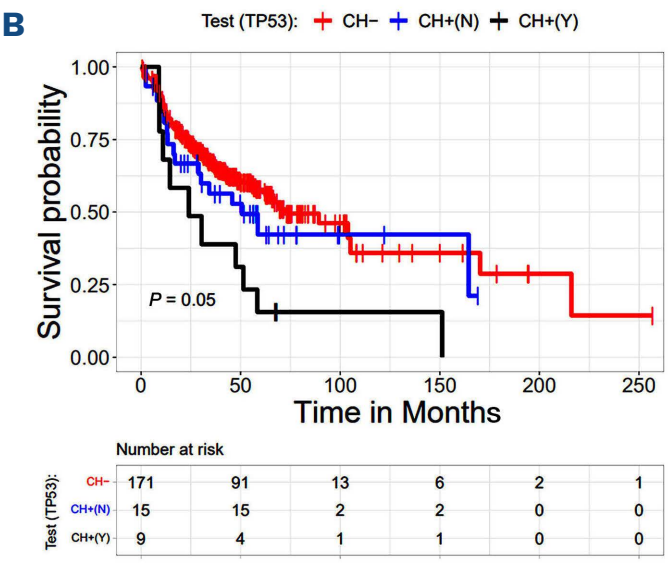
Effects of CH on overall survival (OS) were assessed using a multivariable Cox proportional hazard model adjusted for age at diagnosis (modeled with splines with 4 degrees of freedom) and stratified by CH calling method. Significance of associations was evaluated by likelihood ratio test. A P value <0.05 was considered significant. With a median follow-up of 44 months after treatment initiation, the median OS was 69 months (95% confidence interval [CI]: 57-151) for all patients. CH+ patients had inferior survival compared to CH- patients (median OS 46 months, 95% CI: 17-not reached [NR] *vs*. 72 months, 95% CI: 61-NR). Differences in OS were also observed between CH- patients and CH+ patients without mutations in the DNA repair pathway (51 months, 95% CI: 16-NR), and CH+ patients with mutations in the DNA repair pathway (30 months, 95% CI: 14-NR). Patients harboring a CH with a VAF ≥0.05 had inferior survival compared to those whose VAF were all <0.05 (16 months, 95% CI: 10-NR *vs*. 59 months, 95% CI: 51-NR). Similarly, although cases were limited, >2 CH (24 months, 95% CI: 13-NR *vs*. 58 months, 95% CI: 17-NR) and *TP53* CH (24 months, 95% CI: 11-NR *vs*. 51 months, 95% CI: 17-NR) were associated with inferior outcomes (Figure 1A-D). However, after age adjustment, the presence of CH, DNA repair pathway CH, and *TP53* CH were not significantly associated with OS; although CH with a VAF ≥0.05 and >2 CH showed strong trends towards worse OS (Figure 1E). Analysis on separated paired and unpaired cohorts showed similar impact on survival by CH (data not shown).

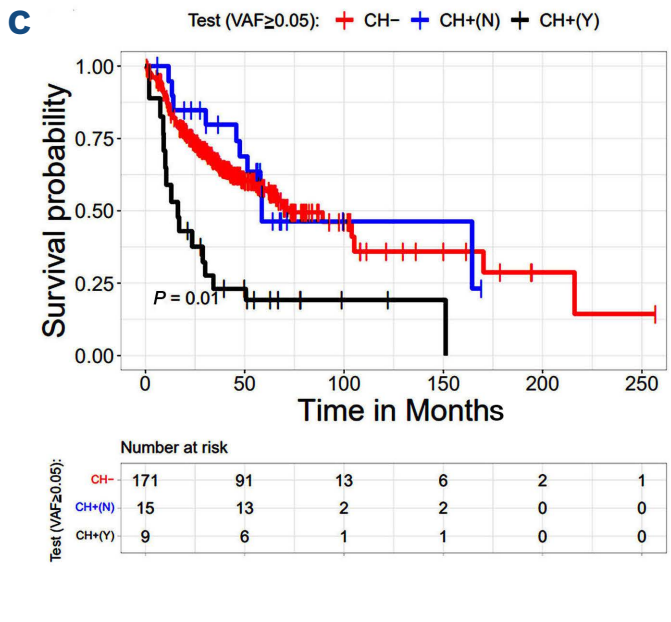
Table 1. Histopathological and clinical features of the 385 cases.

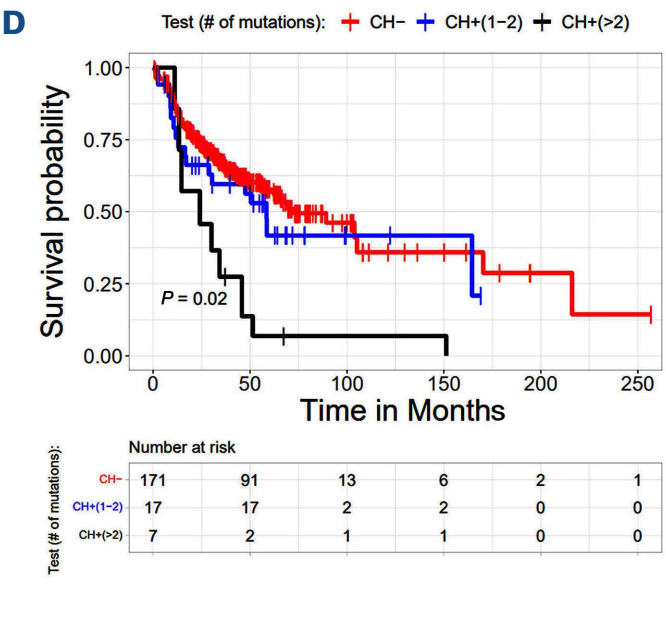
Entire cohort	Total N=385	CH+ N=54	CH- N=331	*P* value
Age in years at DLBCL diagnosis, N (%)				
<55	110	7 (6)	103 (94)	<0.01
55-70	157	21 (13)	136 (87)	
>70	118	26 (22)	92 (78)	
Age in years at CH testing, N (%)				
<55	100	5 (5)	95 (95)	<0.01
55-70	150	20 (13)	130 (87)	
>70	135	29 (21)	106 (79)	
M/F	223/162	33/21	190/141	0.9
CBC at diagnosis, median (range)				
WBC (x10^9/L)	7.0 (0.9-39.5)	7.0 (1.0-39.5)	7.0 (0.9-36.8)	0.7
ANC (x10^9/L)	4.6 (0.1-29.5)	3.8 (0.2-14.7)	4.6 (0.1-29.5)	0.1
HGB (g/dL)	12.6 (6.6-19.0)	12.3 (8.8-16.0)	12.7 (6.6-19.0)	0.4
PLT (x10^9/L)	236 (23-785)	209 (41-753)	237 (23-785)	0.1
Neutropenia post chemotherapy,* N (%)				
Severe	12	1 (8)	11 (92)	0.7
Moderate	12	2 (17)	10 (83)	
Mild	11	3 (27)	8 (73)	
WNL	281	43 (15)	238 (85)	
BM cellularity,** N (%)				
Hypocellular	43	10 (23)	33 (77)	0.1
Normocellular or hypercellular	142	25 (18)	117 (82)	
na	200	19 (10)	181 (90)	
B Symptoms, N (%)				
Yes	110	15 (14)	95 (86)	0.8
No	186	21 (11)	165 (89)	
na	89	18 (20)	71 (80)	
High LDH, N (%)				
Yes	163	24 (15)	139 (85)	0.8
No	145	21 (14)	124 (86)	
na	77	9 (12)	68 (88)	
DLBCL subtypes, N (%)				
GCB	187	22 (12)	165 (88)	0.9
Non-GCB	173	26 (15)	147 (85)	
HGBCL	23	6 (26)	17 (74)	
na	2	0 (0)	2 (100)	
High Ki67 (≥70%), N (%)				
Yes	233	39 (17)	194 (83)	0.4
No	116	12 (10)	104 (90)	
na	36	3 (8)	33 (92)	
Stages at diagnosis, N (%)				
I/II	124	14 (11)	110 (89)	0.4
III/IV	216	38 (18)	178 (82)	
na	45	2 (4)	43 (96)	
≥2 age-adjusted IPI, N (%)				
Yes	173	24 (14)	149 (86)	1.0
No	92	12 (13)	80 (87)	
na	120	18 (15)	102 (85)	
Initial chemotherapy, N (%)				
R-CHOP	248	29 (12)	219 (88)	0.2
DA-R-EPOCH	70	13 (19)	57 (81)	
Clinical trial	28	4 (14)	24 (86)	
Others	39	8 (21)	31 (79)	
CH testing time point (to chemotherapy), N (%)				
Before	179	12 (7)	167 (93)	0.001
After	206	42 (20)	164 (80)	
Auto/allo-SCT, N (%)	87	10 (11)	77 (89)	0.7

Patients' characteristics are summarized by frequency (percentage [%]). Associations between clonal hematopoiesis (CH) status and disease characteristics were tested by Fisher's exact test. High-grade B-cell lymphoma (HGBCL) refers to diffuse large cell pattern but with *MYC*, *BCL2* and/or *BCL6* gene rearrangements. *Severity of neutropenia post first-line diffuse large B-cell lymphoma (DLBCL) chemotherapy when available. Neutropenia: severe (<0.5x10^9/L); moderate (0.5-<1.0x10^9/L); mild (1.0-<1.5x10^9/L); within normal limit (WNL): (≥1.5x10^9/L). **Bone marrow (BM) cellularity (hypocellular: reduced marrow cellularity after age-adjustment; normocellular or hypercellular: normal or increased marrow cellularity after age-adjustment) at the time point of worst neutropenia (nadir) post DLBCL chemotherapy. M: male; F: female; CBC: complete blood count; LDH: lactate dehydrogenase; na: not available; GCB: germinal-center B cell; WBC: white blood cell; ANC: absolute neutrophil count; PLT: platelet; R-CHOP: rituximab plus cyclophosphamide-doxorubicin vincristine prednisone; DA-R-EPOCH: dose-adjusted rituximab etoposide prednisolone vincistrine cyclophosphamide doxorubicin; IPI: International Prognostic Index.









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CH Characteristics	HR (95%CI)	Overall *P*-value
CH status (ref: CH-)		0.73
CH+	1.1 (0.7-1.75)	
DNA repair pathway CH (ref: CH-)		0.60
No	0.91 (0.49-1.71)	
Yes	1.35 (0.71-2.58)	
TP53+ CH (ref: CH-)		0.13
No	0.84 (0.47-1.51)	
Yes	1.99 (1.00-4.28)	
# of CH (ref: CH-)		0.07
1-2	0.84 (0.48-1.49)	
>2	2.36 (1.14-4.88)	
CH with VAF≥5% (ref: CH-)		0.06
No	0.70 (0.36-1.26)	
Yes	1.7 (0.99-3.21)	

Figure 1. Impact of clonal hematopoiesis mutations on patients' survival. Overall survival (OS) was evaluated by Kaplan-Meier method with left truncation at the time of clonal hematopoiesis (CH) testing to account for CH detection performed after the start of first-line chemotherapy for diffuse large B-cell lymphoma (DLBCL) and high-grade B-cell lymphoma (HGBCL). Although CH with a variant allele frequency (VAF) ≥5% and a higher number of CH mutations (>2) appeared to be associated with inferior OS; based on the calculated *P* value (≥0.05) after age adjustment, the presence of CH, any CH in DNA repair pathway, and *TP53* CH did not show a significant association with OS while CH with a VAF ≥5% and a higher number of CH mutations (>2) showed strong trends towards inferior OS. (A) Impact of all CH mutations on OS (CH+: VAF≥1%; CH-: VAF <1% or absent). (B) Impact of *TP53* CH mutations on OS (CH+(Y): CH+ and *TP53* CH present; CH+(N): CH+ and TP53 CH absent; CH-: VAF<1% or absent). (C) Impact of VAF of CH mutations on OS [CH+(Y): CH+ with VAF ≥5% in any CH; CH+(N): CH+ with VAF<5% in all CH; CH-: VAF<1% or absent). (D) Impact of number of CH mutations on OS (CH+(Y): CH+ with >2 CH mutations; CH+(N): CH+ with ≤2 CH mutations; CH-: VAF<1% or absent). (E) Multivariable analysis of CH parameters associated with OS in the 385 DLBCL and HGBCL patients. All statistical analyses were performed using R.

We observed seven patients with concurrent or subsequent diagnosis of MN (t-MN [n=5], CML [n=1], Ph-negative MPN [n=1]). Four of five t-MN cases were CH+ (7% *vs*. 0.3% in CH- cohort) with time to t-MN development significantly shorter for CH+ patients (P<0.001), suggesting an increased risk of t-MN in CH+ patients. Risk of MN development appeared to be higher in CH+ patients with *TP53* CH alone (17% [2/12] *vs*. 5% [2/42]), although statistical analysis was not performed due to limited t-MN cases. Of note, the CH- patient who developed t-MDS 29 months post CH detection harbored a *TP53* mutation (VAF =0.90) in the diagnostic BM. Review of the patient's prior lymphoma and saliva samples demonstrated the same *TP53* with a VAF of 0.002, below the 0.01 cutoff for CH calling (Figure 2).

In order to study the clonal relationship between CH and BCL, corresponding lymphoma tissue were assessed for the CH mutations detected in BM/PB of CH+ patients with paired analysis. Although some CH genes were also mutated in the lymphomas, such as *TP53, ASXL1, TET2, SF3B1* and *ZRSR2*, these mutations were not identical to those detected in BM/PB. All CH mutations present in uninvolved BM/PB were either absent or present at extremely low VAF (<0.005) in lymphoma samples, suggesting blood infiltration of the tissue. In addition, none of the mutations identified in the lymphoma samples were seen in normal BM/PB (Figure 2; *Online Supplementary Table S1*). We also did not detect shared mutations among t-MN and DLBCL in the same patients. These results indicate clonal unrelatedness between CH/t-MN and DLBCL. NGS studies on all lymphoma tissue (Figure 2A; *Online Supplementary Figure 2F*) demonstrated a similar mutational profile to those reported in litereature.[10]

Limited studies are available on the prevalence and clinical significance of CH in DLBCL patients. CH was identified in 12% of HGBCL patients at diagnosis, using a VAF cutoff of 0.05, who demonstrated a trend towards inferior PFS and OS,[4] and in 6.5% of DLBCL patients (VAF cutoff 0.1), which was associated with inferior EFS but not OS.[11] In our cohort, the high CH prevalence (29%) in patients'

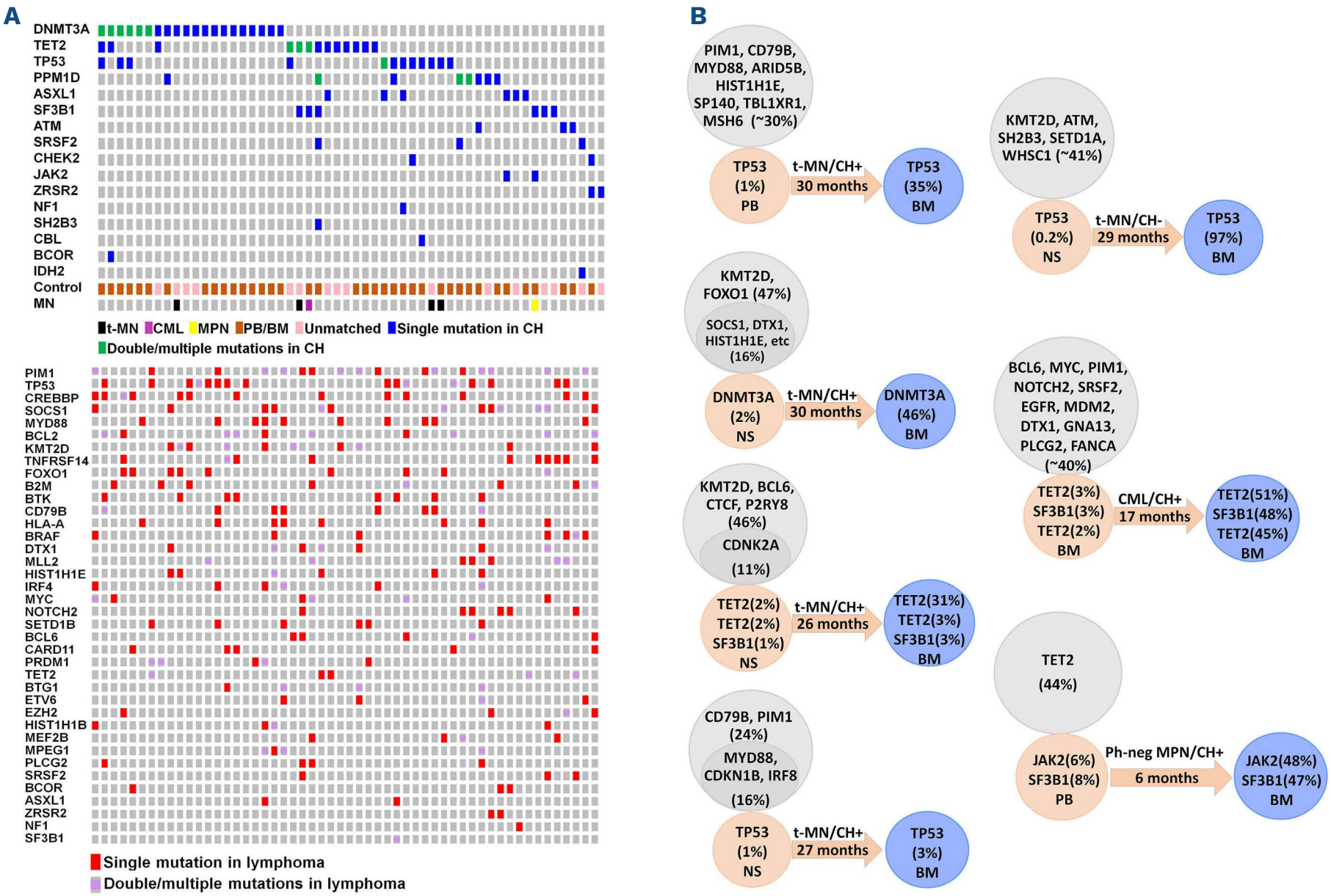

Figure 2. Mutational profiles of lymphoma, clonal hematopoiesis and myeloid neoplasm clones. (A) Mutational profiles of clonal hematopoiesis (CH) and lymphoma clones in 54 CH+ patients. Bone marrow/peripheral blood (BM/PB): cases with paired next-generation sequencing (NGS) study on patients' uninvolved BM or PB samples; unmatched: cases without paired NGS study on patients' BM or PB samples. (B) Mutational profiles of lymphoma (gray), CH (orange) and myeloid neoplasm (MN) (blue) clones in 7 patients with MN. NS: saliva. Time intervals between CH detection and MN development are shown.

uninvolved BM/PB is related to low VAF cutoff (0.01) and post-treatment testing. The negative impact on outcomes by CH was largely attributed to the older age of CH+ compared to CH- patients as shown in previous studies.[4,11] Nevertheless, our data showed evidence suggestive of an association between OS and CH mutations with VAF ≥0.05 and a higher number of CH (>2), which is similar to another study with all types of BCL undergoing autologous stem cell transplant.[12] Although CH was not associated with the degree of post-chemotherapy cytopenia in our study, a recent study has shown that CH mutations especially *DNMT3A, TET2* and *ASXL1* are associated with increased neurotoxicity in BCL patients treated with chimeric antigen receptor (CAR) T-cell therapy.[13] Therefore, the impact of CH on treatment related toxicity warrants further study.

Our study showed that CH may increase the risk for t-MN in DLBCL/HGBCL, consistent with the well-recognized elevated risk of hematologic malignancy in CH.[1,7] Not surprisingly, all five t-MN in our study evolved from CH clones detected at earlier time points at low levels (VAF 0.002-0.02), indicating selective pressure of chemotherapy for expanding pre-existing CH clones, leading to t-MN development. In contrast to the divergent evolution described in AITL,[3] we found no evidence of clonal relatedness between CH/MN and BCL. Although a recent study of CH in classic Hodgkin lymphoma showed one case with *DNMT3A/TET2* double mutations present both as tissue CH and in EBV+ neoplastic clone, evidence of CH in PB/BM myeloid cells was not demonstrated and it is unclear whether EBV infection played an etiologic role.[14] Similarly, although a common stem cell origin of both MDS and plasma cell neoplasm (PCN) has been proposed, a recent study didn't show evidence of shared mutations between MDS and PCN.[15] *DNMT3A* and *TET2* mutations in CH have been differentially detected in normal T cells, but also in myeloid and B-cell lineages at equally high rates.[16] Therefore, the lack of evidence for divergent clonal evolution of CH to myeloid and B neoplasms is intriguing and warrants sequencing studies on purified populations. Of note, a recent study showed incompatibility between *TET2* deficiency and AID-induced demethylation, which may partially explain the lack of clonal relatedness between CH and lymphoma.[17]

The uniform diagnoses, NGS analyses and treatment among our patient cohort is a strength of this study. However, due to its retrospective nature, time points of CH testing were variable and not all patients had sequencing data from uninvolved BM/PB, limiting accurate measurement of CH prevalence and the statistical power of outcome analysis. The prevalence of CH based on lymphoma and saliva sequencing data is likely to be underestimated due to low-level or lack of blood infiltration in such samples. Nevertheless, our study has addressed several questions including: i) the prevalence of CH in DLBCL/HGBCL patients is high; and a higher number of CH mutations (>2) and CH with VAF ≥0.05 show strong trends towards inferior clinical outcome after age adjustment; ii) patients harboring CH mutations have an increased risk of developing t-MN; iii) there is no evidence of clonal relatedness between CH/t-MN and DLBCL. Collectively, our study provides new insights into the impact of CH in DLBCL, risk of t-MN development and the clonal relationships among these entities.

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Contributions

YL, ADo and WX conceived the study; YL, ADe and WX analyzed the data and wrote the manuscript; YL, NL, MZ, MA and GS collected and annotated the data; YZ reviewed cytogenetic data. All the authors approved the final version of the manuscript.

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Data-sharing statement

We will make our original data and protocols available to other investigators without unreasonable restrictions.

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