Distinct Immunogenetic Profiles of Chronic Lymphocytic Leukemia in Asia: A Taiwan Cooperative Oncology Group Registry Study

Chi-Yuan Yao1,2,3, Andreas Agathangelidis4, Shih-Sung Chuang5, Hsiao-Hui Tsou6,7, Wei-Lien Feng6, Ta-Chih Liu6, Tsai-Yun Chen10, Yuan-Bin Yu11, Su-Peng Yeh12, Ming Yao1, Chuan-Cheng Wang13, Johnson Lin14, Wen-Li Hwang15, Jyh-Pnyng Gau16, Wen-Chien Chou1,2, Tsu-Yi Chao17, Liang-In Lin18, Hwei-Fang Tien1, Paolo Ghia19, Shang-Ju Wu1,20

Correspondence: Shang-Ju Wu (wushangju@ntu.edu.tw).

ABSTRACT
Asian patients with chronic lymphocytic leukemia (CLL) exhibit immunoglobulin heavy variable (IGHV) gene repertoires that are distinct from those observed in Western populations, and a higher proportion of Asian CLL patients carry heavy loads of somatic hypermutations (SHM) within the B-cell receptor immunoglobulins (BcR IG). Due to the low regional incidence of CLL in Asia, only a limited number of studies had attempted to probe the phenomenon of BcR IG stereotypy in Asian populations. In this study, we analyzed the IGHV-IGHD-IGHJ gene rearrangements from a series of 255 CLL patients recruited in a nationwide, multicenter study in Taiwan. Our analysis revealed that the IGHV gene repertoire was characterized by evident biases, with IGHV3-7, IGHV4-34, and IGHV3-23 being the most frequent rearranged IGHV genes, and a higher proportion of cases carrying mutated IGHV genes. In terms of BcR stereotypy, the incidence of major subsets was less frequent in this cohort, with subsets #77 and #28A being the most common, while the incidence of minor subsets was approximately equivalent to that reported in the Western cohorts. With this study, we provide evidence that CLL in Asia is indeed associated with distinct immunogenetic characteristics regarding IGHV gene usage, SHM status, and BcR IG stereotypy.

INTRODUCTION
The B-cell receptor immunoglobulin (BcR IG) mediates the highly specific antigen recognition by B cells, and has been shown to play important roles in the molecular pathogenesis of chronic lymphocytic leukemia (CLL).1,2 More than 2 decades ago, researchers discovered that the immunoglobulin heavy variable (IGHV) gene usage in CLL was biased and distinct from that of normal B-cell populations.1,3 These studies provided the first evidence that chronic stimulation by specific antigen(s) may...
result in the clonal selection and expansion of certain B cells, eventually leading to the onset of full-blown CLL. Later studies then identified the prognostic value of the somatic hypermutation (SHM) status of rearranged IGHV genes, leading to its establishment as one of the most accurate and straightforward prognostic biomarkers for CLL patients. As researchers gained a deeper understanding of CLL immunogenetics, the discovery of BcR IG stereotypy in a substantial fraction of CLL patients introduced an additional layer of complexity in the molecular classification of CLL. The 2 main criteria for BcR IG stereotypy assignment are as follows: (1) IGHV genes should derive from the same phylogenetic clan and (2) VH complementarity-determining region 3 (CDR3) amino acid (aa) sequences should have the same lengths and same offsets of shared sequence pattern, as well as a high level of identity (50%) and similarity (70%). CLL patients belonging to a particular stereotyped subset are characterized by substantial similarities in both biological characteristics and clinical outcomes, indicating that in-depth studies of BcR IG stereotypy may not only improve our understanding of CLL pathogenesis but also hold potential to guide clinical decision-making.

It is widely known that CLL is significantly less prevalent in Asian populations than in Western ones; however, the underlying causes of this difference remain to be fully elucidated. In this context, a number of studies have identified distinct characteristics of CLL in Asian populations, including different clinical characteristics, genetic profiles, and immunogenetic repertoires. Despite the inevitable limitation of small sample size in most of these studies, their findings consistently alluded to the possibility that a different set of antigens could contribute to clonal selection of CLL in Asia. Therefore, further exploration of the immunogenetic differences between Asian and Western CLL cohorts may provide important insights into the geographical differences of CLL pathogenesis.

In a recent comprehensive study by the European Research Initiative on CLL (ERIC), Agathangelidis et al performed an in-depth analysis of BcR IG stereotypy in a cohort of nearly 30,000 Western CLL patients, leading to the identification of 2 major features. First, the authors found a significant increase in the proportion of BcR IG stereotypy, reaching 41% of the entire cohort. In addition, the study revealed a series of novel stereotyped subsets, including 10 major ones. Based on these advances in CLL immunogenetics, we revisited the geographical differences in CLL pathogenesis and investigated the immunogenetic repertoire of 235 Asian CLL patients in the national registry of CLL in Taiwan. Our analysis comprised a systematic profiling of BcR IG sequence characteristics, such as IGH gene repertoire, SHM status, and stereotypy, accompanied by genetic mutation characterization.

### RESULTS

The BcR IG gene repertoire of CLL in Taiwan is characterized by distinct biases. A total of 255 CLL patients were enrolled into the TCOG T1914 study. Among this group, 243 patients (95.3%) carried single, productive IGHV-IGHD-IGHJ rearrangements, and these sequences were subjected to downstream analysis.

The clinical characteristics of the 243 patients are listed in Suppl. Table S1. The distribution of IGHV gene usage is shown in Figure 1A, while IGHD and IGHJ gene repertoires are shown in Suppl. Figure S1. The most prevalent IGHV gene in our cohort was IGHV3-7 (n = 32, 13.2%), followed by IGHV4-34 (n = 29, 11.9%) and IGHV3-23 (n = 27, 11.1%). Notably, IGHV1-69, which is regarded the most frequently rearranged IGHV gene in Western cohorts, was much less prevalent in this cohort (n = 4, 1.6%). The most prevalent IGHD gene in our cohort was IGHD3-10 (n = 24, 9.9%), and the IGHJ gene repertoire was dominated by IGHJ4 (n = 133, 54.7%).

The prevalence of mutated CLL is significantly higher in the Taiwan cohort.

SHM status was assessed using the established 98% cutoff for germline identity (GI) of the clonotypic rearranged IGHV gene. In detail, 189 cases (77.8%) were categorized as mutated CLL (M-CLL, <98% GI), whereas 54 patients (22.2%) were assigned to unmutated CLL (U-CLL, ≥98% GI). Of the total, only 13 of 243 cases (5.3%) were truly unmutated (100% GI) (Table 1). In addition, differences were evident in the distribution of the SHM status among cases expressing different IGHV genes (Figure 1A). The vast majority of cases carrying one of the 3 most prevalent IGHV genes, namely IGHV3-7, IGHV 4-34, and
IGHV3-23, belonged to M-CLL (93.8%, 89.7%, and 92.6%, respectively). Among cases expressing other IGHV genes, those carrying the IGHV4-59 and IGHV4-4 genes were exclusively M-CLL. On the other hand, an unmutated SHM status was enriched in groups carrying the IGHV1-2 and IGHV1-69 genes. Similar to previous reports, the IGHV3-21 gene accounted for around 2.5% of the total repertoire, with most cases belonging to M-CLL. As expected, with a median follow-up time of 4.3 years, U-CLL patients exhibited a shorter overall survival (OS) compared to M-CLL patients (median: 9.3 years versus not reached, \( p < 0.001 \), Figure 1B).

Restrictions in the combinations of IGHV, IGHD, and IGHJ genes were evident in the Taiwan CLL cohort

Next, we looked at the distributions of IGHV, IGHD, and IGHJ gene combinations, as depicted in Figure 2, with the partnerships at the individual gene level displayed in Suppl. Figure
S2. In terms of IGHV-IGHD gene combinations, IGHV3 subgroup genes were more commonly rearranged with IGHD3 genes (34.8% of IGHV3 partners), while those belonging to the IGHV4 subgroup were more commonly rearranged with genes belonging to the IGHD3, IGHD6, and IGHD2 gene subgroups (respectively, 30.9%, 22.1%, and 20.6% of IGHV4 partners). Concerning the IGHD-IGHJ gene combinations, genes belonging to the IGHD1, IGHD2, IGHD3, IGHD4, and IGHD6 subgroups were more commonly rearranged with the IGHD4 gene (respectively, 51.5%, 54.2%, 59.3%, 62.5%, and 58.1% of partners for each IGHD gene). In contrast, IGHD5 subgroup genes were found to be evenly rearranged with the IGHD4 and IGHD6 genes (respectively, 31.6% and 31.6% of IGHD5 partners). Although IGHV and IGHJ genes are not physically adjoined in the context of immunoglobulin heavy chain recombination, we noted that genes from the IGHV1, IGHV3, IGHV4 gene subgroups were more commonly associated with IGHJ4 (respectively, 55.0%, 57.7%, and 47.1% of partners for each IGHV).

**Major stereotyped subsets are infrequent in the Taiwan CLL cohort**

The presence of BcR IG stereotypy was analyzed based on a robust bioinformatic algorithm with updated classification criteria.26 Among the 243 productive IGHV-IGHD-IGHJ gene rearrangements, the lengths of VH CDR3 ranged from 5 to 26 aa (median, 14). Similar to prior reports on Western cohorts, the lengths of VH CDR3 were longer in U-CLL than M-CLL (Wilcoxon rank sum \( P < 0.001 \), Figure 3A).

In terms of BcR IG stereotypy, 83 cases (34.2%) could be assigned to stereotyped subsets: 15 (6.2%) belonged to the major subsets, and 68 (28.0%) were assigned to the minor subsets (Figure 3B). The cumulative incidence of the minor subsets was similar to that reported in Western populations (27.7%), but the incidence of the major subsets was significantly less than that in Western cohorts (13.5%) \( (P < 0.001) \).26 Focusing on the major stereotyped subsets, the relative sizes differed from those reported in Western cohorts. As such, subsets #77 and #28A were the most prevalent in our cohort; the VH CDR3 aa sequence patterns matched those in the literature16 (Suppl. Figure S3). On the other hand, subset #2, which is the most frequent major subset in the Western CLL cohorts, was absent in our dataset.

The fractions of patients carrying stereotyped BcR IG were different in U-CLL and M-CLL groups, with the trend being similar to previous reports on Western patients.26 As shown in Figure 3C, 51.9% of U-CLL BcR IGs could be assigned to stereotyped subsets: 13% belonged to major subsets and 38.9% belonged to minor subsets. In contrast, the incidence of stereotyped BcR IG in M-CLL was only 29.1%, including 4.2% belonging to major subsets and 24.9% belonging to minor subsets.

**Stereotyped fraction of CLL in the Taiwan cohort is characterized by unique immunogenetic properties**

In addition to biases in the IGHV gene repertoire of our CLL cohort (Figure 1A), IGHV gene utilization in the stereotyped fraction was also characterized by restrictions distinct from those in the total cohort (Figure 4). In particular, the IGHV4-4, IGHV1-69, and IGHV1-3 genes were overrepresented in the stereotyped fraction of CLL. Cases expressing the IGHV3-23, IGHV3-15, IGHV3-74, and IGHV3-9 genes showed the opposite trend, exhibiting lower frequencies in the stereotyped group compared to the group with heterogeneous BcR IG.

**Table 1**

| Somatic Hypermutation Status in the TCOG Cohort | Number | % |
|-----------------------------------------------|--------|---|
| Truly unmutated (GI = 100%)                   | 13     | 5.3|
| Unmutated (98% ≤ GI < 99.9%)                  | 41     | 16.9|
| Mutated (GI < 98%)                            | 189    | 77.8|

GI = germline identity; IGHV = immunoglobulin heavy variable; TCOG = Taiwan Cooperative Oncology Group.
The immunogenetic characteristics of patients belonging to major and minor stereotyped subsets are listed in Table 2 and Suppl. Table S2, respectively. Similar to the findings in previous reports, all 4 cases of subset #77 belonged to M-CLL, with three of them (75%) expressing the IGHV4-4 gene. Along the same lines, 2 of 3 (66.7%) cases in subset #28A expressed the IGHV1-3 gene.

The clonotypic light chain (IGKV-IGKJ or IGLV-IGLJ) gene rearrangement was identified in 11 cases assigned to the major stereotyped subsets; 2 belonged to subset #1 and 3 to subset #28A. A productive rearrangement was obtained in nine of the 11 cases (81.8%) (Suppl. Table S3). Both of the subset #1 cases exhibited the IGKV1-39/IGKJ1 gene recombination, while IGKV4-1 and IGKJ4 gene usage was identified in 2 of the 3 subset #28A cases.

Driver genetic lesions are less frequent in the Taiwan CLL cohort

The mutation statuses of TP53, MYD88, SF3B1, and BIRC3, and cytogenetic abnormalities detected by FISH in the TCOG cohort are summarized in Suppl. Figure S4A. The mutation incidences for the TP53, MYD88, SF3B1, and BIRC3 genes were 9.1%, 3.7%, 3.3%, and 0.8%, respectively. We noted that most mutational events were mutually exclusive, except for one patient harboring concurrent TP53 and MYD88 mutations; another patient exhibited concurrent TP53 and SF3B1 mutations. Similar to the findings in a Western CLL cohort, the TP53 mutation mainly occurred in U-CLL ($P < 0.001$), while MYD88 mutations were enriched in M-CLL (88.9%). With regard to cytogenetic abnormalities, del(13q), trisomy 12, del(17p), del(11q), and trisomy 3 were detected in 55.8%, 16.1%, 8.3%, 6.6%, and 3.7% of patients, respectively. Most of the del(17p) events occurred in the TP53-mutated patients ($P < 0.001$). For the more frequent genetic events (those that occurred in more than 5% of the 243 patients in the TOCG cohort), the impacts on the survival outcomes were examined. Similar to the findings from prior studies, both the TP53 mutations and del(17p) conferred the strongest poor prognostic value (Suppl. Figure S4B, log-rank $P < 0.001$ and $P = 0.001$, respectively). Furthermore, the prognostic values of TP53 mutations and del(17p) remained significant when applied only for stereotyped or nonstereotyped CLL patients (Suppl. Figure S4C).
DISCUSSION

We presented the analysis results of BcR IG immunogenetics profiling in a cohort of Asian CLL patients recruited in the TCOG T1914 study, a nationwide, multicenter, observational study in Taiwan. According to our findings, the overall IGHV gene usage in our cohort was consistently distinct from that of the Western cohorts, with IGHV3-7, IGHV4-34, and IGHV3-23 being the most predominant rearranged IGHV genes. Additionally, the phenomenon of biased IG gene usage could also be observed in the IGHD and IGHJ gene repertoires, even though these regions exhibited relatively low genetic variability.

Regarding the analysis of BcR IG stereotypy, the proportion of cases belonging to the major subsets was smaller than that in the Western cohorts, and the more frequent major subsets were different.16,26 In our dataset, subsets #77 and #28A accounted for the majority of the major subsets cases (Table 2). Subset #2, while being the most frequent major subset in Western CLL cohorts, was nevertheless absent in our dataset. These observations therefore support the distinctive composition of stereotyped subsets in Asian CLL patients, and indicate the inherent differences in the nature of chronic antigenic interactions during CLL pathogenesis, between Asia and the West.

Focusing on major stereotyped subsets, we noted that the 2 most frequently expressed IGHV genes in cases assigned to subset #28A were IGHV1-2 and IGHV7-4-1. This finding was in contrast to previous studies on Western patients in which the most frequently used IGHV gene in subset #28A is IGHV1-2.16 Thus, in addition to our observation of restricted IGHV usage at the cohort level, the phenomenon of single- or oligo-IGHV gene predominance likely exists within stereotyped subsets as well, which is further compounded with geographical diversity. Moreover, although the number of cases sequenced for IG light chain was limited, our data provide preliminary evidence that
Table 3

| Reference               | Ethnicity | Patient Number | M-CLL: U-CLL % | Prominent IGHV Genes | Proportion With Stereotypy (Major/Minor) % |
|-------------------------|-----------|----------------|----------------|-----------------------|------------------------------------------|
| Agathangelidis et al13  | Caucasian | 7424 (7596 productive) | 54.9: 45.1 | V1-69, V4-34, V3-23   | 30.4 (12.4/18.0) |
| Marinelli et al20       | Caucasian (Italy) | 789 (792 productive) | 48.9: 51.1 | V1-69, V4-34, V3-23   | 25.7 (14.5/11.2) |
| Wu et al21             | Asian (Taiwan) | 83 (58 productive) | 65.7: 34.2 | V3-23, V3-7, V3-74   | 19.7 (10.6/9.9) |
| Huang et al24          | Asian (Taiwan) | 194 (191 productive) | 63.8: 36.2 | V3-23, V3-7, V3-74   | 22.4 (6.9/15.5) |
| Agathangelidis et al25  | Caucasian | 20,856 (30,413 productive) | 54.1: 45.9 | V1-69, V4-34, V3-23   | 41.2 (13.5/27.7) |
| TCOG-T1914 (current study) | Asian (Taiwan) | 255 (243 productive) | 77.8: 22.2 | V3-7, V4-34, V3-23   | 34.2 (6.2/28.0) |

CLL = chronic lymphocytic leukemia; IGHV = immunoglobulin heavy variable; M-CLL = mutated CLL; U-CLL = unmutated CLL; TCOG = Taiwan Cooperative Oncology Group.

preferential usage of certain IGLV and IGLJ genes within the stereotyped subsets is likely.

Similar to the findings from the most recent, a large-scale CLL immunogenetic study by Agathangelidis et al.,26 the IGHV gene repertoire of stereotyped CLL in our cohort was notably different from that of the overall cohort. Specifically, cases carrying the IGHV4-4, IGHV1-69, and IGHV1-3 genes were over-represented in the stereotyped fraction, while cases carrying the IGHV3-23, IGHV3-15, IGHV3-74, and IGHV3-9 genes were under-represented. Based on the findings of the present study, several important observations regarding CLL immunogenetic similarities and differences between Asian and Western cohorts could be made. First, although IGHV4-4 was not a particularly frequent IGHV gene at the level of our entire cohort, it was obviously over-represented in the stereotyped fraction. In contrast, although the IGHV3-23 gene was among the most prevalent in the total cohort, it was relatively under-represented among stereotyped cases. Similar to Western CLL cohorts, an over-representation of the IGHV1-69 gene was observed in the stereotyped fraction of the present cohort. To summarize, these findings suggest that there may exist a unified theme of CLL pathogenesis in both Asian and Western CLL, which is diversified by geographically specific immune repertoire compositions.

To further compare the differences of immunogenetic profiles between Asian and Western CLL patients, data derived from relevant publications were compiled in Table 3. Compared to the Western CLL cohorts, Asian CLL cohorts are characterized by a larger proportion of M-CLL, more frequent IGHV3-7 gene usage, and less frequent IGHV1-69 gene usage. In terms of BcR IG stereotypy, Asian CLL patients seem to be less often assigned to stereotyped subsets, although this result might have been influenced by the generally smaller cohort sizes in Asian studies, as suggested by the random data subsampling analysis by Agathangelidis et al.24 Additionally, in the current study, with the application of a well-established, purpose-built pattern discovery algorithm, we demonstrated that the proportion of cases assigned to minor subsets was nearly equivalent to that reported by Agathangelidis et al.,26 while the proportion of cases assigned to major subsets was still lower than that of the Western cohorts, further supporting the characteristic immunogenetic profiles in Asian CLL patients.

Although the size of the current cohort was not powered for detailed prognostic impact analysis of gene mutations and cytogenetic abnormalities, we provide evidence that for both stereotyped and nonsterotyped CLL patients, TP53 mutation and del(17p) status could risk-stratify clinical outcomes. Thus, the genetic mutation and cytogenetic analysis seems to remain pertinent even in the contemporary immunogenetic era.

In summary, we report the comprehensive immunogenetic characterization of an Asian CLL cohort, analyzed with state-of-the-art bioinformatic algorithms. Although the sample size of our cohort was smaller than studies derived from the Western cohorts, we provide convincing evidence that CLL in Asia is associated with distinct immunogenetic characteristics in terms of IGHV gene usage, SHM status, and BcR IG stereotypy. We believe that further research efforts on BcR IG repertoires in larger Asian CLL cohorts would advance our understanding of how geographical differences may shape the molecular pathogenesis and clinical behavior of CLL.

ACKNOWLEDGMENTS

The authors thank Mr. Chao-Tung Lee, Ms. Wei-Lan Yu, Ms. Sy-Ying Lee, Ms. Yi-Fang Chen, Ms. Ching, Tsai-Rong, and Ms. Ya-Ling Wu at TCOG for their assistance with data management, statistical analyses, and administrative support. The authors thank co-investigators for help in patient enrollment, treatment, and follow-up.

AUTHOR CONTRIBUTIONS

C-YY and AA were responsible for data management, statistical analysis and interpretation, literature research, and manuscript writing; S-SC was responsible for pathological review of patient specimens and manuscript writing; H-HT assisted in statistical analysis; W-LF, T-CL, T-YC, C-YY and AA were responsible for data management, statistical analyses, and administration. The authors have no conflicts of interest to disclose.

DISCLOSURES

This work was supported by grants from Ministry of Science and Technology, Taiwan (104-2314-B-002-104-MY3), TCOG, National Health Research Institute, Taiwan (TCOG T1914), Roche Pharmaceutical, Taiwan, and Abbvie Pharmaceutical, Taiwan.

REFERENCES

1. Vardi A, Agathangelidis A, Sutton LA, et al. Immunogenetic studies of chronic lymphocytic leukemia: revelations and speculations about ontogeny and clinical evolution. Cancer Res. 2014;74:4211–4216.
2. Sutton LA, Rosenquist R. The complex interplay between cell-intrinsic and cell-extrinsic factors driving the evolution of chronic lymphocytic leukemia. Semin Cancer Biol. 2015;34:22–35.
3. Efremov DG, Ivanovski M, Siljanovski N, et al. Restricted immunoglobulin VH region repertoire in chronic lymphocytic leukemia patients with autoimmune hemolytic anemia. Blood. 1996;87:3869–3876.
4. Johnson TA, Rassenti LZ, Kipp TJ. IGHV genes expressed in B cell chronic lymphocytic leukemia exhibit distinctive molecular features. J Immunol. 1997;158:235–246.
5. Fais F, Ghiotto F, Hashimoto S, et al. Chronic lymphocytic leukemia B cells express restricted sets of mutated and unmutated antigen receptors. J Clin Invest. 1998;102:1515–1525.
6. Chu CC, Catra R, Hatzi K, et al. Chronic lymphocytic leukemia antibodies with a common stereotypic rearrangement recognize nonmuscle myosin heavy chain IIIA. Blood. 2008;112:5122–5129.

7. Gounari M, Ntoufa S, Apollonio B, et al. Excessive antigen reactivity may underlie the clinical aggressiveness of chronic lymphocytic leukemia stereotyped subset #8. Blood. 2015;125:3380–3387.

8. Seiler T, Wolfle M, Yancopoulos S, et al. Characterization of structurally defined epitopes recognized by monoclonal antibodies produced by chronic lymphocytic leukemia B cells. Blood. 2009;114:3615–3624.

9. Hamblin TJ, Davis Z, Gardiner A, et al. Unmutated Ig V(H) genes are associated with a more aggressive form of chronic lymphocytic leukemia. Blood. 1999;94:1848–1854.

10. Damle RN, Wasil T, Fais F, et al. Ig V gene mutation status and CD38 expression as novel prognostic indicators in chronic lymphocytic leukemia. Blood. 1999;94:1840–1847.

11. Ghiotto F, Fais F, Valetto A, et al. Remarkably similar antigen receptors among a subset of patients with chronic lymphocytic leukemia. J Clin Invest. 2004;113:1008–1016.

12. Messmer BT, Albesiano E, Efremov DG, et al. Multiple distinct sets of stereotyped antigen receptors indicate a role for antigen in promoting chronic lymphocytic leukemia. J Exp Med. 2004;200:519–525.

13. Widdop GF, 2nd, Rassenti LZ, Toy TL, et al. Chronic lymphocytic leukemia B cells of more than 1% of patients express virtually identical immunoglobulins. Blood. 2004;104:2499–2504.

14. Tobin G, Thunberg U, Karlsson K, et al. Subsets with restricted immunoglobulin gene rearrangement features indicate a role for antigen selection in the development of chronic lymphocytic leukemia. Blood. 2004;104:2879–2885.

15. Stamatopoulos K, Belessi C, Moreno C, et al. Over 20% of patients with chronic lymphocytic leukemia carry stereotyped receptors: pathogenetic implications and clinical correlations. Blood. 2007;109:259–270.

16. Agathangelidis A, Darzentas N, Hadzidimitriou A, et al. Stereotyped B-cell receptors in one-third of chronic lymphocytic leukemia: a molecular classification with implications for targeted therapies. Blood. 2012;119:4467–4475.

17. Bystrzy, Agathangelidis A, Bikos V, et al; European Research Initiative on CLL. ARReSt/AssignSubsets: a novel application for robust subclassification of chronic lymphocytic leukemia based on B cell receptor IG stereotypy. Bioinformatics. 2015;31:3844–3846.

18. Bialiakas P, Agathangelidis A, Hadzidimitriou A, et al. Not all IGHV3-21 chronic lymphocytic leukemias are equal: prognostic considerations. Blood. 2015;125:856–859.

19. Bialiakas P, Hadzidimitriou A, Sutton LA, et al. Clinical effect of stereotyped B-cell receptor immunoglobulins in chronic lymphocytic leukemia: a retrospective multicentre study. Lancet Haematol. 2014;1:e74–e84.

20. Rossi D, Spina V, Cerri M, et al. Stereotyped B-cell receptor is an independent risk factor of chronic lymphocytic leukemia transformation to Richter syndrome. Clin Cancer Res. 2009;15:4415–4422.

21. Wu SJ, Huang SY, Lin CT, et al. The incidence of chronic lymphocytic leukemia in Taiwan, 1986-2005: a distinct increasing trend with birth-cohort effect. Blood. 2010;116:4430–4435.

22. Wu SJ, Chang CJ, Lin CT, et al. A nationwide population-based cross-sectional comparison of hematological malignancies incidences between Taiwan and the United States of America. Ann Hematol. 2016;95:165–167.

23. Wu SJ, Lin CT, Agathangelidis A, et al. Distinct molecular genetics of chronic lymphocytic leukemia in Taiwan: clinical and pathogenetic implications. Haematologica. 2017;102:1085–1090.

24. Huang YJ, Kuo MC, Chang H, et al. Distinct immunoglobulin heavy chain variable region gene repertoire and lower frequency of del(11q) in Taiwanese patients with chronic lymphocytic leukaemia. Br J Haematol. 2019;187:82–92.

25. Marinelli M, Ilari C, Xia Y, et al. Immunoglobulin gene rearrangements in Chinese and Italian patients with chronic lymphocytic leukemia. Oncotarget. 2016;7:20520–20531.

26. Agathangelidis A, Chatzidimitriou A, Gemenetzki, et al. Higher-order connections between stereotyped subsets: implications for improved patient classification in CLL. Blood. 2021;137:1365–1376.

27. Swerdlow SH, Campo E, Pileri SA, et al. The 2016 revision of the World Health Organization classification of lymphoid neoplasms. Blood. 2016;127:237e–2390.

28. Ghia P, Stamatopoulos K, Belessi C, et al. ERIC recommendations on IGHV gene mutational status analysis in chronic lymphocytic leukaemia. Leukemia. 2007;21:1–3.

29. Rosenquist R, Ghia P, Hadzidimitriou A, et al. Immunoglobulin gene sequence analysis in chronic lymphocytic leukemia: updated ERIC recommendations. Leukemia. 2017;31:1477–1481.

30. Alamyar E, Duroux P, Lefranc MP, et al. IMGT® tools for the nucleotide analysis of immunoglobulin (IG) and T cell receptor (TR) V-(D)-J repertoires, polymorphisms, and IG mutations: IMGT/V-QUEST and IMGT/HighV-QUEST for NGS. Methods Mol Biol. 2012;882:569–604.

31. Tobin G, Thunberg U, Johnson A, et al. Somatically mutated Ig V(H)3-21 genes characterize a new subset of chronic lymphocytic leukemia. Blood. 2002;99:2262–2264.

32. Tobin G, Thunberg U, Johnson A, et al. Chronic lymphocytic leukemias utilizing the VH3-21 gene display highly restricted Vlambda2-14 gene use and homologous CDR3s: implicating recognition of a common antigen epitope. Blood. 2003;101:4952–4957.

33. Tausch E, Beck P, Schlenk RF, et al. Prognostic and predictive role of gene mutations in chronic lymphocytic leukemia: results from the pivotal phase III study COMPLEMENT1. Haematologica. 2020;105:2440–2447.