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by Yuting Yan, Yongxin Xia, Rui Cui, Weihao Chen, Tingyu Wang, Ying Yu, Wenjie Xiong, Gang An, Dehui Zou, Lugui Qiu, Liang Wang and Shuhua Yi

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The prognostic implications of autoimmune hemolytic anemia in chronic lymphocytic leukemia across treatment eras

Yuting Yan*[1,2], Yongxin Xia*[1,2,3,4], Rui Cui*[1,2,5], Weihao Chen[1,2], Tingyu Wang[1,2], Ying Yu[1,2], Wenjie Xiong[1,2], Gang An[1,2], Dehui Zou[1,2], Lugui Qiu[1,2], Liang Wang#[1,2,3,4], Shuhua Yi#[1,2] for the Chinese Workshop of Indolent Lymphomas

1. State Key Laboratory of Experimental Hematology, National Clinical Research Center for Blood Diseases, Haihe Laboratory of Cell Ecosystem, Institute of Hematology & Blood Diseases Hospital, Chinese Academy of Medical Sciences & Peking Union Medical College, Tianjin, China

2. Tianjin Institutes of Health Science, Tianjin, China

3. School of Clinical Medicine, Shandong Second Medical University, Weifang, China

4. Shengli Oilfield Central Hospital, Shandong, China

5. Tianjin First Central Hospital, Tianjin, China

* These authors contributed equally as co-first authors

# These authors contributed equally as co-corresponding authors

Corresponding authors

Shuhua Yi

State Key Laboratory of Experimental Hematology, National Clinical Research Center for Blood Diseases, Haihe Laboratory of Cell Ecosystem, Institute of Hematology & Blood Diseases Hospital, Chinese Academy of Medical Sciences & Peking Union Medical College, Tianjin, China

Tianjin Institutes of Health Science, Tianjin, China

Email: yishuhua@ihcams.ac.cn

Liang Wang

School of Clinical Medicine, Shandong Second Medical University, Weifang, China

Shengli Oilfield Central Hospital, Shandong, China

Email: wangliang235@gmail.com

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding authors.

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Authorship Contributions

Y. Yan and Y. Xia conceived and designed the study, acquired data, analyzed and interpreted data, and drafted and critically revised the manuscript. R. Cui analyzed the data and revised the manuscript. W. Chen, T. Wang, G. An and D. Zou critically revised the manuscript for important intellectual content and approved the final version. Y. Yu and W. Xiong acquired data and approved the final version. L. Wang and S. Yi conceived and designed the study, analyzed and interpreted data, critically revised the manuscript, and approved the final version.

Conflict-of-interest disclosure

The authors declare no competing financial interests.

Abstract

Approximately 5%-10% of patients with chronic lymphocytic leukemia (CLL) develop autoimmune hemolytic anemia (AIHA). However, its pathogenesis and prognostic significance remain heterogeneous and incompletely defined. This study investigated the clinical and molecular features of CLL-associated AIHA and aimed to clarify its prognostic impact. We retrospectively analyzed baseline characteristics, first-line treatments, and survival outcomes in 1,404 patients with CLL. The incidence of AIHA was 10.4%, with 69.2% of cases classified as warm-antibody AIHA (wAIHA). CLL patients with AIHA were characterized by male predominance, advanced disease stage, IGHV4-34 usage, and other adverse biological features. Among the tested genes, *DNMT3A* mutations were more frequent in patients with AIHA, while *MYD88* mutations were enriched in cold-antibody AIHA (cAIHA). Although AIHA conferred a significantly adverse prognostic impact on CLL outcomes, this effect was markedly attenuated with targeted therapies. Unmutated IGHV status predicted inferior outcomes in the overall CLL cohort, but not among patients with AIHA. Our findings underscore the importance of routine AIHA screening in high-risk CLL and support consideration of targeted therapies to mitigate the adverse impact of AIHA on long-term survival.

Keywords: Chronic lymphocytic leukemia; Autoimmune hemolytic anemia; IGHV; IGHV4-34; *DNMT3A*; *MYD88*; Targeted therapy

Introduction

Chronic lymphocytic leukemia (CLL) is characterized by the progressive accumulation of monoclonal CD5+ B lymphocytes and by profound immune dysregulation.[1-3] In this context, autoimmune hemolytic anemia (AIHA) is among the most clinically significant autoimmune complications and occurs in approximately 5%-10% of patients.[4-8] Previous studies have shown that CLL patients with AIHA more often present with advanced disease and adverse biological features,[4, 7, 9, 10] suggesting that AIHA may mark a more aggressive and immunologically active disease state. Although AIHA has been regarded as a marker of adverse prognosis in CLL, its biological basis and prognostic significance in the era of targeted therapy remain incompletely understood.

Recent therapeutic advances have substantially changed the management of CLL. In particular, targeted agents such as Bruton tyrosine kinase inhibitors (BTKi) and BCL2 inhibitors have improved disease control and may also modify the clinical course of autoimmune complications.[11-16] The historical link between AIHA and inferior outcomes therefore warrants re-evaluation. Moreover, large studies of CLL-associated AIHA have been conducted in Western populations.[17] Asian CLL cohorts, however, exhibit distinct immunogenetic and molecular features, including a higher frequency of MYD88 mutations and differences in IGHV repertoire usage.[18-20] Whether these differences shape the biology of AIHA in Asian patients has not been systematically examined.

To address these gaps, we conducted a retrospective analysis of 1,404 CLL patients from a single institution in China. We characterized the incidence, clinical and immunogenetic features, somatic mutation landscape, and prognostic relevance of AIHA. We further examined whether established CLL risk markers retain their prognostic value in patients with AIHA, and whether targeted therapies modify the adverse impact of AIHA on long-term outcomes.

Methods

Study design and patients

We retrospectively identified 1,404 consecutive patients with CLL who were evaluated at the Institute of Hematology, Chinese Academy of Medical Sciences, between June 1994 and June 2024. The protocol was approved by the institutional ethics committee, and informed consent was obtained in accordance with the Declaration of Helsinki. All patients underwent direct antiglobulin test (DAT) and/or cold agglutinin testing. The patient selection process is shown in Supplementary Figure S1. Clinical and laboratory data collected included age, sex, hemoglobin, platelet count, white blood cell count, lactate dehydrogenase, β2-microglobulin, albumin, immunoglobulin levels, immunofixation electrophoresis, cytogenetic abnormalities, IGHV mutational status, treatment, and survival outcomes. Patients receiving therapy were assessed approximately monthly for disease status and treatment indications, whereas those managed with a watch-and-wait strategy were followed every 3 months. First-line therapies were categorized as chemotherapy only, chemoimmunotherapy, or targeted therapy.

Definitions of CLL, AIHA, and AIHA subtypes

The diagnosis of CLL was established strictly in accordance with the iwCLL diagnostic criteria.[11]

Diagnostic evaluation included immunophenotyping, conventional karyotyping, fluorescence in situ hybridization (FISH) for del(17p13), del(13q14.3), trisomy 12, and del(11q22), and IGHV somatic hypermutation analysis when samples were available. IGHV mutational status was defined as unmutated (UM) at ≥98% germline identity and mutated (M) at <98%. In patients with atypical features suggestive of Waldenström macroglobulinemia/lymphoplasmacytic lymphoma (WM/LPL), particularly elevated serum IgM levels and/or MYD88 mutations, additional studies were performed to exclude WM/LPL. These included bone marrow biopsy with morphologic review and immunohistochemical analysis, flow-cytometric immunophenotyping, serum immunofixation electrophoresis, quantitative immunoglobulin testing, and clinicopathologic correlation. The immunophenotypic features of patients with elevated IgM levels and/or MYD88 mutations are summarized in Supplementary Table S1. After exclusion of findings supporting WM/LPL, the diagnosis of CLL was confirmed according to iwCLL criteria. Detailed laboratory procedures are provided in the Supplementary Methods.

AIHA in patients with CLL was defined according to the following criteria:[13, 21, 22] (1) hemoglobin ≤110 g/L without chemotherapy in the prior month, excluding other causes of anemia (including iron/vitamin deficiency, occult bleeding, chronic inflammation, and sepsis); (2) a positive DAT and/or cold agglutinin test; (3) at least one laboratory feature consistent with hemolysis (elevated total bilirubin ≥17.1 μmol/L, predominantly unconjugated; elevated LDH; reduced haptoglobin <250 mg/L; reticulocyte percentage >4% or absolute reticulocyte count >120×10^9/L). AIHA was subclassified as wAIHA (a negative cold agglutinin test; a positive DAT for IgG or IgG+C3d, less commonly IgA) or cAIHA (a positive cold agglutinin test; DAT typically C3d positive). Patients were further categorized into three subgroups according to the timing of AIHA relative to CLL diagnosis: (1) concurrent, defined as AIHA diagnosed within 1 month of CLL diagnosis; (2) preceding CLL, defined as AIHA diagnosed more than 1 month before CLL diagnosis, after exclusion of alternative causes; (3) following CLL, defined as AIHA diagnosed more than 1 month after CLL diagnosis, irrespective of ongoing CLL-directed therapy, including BTKi.[5, 23]

Statistical analysis

Time to first treatment (TTFT) was defined as the interval from diagnosis to initiation of first-line therapy. Progression-free survival (PFS) was defined as the interval from treatment initiation to disease progression, relapse, death from any cause, or last follow-up. Overall survival (OS) was defined as the interval from treatment initiation to death from any cause or last follow-up. Data were analyzed using SPSS Statistics (v25) and GraphPad Prism (v10). Continuous variables are presented as median (range) or mean ± standard deviation, and categorical variables as counts and percentages. Continuous variables were compared using the independent-samples *t* test or Wilcoxon rank-sum test, and categorical variables using the Chi-square test or Fisher's exact test, as appropriate. TTFT, PFS, and OS were estimated by the Kaplan-Meier method and compared with the log-rank test. A two-tailed $P \leq 0.05$ was considered statistically significant.

Results

Baseline characteristics

Among 1,404 patients with CLL, 146 (10.4%) developed AIHA, including 101 (69.2%) with wAIHA and 45 (30.8%) with cAIHA. AIHA was concurrent with CLL diagnosis in 63 patients (43.2%),

preceding CLL in 2 (1.4%; median 50 months earlier), and following CLL in 81 (55.5%; median 25 months later). The median cohort follow-up was 45.5 months.

Compared with patients without AIHA, those with AIHA were more often male and presented with a more advanced disease phenotype (Table 1). They more frequently had advanced Rai stage (III–IV) and Binet stage C, as well as splenomegaly and greater nodal burden. AIHA was also associated with higher β2-microglobulin and IgM levels, more frequent positive immunofixation, and lower albumin levels. In contrast, trisomy 12 was less frequent in the AIHA group, whereas age at diagnosis and IGHV mutational status did not differ significantly between the two groups.

We further compared baseline characteristics between the wAIHA and cAIHA subgroups. Overall, the two subgroups shared a broadly similar adverse clinical profile. However, wAIHA was associated with more frequent extreme leukocytosis, whereas cAIHA tended to cluster in lower-risk CLL-IPI categories and showed higher serum IgM levels (Supplementary Table S2).

Patient disposition and follow-up

In the overall cohort of 1,404 patients who underwent DAT and/or cold agglutinin testing, 313 (22.3%) were lost to follow-up. During follow-up, 518 patients (36.9%) experienced disease progression and 345 (24.6%) died, these categories were not mutually exclusive. The remaining patients were alive and in continued follow-up without documented progression at the time of data cut-off. CLL-related causes accounted for the majority of these deaths (203/345, 58.8%), with the remaining causes detailed in Supplementary Table S3.

IGHV mutational status and repertoire

Among the 146 AIHA patients, IGHV mutational status was successfully determined in 89 patients (61.0%), and unambiguous IGHV gene segment assignment was obtained in 87 patients (59.6%). No significant difference in overall IGHV mutational status was observed between AIHA and non-AIHA patients (Supplementary Figure S2A, B). However, AIHA cases showed a relative enrichment in the lowest IGHV mutation-load category (≤ 2.00%), whereas non-AIHA cases more often exhibited intermediate mutation levels (5.01%-10.00%; Supplementary Figure S2C). Notably, IGHV4-34 usage was significantly enriched in patients with AIHA, particularly in those with cAIHA (Figure 1A, B). In contrast, IGHD/IGHJ gene usage and CDR3 length did not differ by AIHA status (Supplementary Figure S3). Among the 87 AIHA patients with evaluable IGHV gene segment data, 19 (21.8%) used IGHV4-34, and these IGHV4-34-positive cases were predominantly M-IGHV and tended to fall into lower CLL-IPI risk categories (Supplementary Table S4).

Somatic mutations in CLL with AIHA

Targeted next-generation sequencing (NGS) data were available in 683 CLL patients, including 56 with AIHA and 627 without AIHA. Overall, the most frequently mutated genes in our CLL cohort were *TP53, MYD88, NOTCH1, SF3B1,* and *ATM*, consistent with the established mutational landscape of CLL.[19] Most gene alterations did not differ significantly between patients with and without AIHA. However, *DNMT3A* mutations were more frequent in the AIHA group (P = 0.046; Figure 2A). As NGS was performed on unsorted peripheral blood or bone marrow samples and the observed *DNMT3A variant allele frequencies* (VAFs) were generally low (Supplementary Table S5), we cannot exclude the possibility that these mutations represent clonal hematopoiesis of

indeterminate potential (CHIP) rather than CLL-specific alterations. Accordingly, this association should be regarded as hypothesis-generating and warrants validation in studies using purified cell populations. In the subtype analysis, *MYD88* mutations were significantly more common in patients with cAIHA compared to wAIHA ($P = 0.019$), suggesting distinct molecular drivers across AIHA subtypes (Figure 2B).

Impact of AIHA on treatment timing and survival outcomes

We next evaluated the impact of AIHA on clinical outcomes in CLL. As expected, patients with AIHA had a shorter TTFT than those without AIHA (median TTFT: 1.7 months vs 10.2 months). More importantly, AIHA was also associated with inferior survival, including shorter PFS (median PFS: 42.7 vs 49.2 months; $P = 0.009$; Figure 3A) and OS (median OS: 70.6 vs 105.4 months; $P < 0.001$; Figure 3B).

Subgroup analysis based on the timing of AIHA diagnosis demonstrated that patients with concurrent CLL and AIHA had the poorest outcomes, with shorter TTFT (median TTFT: 0.3 months vs 10.7 months, $P = 0.001$) and OS (median OS: 48.0 months vs 82.0 months, $P = 0.017$) compared with those who developed AIHA after CLL diagnosis. However, PFS was similar between the two groups (Figure 3C-D). No significant differences in survival were observed between patients with wAIHA and cAIHA (Figure 4A-C).

Given that AIHA frequently constitutes a direct indication for CLL-directed therapy, we further examined the reasons for treatment initiation among patients without AIHA. In this group, treatment initiation largely followed iwCLL guidelines, most commonly due to progressive lymphocytosis or high tumor burden (Supplementary Table S6).

As expected, UM-IGHV status was associated with inferior TTFT (median TTFT: 2.3 months vs 17.1 months, $P < 0.001$), PFS (median PFS: 38.9 months vs 60.9 months, $P < 0.001$), and OS (median OS: 82.4 months vs 117.8 months, $P = 0.024$) in the overall cohort. In contrast, in the subgroup of patients with AIHA, IGHV status did not retain prognostic significance for all three survival endpoints. Among patients without AIHA, however, UM-IGHV status remained a strong predictor of adverse outcomes (Supplementary Figure S4). Similarly, IGHV4-34 gene usage did not significantly impact TTFT, PFS, or OS in patients with AIHA (Supplementary Figure S5). These findings suggest that, in the presence of AIHA, the prognostic stratification provided by IGHV status may be less informative.

Impact of treatment modality on CLL outcomes

We next assessed outcomes according to first-line treatment modality. Among patients treated with conventional chemotherapy or chemoimmunotherapy, the presence of AIHA was associated with inferior PFS and OS. By contrast, among patients receiving targeted therapy, neither PFS nor OS differed significantly according to AIHA status (Figure 5A-F). These results indicate that targeted agents may mitigate the adverse prognostic impact of AIHA. Response data after first-line therapy were available for 619 patients. Overall, 20.7% achieved complete remission (CR) and 56.9% achieved partial remission (PR). Among the 115 treated patients with AIHA, responses were predominantly partial, with 59 patients (51.3%) achieving PR (Supplementary Figure S6A,B). In addition, a small subset of patients with AIHA (14/146, 9.6%) received corticosteroids alone as initial therapy, achieving an overall AIHA response rate of 64.3% and a median first remission

duration of 13.2 months. The decision to attempt corticosteroid therapy alone was based on treating-physician judgment rather than on predefined clinical or laboratory criteria; accordingly, this should not be interpreted as reflecting the proportion of AIHA patients who would have been expected to respond to steroids.

When stratified by first-line treatment modality, targeted therapy produced the highest response rates in both patients with and without AIHA, followed by chemoimmunotherapy and chemotherapy alone. In patients with AIHA, the ORR was 92.9% with targeted therapy, 78.6% with chemoimmunotherapy, and 51.1% with chemotherapy (Supplementary Table S7).

AIHA outcomes during treatment and relapse

We evaluated the AIHA response in 109 AIHA patients for whom detailed hematologic follow-up was available. Following CLL-directed and supportive treatment, AIHA responses were predominantly PR or NR (Supplementary Figure S6C). Notably, a close association was observed between the effectiveness of CLL treatment and the control of AIHA (Supplementary Figure S7). Among the AIHA patients who achieved a CR of CLL, 87.0% also achieved CR of AIHA. By contrast, among patients with poor CLL responses, a substantial proportion (46.7%) continued to exhibit refractory hemolysis. These findings indicate that the depth of CLL response is a key determinant of AIHA control, thereby motivating further analyses stratified by treatment modality.

We further examined the dynamics of AIHA over the disease course, with particular attention to CLL relapse. AIHA recurrence at relapse increased stepwise across successive lines of therapy, rising from 7.7% after first-line treatment to 11.8% after second-line treatment and 16.7% after third-line or later treatment (Supplementary Table S8). When analyzed by treatment regimen, targeted therapy-based regimens were associated with the most favorable control of AIHA, followed by chemoimmunotherapy, whereas chemotherapy-based regimens showed the least favorable outcomes (Supplementary Table S9). Overall, these findings indicate that more effective CLL-directed regimens, particularly targeted therapies, are associated with improved control of secondary autoimmune hemolysis.

Discussion

In this large single-center retrospective study of 1,404 Chinese patients with CLL, we characterized the incidence and the clinical, molecular, and prognostic features of AIHA. We observed an AIHA incidence of 10.4%, consistent with the 5-10% rate reported in Western populations,[5, 8, 24] indicating a comparable prevalence of this complication across different ethnic groups. Importantly, we also found that AIHA was associated with distinct molecular features, had adverse prognostic significance in the pre-targeted therapy era, and that its negative impact appeared to be attenuated in patients treated with novel agents.

CLL with AIHA was characterized by more aggressive clinical features, including advanced stage, higher tumor burden, elevated β2-microglobulin, hypoalbuminemia, and male predominance. These findings are consistent with previous Western cohorts.[8, 25] By contrast, AIHA was not associated with IGHV mutational status or with classic adverse cytogenetic abnormalities, such as del(11q) and del(17p), whereas trisomy 12 was less frequent in patients with AIHA. These observations suggest that the development of AIHA may be shaped less by conventional genetic risk features and more by immune dysregulation and selected biologic pathways. Notably, 33% of

patients in this study exhibited no significant abnormalities on FISH analysis, which may also reflect known differences in the genetic landscape of CLL between East Asian and Western populations.[26]

IGHV4-34 emerged as a key immunogenetic marker of AIHA susceptibility, particularly in the cold-antibody subtype. IGHV4-34 is well recognized as an inherently autoreactive immunoglobulin gene, and antibodies encoded by IGHV4-34 can intrinsically bind I/i carbohydrate antigens on red blood cells.[22, 27] In the dysregulated immune environment of CLL, such clones may escape tolerance and contribute to the development of AIHA.[28] This is biologically consistent with primary cold agglutinin disease, in which pathogenic cold agglutinins commonly use IGHV4-34.[29, 30] Notably, the enrichment of IGHV4-34 within M-IGHV cases suggests that somatic hypermutation may not fully eliminate its intrinsic autoreactive potential.[9, 31, 32]

Our mutation analysis highlighted *DNMT3A* mutations as being more frequent in patients with CLL-associated AIHA. *DNMT3A* mutations are common in clonal hematopoiesis and certain myeloid malignancies, but are relatively uncommon in CLL.[33] In our cohort, *DNMT3A* mutations were characterized by low VAFs, favoring an origin from clonal hematopoiesis rather than the leukemic CLL clone. Accordingly, this association may reflect age-related clonal hematopoiesis in an elderly population, rather than a direct pathogenic role in CLL. Nevertheless, given the links between *DNMT3A* dysfunction, epigenetic dysregulation, and immune imbalance,[34-36] an indirect contribution to autoimmune susceptibility cannot be excluded. In light of the limited number of *DNMT3A*-mutated cases and the low VAFs observed, these findings should be interpreted cautiously.

We also observed an enrichment of *MYD88* mutations in cAIHA. *MYD88* is a central adaptor in Toll-like receptor signaling and is more frequently mutated in Chinese patients with CLL than in Western CLL cohorts.[19, 37] Our findings raise the possibility that MYD88-mutant CLL may be associated with a more immunologically active phenotype that predisposes to cold-antibody hemolysis. Given the role of MYD88 in NF-κB activation and downstream inflammatory signaling, this association is biologically plausible.[38, 39] However, the mechanistic implications of this association remain speculative, particularly given the limited number of affected cases, and require validation in larger studies.

IGHV mutational status, a cornerstone of CLL risk stratification, showed attenuated prognostic significance in our AIHA cohort. This masking effect suggests that, in the presence of AIHA, disease progression may be driven more by immunological and microenvironmental factors than by the intrinsic genetic features of the tumor clone, underscoring the need for AIHA-specific risk assessment tools in this patient subgroup.

AIHA was an adverse prognostic factor in CLL patients treated with conventional regimens, confirming earlier literature.[5, 7, 17] Our data further suggest that the timing of AIHA is clinically relevant. Patients who presented with AIHA at the time of CLL diagnosis experienced the poorest outcomes, consistent with prior reports identifying concurrent AIHA as a high-risk feature.[7, 24] Compared with primary (idiopathic) AIHA,[21, 40] CLL-associated AIHA may respond less favorably to standard immunosuppressive treatment, likely because the autoimmune process is sustained by the leukemic clone itself. Although corticosteroids may provide initial symptomatic control in

selected patients,[11] the decision to attempt steroid-only therapy in this retrospective cohort was based on treating-physician judgment rather than predefined criteria. Therefore, the low proportion of patients managed initially with corticosteroids alone should not be interpreted as indicating that the remaining patients were necessarily unlikely to respond to steroids. This also represents a limitation in the interpretation of TTFT, which may have been influenced in part by investigator-dependent treatment decisions.

Notably, although AIHA was associated with inferior survival in the pre-targeted therapy era, this adverse prognostic effect appeared to be attenuated in patients treated with targeted therapies. Novel agents may benefit these patients by simultaneously controlling the leukemic clone and improving immune dysregulation. This observation is consistent with previous studies showing that BTK inhibitors rarely induce new-onset AIHA and can lead to resolution of pre-existing AIHA or ITP in many patients.[14, 16, 41, 42] In our cohort, achievement of deeper CLL responses was also closely associated with improved AIHA control. Together, these findings support the concept that effective control of the leukemic clone is central to long-term control of CLL-associated AIHA and provide real-world evidence supporting the use of targeted therapies in this setting.

In conclusion, this large single-center study characterizes the clinical, immunogenetic, and prognostic features of AIHA in Chinese patients with CLL. We demonstrate that AIHA is associated with advanced disease, IGHV4-34 usage, and *MYD88* mutations in the cold-antibody subtype. While AIHA confers an adverse prognosis in patients treated with conventional therapies, this disadvantage is largely abrogated with targeted agents. These findings support the preferential use of targeted therapies in CLL patients with AIHA and highlight the need for routine AIHA screening in high-risk populations. Future studies using purified cell populations and functional assays are warranted to elucidate the mechanistic links between specific immunogenetic features and autoimmune pathogenesis in CLL.

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Table 1. The clinical and biological characteristics at diagnosis in CLL patients with or without AIHA.

Characteristics, n (%)	Without AIHA (n=1258)	With AIHA (n=146)	P-value
Male/Female	805/449 (64.2%)	108/38 (74.0%)	0.019
Age at diagnosis, mean ± SD	59.4 ± 10.5	59.7 ± 9.7	0.780
Age >65 years	367/1250 (29.4%)	39/146 (26.7%)	0.505
Splenomegaly	648/988 (65.6%)	109/132 (82.6%)	<0.001
Region of lymph node enlargement ≥3	242/438 (55.3%)	66/83 (79.5%)	<0.001
WBC ≥100x10^9/L	142/1174 (12.1%)	26/144 (18.1%)	0.043
ALB <35 g/L	74/1137 (6.5%)	21/145 (14.5%)	0.001
β2-microglobulin >3.5 mg/L	390/924 (42.2%)	52/91 (57.1%)	0.006
IgA >4 g/L	26/1063 (2.4%)	4/113 (3.5%)	0.698
IgM >3.04 g/L	38/1063 (3.6%)	12/112 (10.7%)	0.001
IgG >15.6 g/L	137/1066 (12.9%)	15/117 (12.8%)	0.992
Positive Immunofixation Electrophoresis	104/617 (16.9%)	24/74 (32.4%)	0.001
FISH examination			
Del (17p)	111/1011 (11.0%)	14/134 (10.4%)	0.853
Del (11q)	119/967 (12.3%)	8/115 (7.0%)	0.092
Del (13q)	166/718 (23.1%)	23/110 (20.9%)	0.607
+12	168/797 (21.1%)	10/86 (11.6%)	0.038
With mutated IGHV	388/589 (65.9%)	53/89 (59.6%)	0.244
Abnormal karyotype	482/1028 (46.9%)	54/130 (41.5%)	0.249
Rai stage			<0.001
0	145/1108 (13.1%)	6/134 (4.5%)	
□	312/1108 (28.2%)	14/134 (10.4%)	
□	243/1108 (21.9%)	23/134 (17.2%)	
□	137/1108 (12.4%)	54/134 (40.3%)	
□	271/1108 (24.5%)	37/134 (27.6%)	
Binet stage			<0.001
A	248/878 (28.2%)	15/113 (13.3%)	
B	323/878 (36.8%)	28/113 (24.8%)	
C	307/878 (35.0%)	70/113 (61.9%)	
CLL-IPI stage			0.152
Low	367/903 (40.6%)	30/97 (30.9%)	
Intermediate	286/903 (31.7%)	35/97 (36.1%)	
High	168/903 (18.6%)	25/97 (25.8%)	
Very high	82/903 (9.1%)	7/97 (7.2%)	

Abbreviation: CLL Chronic Lymphocytic Leukemia; AIHA Autoimmune hemolytic anemia; CLL-IPI Chronic Lymphocytic Leukemia International Prognostic Index; WBC White Blood Cell count; ALB

Albumin; FISH Fluorescence In Situ Hybridization; IGHV Immunoglobulin Heavy Variable.

Table legend

Table 1. The clinical and biological characteristics at diagnosis in CLL patients with or without AIHA.

Figure legends

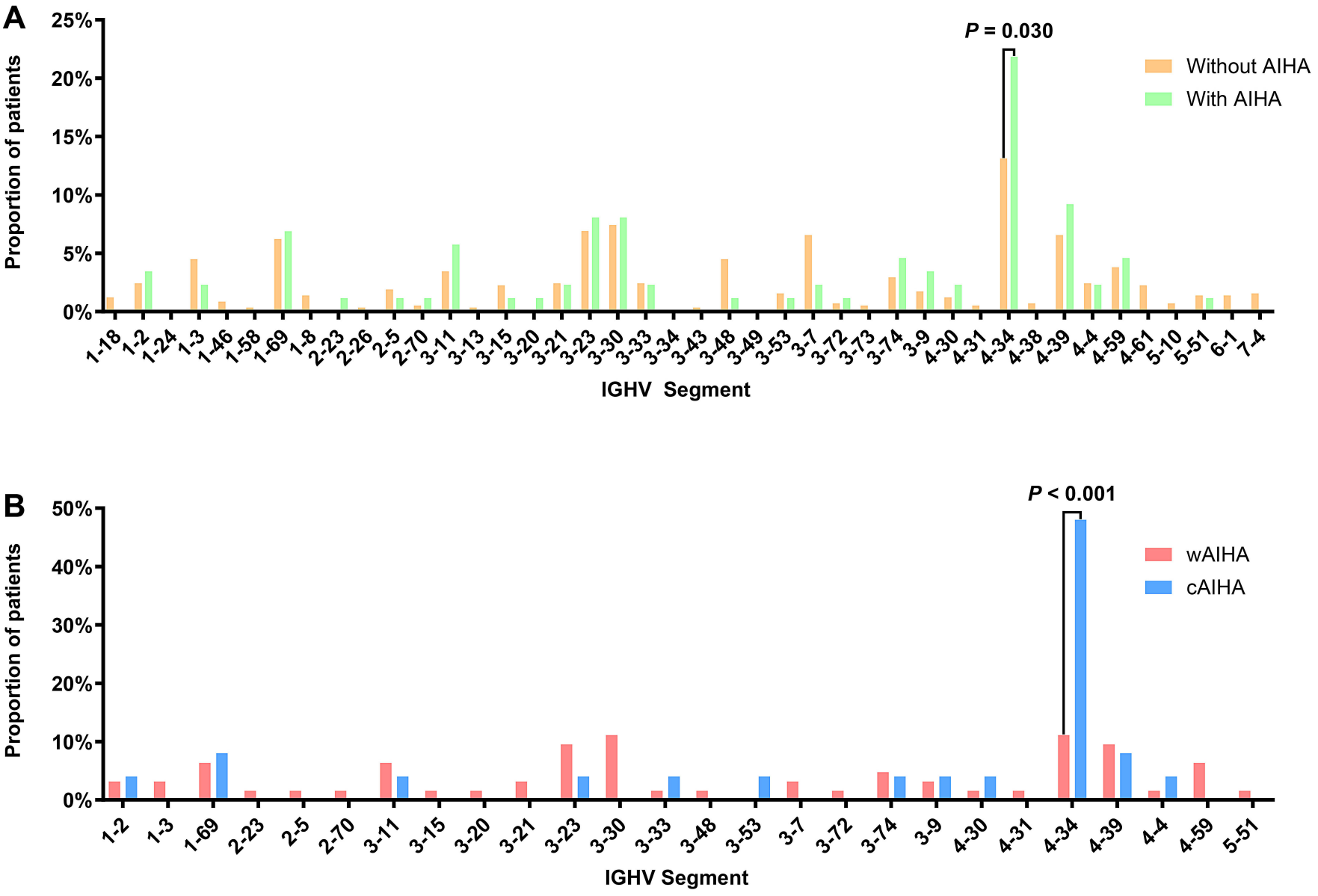
Figure 1. Proportions of IGHV segments in CLL patients with different AIHA-related statuses. (A) Proportions of IGHV Segments in CLL Patients with and without AIHA. (B) Proportions of IGHV Segments in CLL Patients with wAIHA and cAIHA.

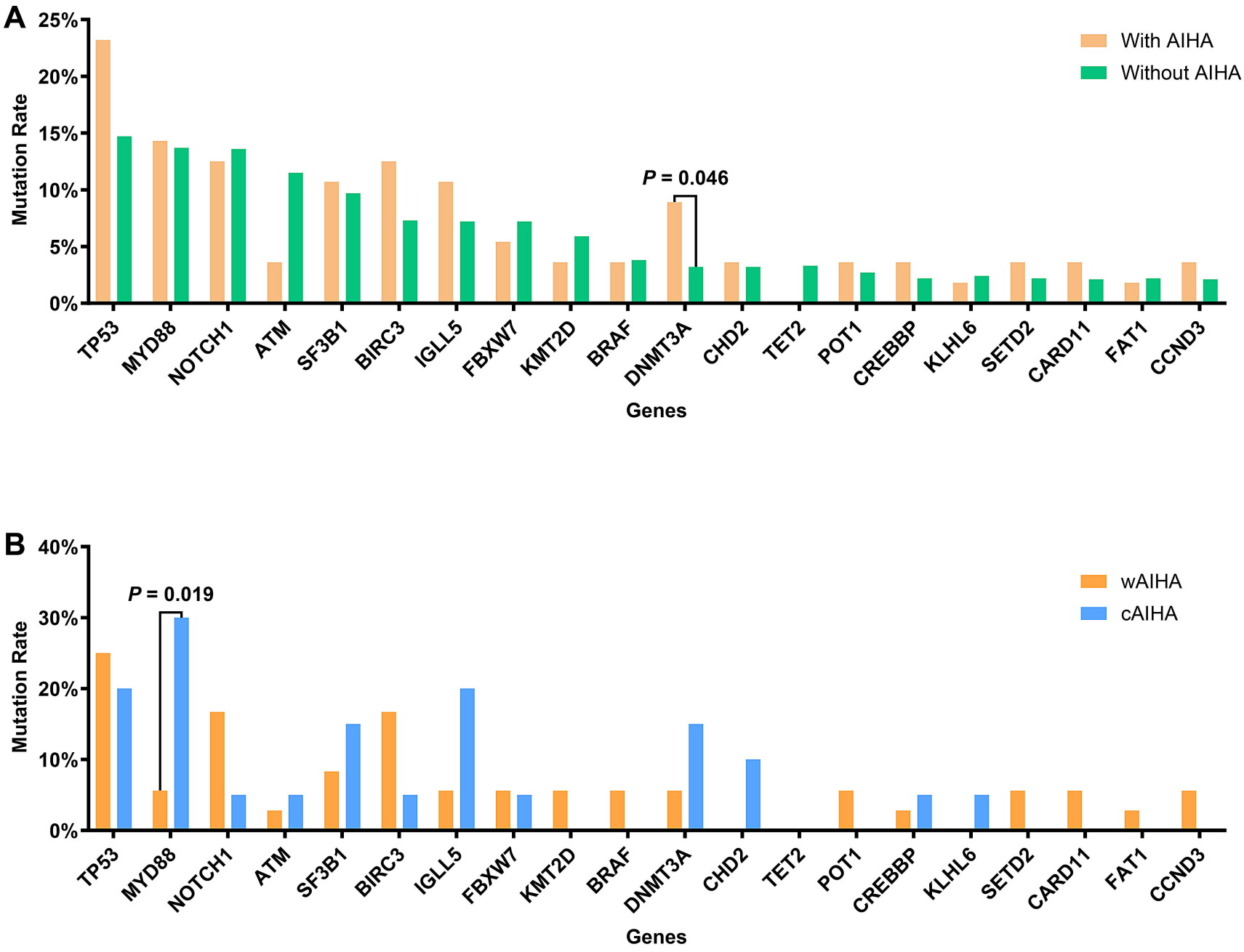
Figure 2. Analysis of gene mutations associated with AIHA in CLL. (A) Comparison of gene mutation rates in CLL patients with and without AIHA. (B) Comparison of gene mutation rates in CLL patients with warm-antibody versus cold-antibody AIHA.

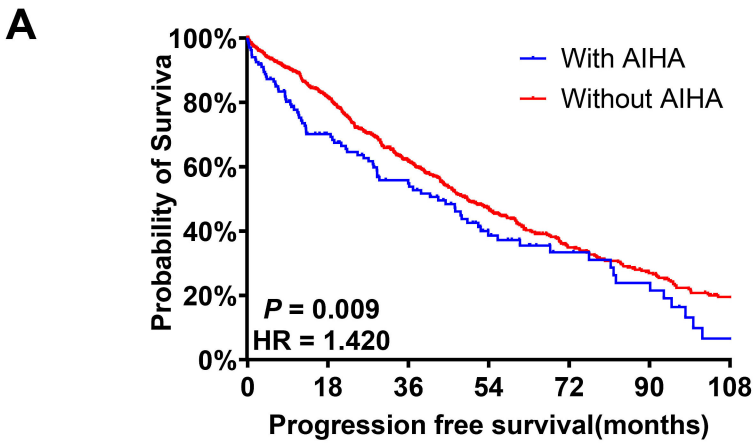
Figure 3. Survival analysis of CLL complicated by AIHA. Progression-free survival (A) and overall survival (B) in CLL patients with and without AIHA. Progression-free survival (C) and overall survival (D) based on the temporal relationship between CLL and AIHA diagnosis.

Figure 4. Survival analysis of patients with different types of AIHA. Time to first treatment (A), progression-free survival (B) and overall survival (C) in patients with cAIHA versus wAIHA.

Figure 5. Survival analysis of different treatment modalities in CLL patients with or without AIHA. (A) Progression-free survival for patients treated with chemotherapy. (B) Overall survival for patients treated with chemotherapy. (C) Progression-free survival for patients treated with chemoimmunotherapy. (D) Overall survival for patients treated with chemoimmunotherapy. (E) Progression-free survival for patients treated with targeted therapy. (F) Overall survival for patients treated with targeted therapy.

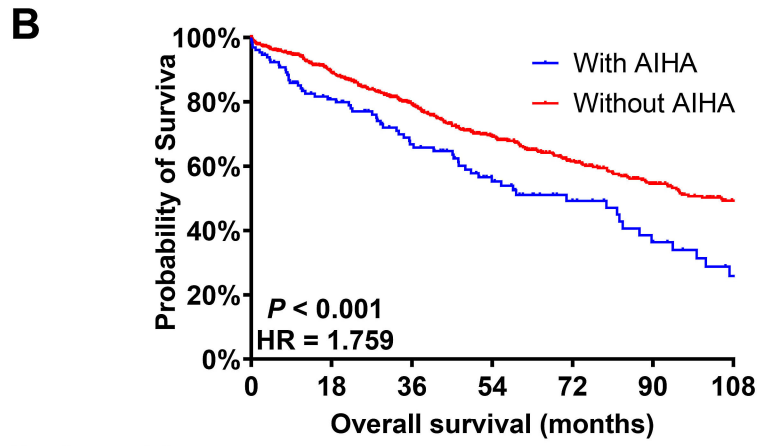

A
Proportion of patients
25%
20%
15%
10%
5%
0%
P = 0.030
Without AIHA
With AIHA
1-18 1-2 1-24 1-3 1-46 1-58 1-69 1-8 2-23 2-26 2-5 2-70 3-11 3-13 3-15 3-20 3-21 3-23 3-30 3-33 3-34 3-43 3-48 3-49 3-53 3-7 3-72 3-73 3-74 3-9 4-30 4-31 4-34 4-38 4-39 4-4 4-59 4-61 5-10 5-51 6-1 7-4
IGHV Segment
B
Proportion of patients
50%
40%
30%
20%
10%
0%
P < 0.001
wAIHA
cAIHA
1-2 1-3 1-69 2-23 2-5 2-70 3-11 3-15 3-20 3-21 3-23 3-30 3-33 3-48 3-53 3-7 3-72 3-74 3-9 4-30 4-31 4-34 4-39 4-4 4-59 5-51
IGHV Segment


A
25%
20%
15%
10%
5%
0%
Mutation Rate
With AIHA
Without AIHA
P = 0.046
TP53
MYD88
NOTCH1
ATM
SF3B1
BIRC3
IGLL5
FBXW7
KMT2D
BRAF
DNMT3A
CHD2
TET2
POT1
CREBBP
KLHL6
SETD2
CARD11
FAT1
CCND3
Genes
B
40%
30%
20%
10%
0%
Mutation Rate
wAIHA
cAIHA
P = 0.019
TP53
MYD88
NOTCH1
ATM
SF3B1
BIRC3
IGLL5
FBXW7
KMT2D
BRAF
DNMT3A
CHD2
TET2
POT1
CREBBP
KLHL6
SETD2
CARD11
FAT1
CCND3
Genes



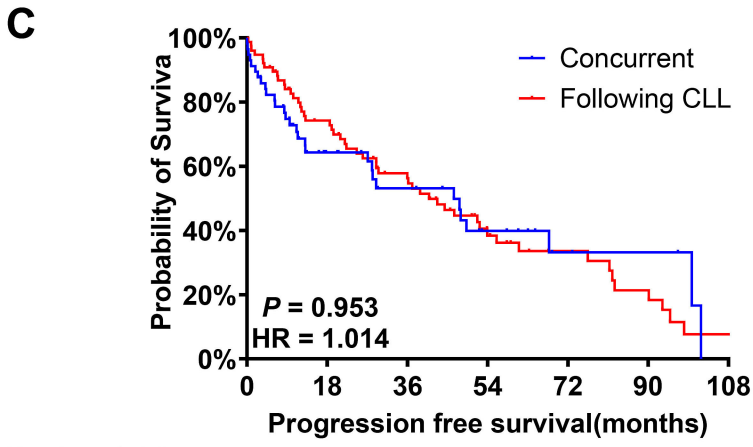
Number at risk

With AIHA	134	78	53	28	15	10	2
Without AIHA	824	512	310	184	114	73	45



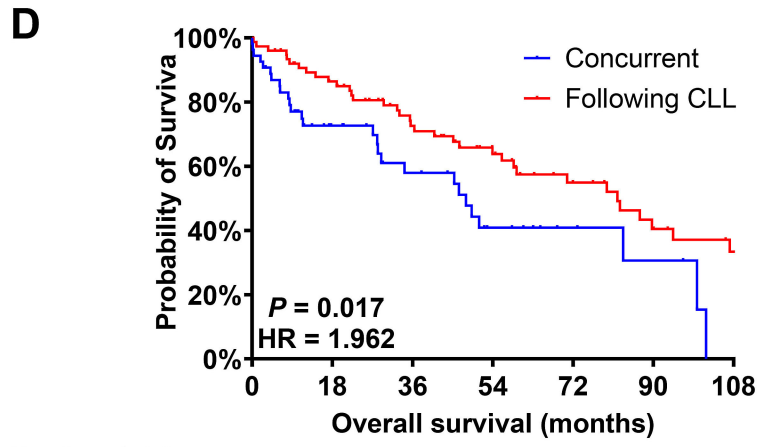
Number at risk

With AIHA	131	89	64	42	26	17	9
Without AIHA	825	558	393	272	196	136	97



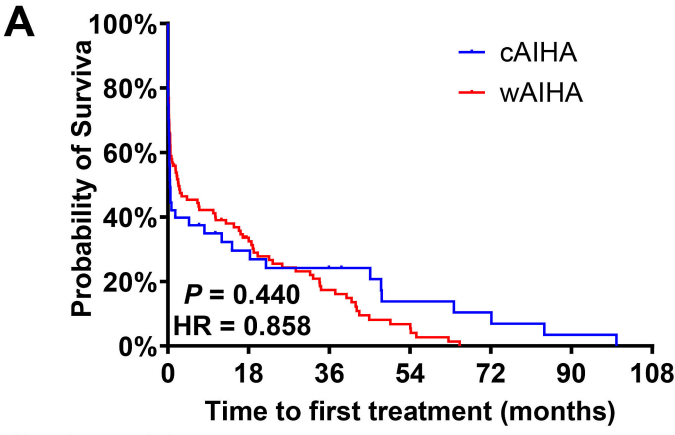
Number at risk

Concurrent	57	26	18	10	4	3	0
Following CLL	76	52	35	18	11	7	2



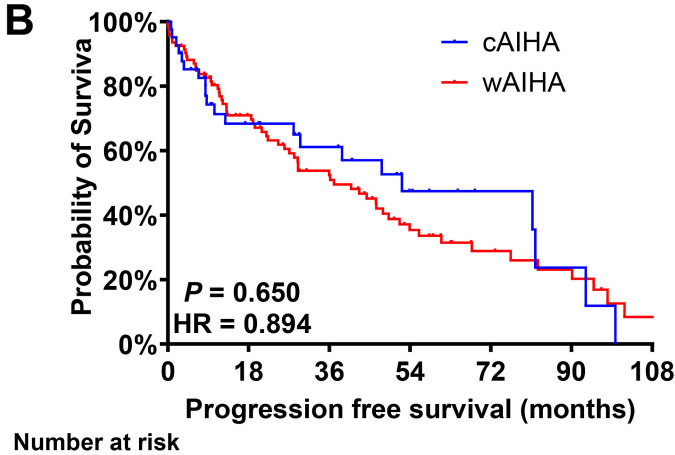
Number at risk

Concurrent	54	28	19	10	5	3	0
Following CLL	76	61	45	32	21	14	9



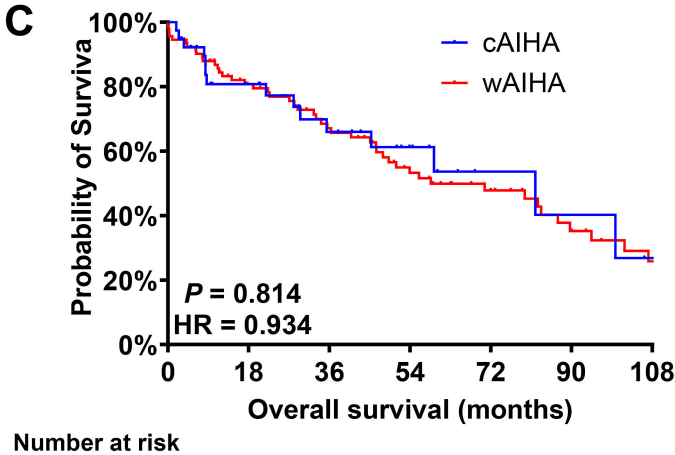
Number at risk

cAIHA	44	11	8	4	3	1	0
wAIHA	100	29	14	5	0	0	0



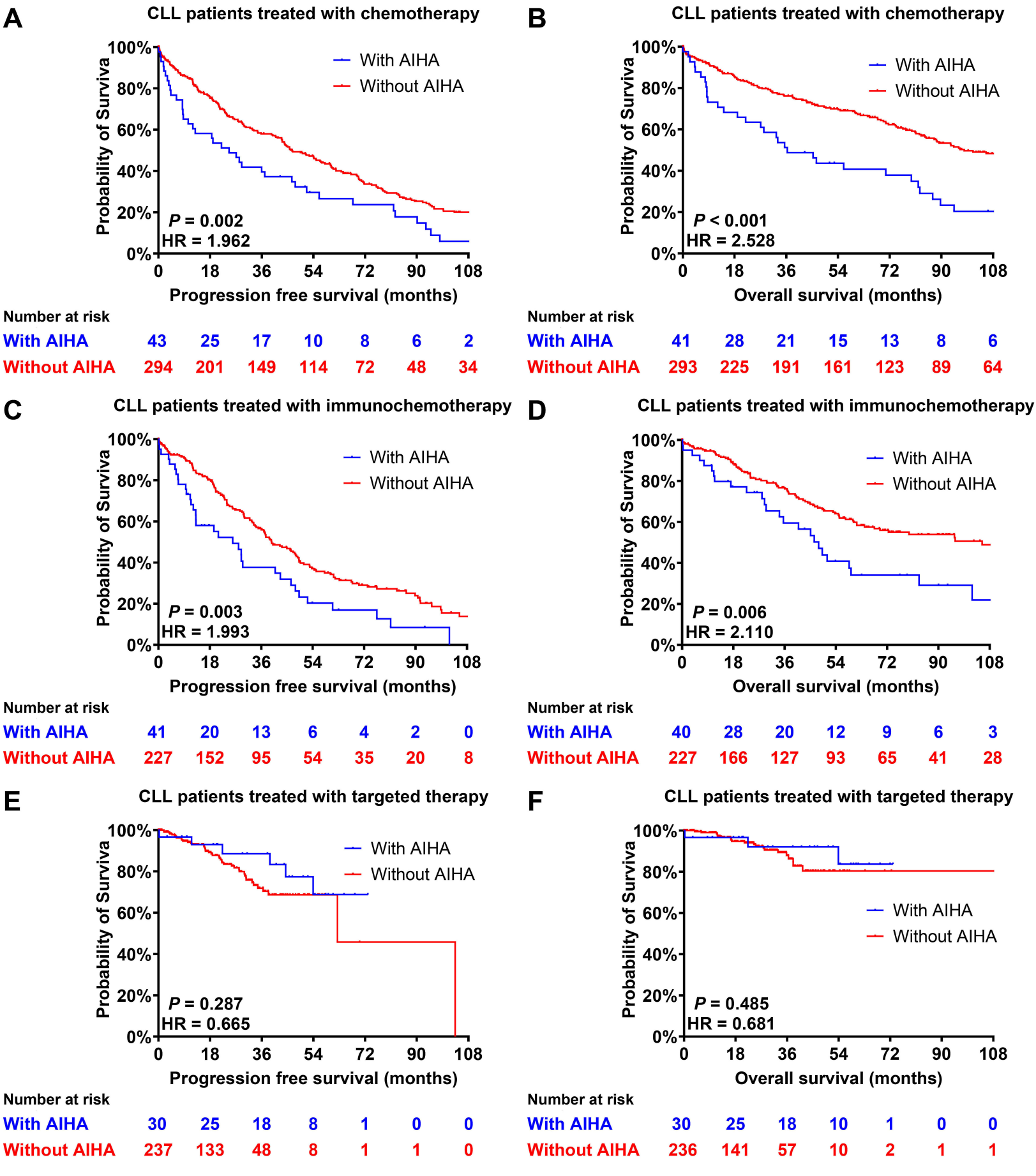
Number at risk

cAIHA	41	22	16	8	4	2	0
wAIHA	93	56	37	20	11	8	2



Number at risk

cAIHA	39	25	17	10	4	3	1
wAIHA	92	64	47	32	22	14	8


A
CLL patients treated with chemotherapy
Probability of Survival
100%
80%
60%
40%
20%
0%
With AIHA
Without AIHA
P = 0.002
HR = 1.962
0
18
36
54
72
90
108
Progression free survival (months)
Number at risk
With AIHA 43 25 17 10 8 6 2
Without AIHA 294 201 149 114 72 48 34
B
CLL patients treated with chemotherapy
Probability of Survival
With AIHA
Without AIHA
P < 0.001
HR = 2.528
Overall survival (months)
Number at risk
With AIHA 41 28 21 15 13 8 6
Without AIHA 293 225 191 161 123 89 64
C
CLL patients treated with immunochemotherapy
Probability of Survival
With AIHA
Without AIHA
P = 0.003
HR = 1.993
Progression free survival (months)
Number at risk
With AIHA 41 20 13 6 4 2 0
Without AIHA 227 152 95 54 35 20 8
D
CLL patients treated with immunochemotherapy
Probability of Survival
With AIHA
Without AIHA
P = 0.006
HR = 2.110
Overall survival (months)
Number at risk
With AIHA 40 28 20 12 9 6 3
Without AIHA 227 166 127 93 65 41 28
E
CLL patients treated with targeted therapy
Probability of Survival
With AIHA
Without AIHA
P = 0.287
HR = 0.665
Progression free survival (months)
Number at risk
With AIHA 30 25 18 8 1 0 0
Without AIHA 237 133 48 8 1 1 0
F
CLL patients treated with targeted therapy
Probability of Survival
With AIHA
Without AIHA
P = 0.485
HR = 0.681
Overall survival (months)
Number at risk
With AIHA 30 25 18 10 1 0 0
Without AIHA 236 141 57 10 2 1 1

Supplementary Data

Supplementary Methods

Patient cohort

The diagnosis of chronic lymphocytic leukemia (CLL) was established strictly in accordance with the iwCLL diagnostic criteria. All cases fulfilled the following requirements: (1) a persistent peripheral blood clonal B-cell count ≥5×10^9/L; (2) characteristic lymphocyte morphology on peripheral blood smear; and (3) a typical CLL immunophenotype confirmed by flow cytometry, including expression of CD19, CD5, and CD23, with dim CD20 and surface immunoglobulin expression, and negativity for markers suggestive of alternative B-cell lymphoproliferative disorders (e.g., CD10 and CD103).

All patients were followed from study entry until the last follow-up or death. In general, patients receiving active treatment were assessed at approximately monthly intervals, whereas patients managed with a watch-and-wait strategy were followed every three months. The duration of observation depended on treatment completion, disease progression, or initiation of subsequent therapy.

Disease status was evaluated through regular physical examinations, focusing on clinical signs of progression such as lymphadenopathy, splenomegaly, or hepatomegaly. Complete blood counts were performed routinely to monitor leukocyte and lymphocyte counts, as well as hemoglobin levels and hemolytic parameters in patients with AIHA. Imaging studies (computed tomography or ultrasound) were performed in selected patients, particularly those with suspected lymphadenopathy or splenomegaly, to assess disease burden or progression. Flow cytometry was used to evaluate CLL immunophenotype during treatment, at the end of therapy, during follow-up, and at relapse; it was also employed for minimal residual disease (MRD) assessment when clinically indicated. Bone marrow aspiration was performed in patients with suspected disease progression or relapse to evaluate marrow involvement.

Treatment regimen

The study included 902 patients who received first-line therapy, 307 patients who received second-line therapy, and 69 patients who received third-line therapy. First-line therapies were classified into chemotherapy, chemoimmunotherapy, and targeted therapy. Chemotherapy regimens included options like Chlorambucil, CHOP/CHOP-like, FC, and Bendamustine. For chemoimmunotherapy, the most commonly used regimens were R-CHOP/R-CHOP-like, FCR, R + Chlorambucil, and BR. In this study, the BTKi inhibitors primarily used were ibrutinib, zanubrutinib, and orelabrutinib, with rituximab almost exclusively administered as the CD20 monoclonal antibody. Venetoclax was predominantly used for BCL2 inhibition.

Immunophenotyping

For sample preparation, bone marrow aspirate fluid was collected in tubes containing a mix of heparin and EDTA. Mononuclear Cells were extracted using the red cell lysis method. Staining was done with precise volume of mononuclear cells at a concentration of 5×10^8/ml. Each

analysis involved loading a total of 20,000 - 50,000 cells. Gating Strategy was CD45/SSC; Patients with available samples were immunophenotyped by FCM using five combinations of eight-color monoclonal antibodies: CD103/ CD25 /CD23 /CD11c /CD200 /CD19 /FMC7 /CD45, CD57 /TCRgd+ CD22 /CD56 /CD5 /CD7 /CD3 /CD16 /CD45, CD26 /CD30 /CD4 /CD45RA /CD45RO /CD8 /CD3 /CD45, Lambda /CD10 /CD5 /CD38 /Kappa /CD20 /CD19 /CD45, CD81 /CD79b /sIgD /CD2 /sIgM /CD19 /CD45. The measurements were performed on a cell analyzer (BD FACSCanto II) after implementation of the EuroFlow Standard Operating Protocol for Instrument Setup and Compensation.

Cytogenetic analysis and FISH

FISH analysis was performed on bone marrow and/or peripheral blood samples obtained at diagnosis or prior to treatment. Interphase FISH was conducted according to standard procedures for CLL. Cells were treated with 0.075 mol/L KCl, fixed in methanol-acetic acid (3:1), and processed for slide preparation. Slides were denatured in 70% formamide/2× standard saline citrate (SSC) at 73°C before hybridization. A CLL-specific FISH probe panel targeting 17p13 (LSI TP53), 13q14.3 (LSI D13S25 and RB1), chromosome 12 centromere (CEP12), and 11q22 (LSI ATM) was applied (Vysis; Abbott Molecular, Downers Grove, IL, USA). Probes were hybridized overnight at 37°C, and post-hybridization processing was performed according to the manufacturer's instructions. Hybridization signals were assessed by fluorescence microscopy, with at least 200 interphase nuclei analyzed per probe. A cutoff value of 10% was used to define FISH positivity. All sample preparation and laboratory procedures followed the manufacturers' instructions and previously published protocols.[1, 2]

IGHV sequencing and analysis

IGHV somatic hypermutation status was assessed using the IGH Somatic Hypermutation Assay v2.0 (Invivoscribe Technologies, San Diego, CA, USA), which amplifies IGHV-IGHD-IGHJ rearrangements from genomic DNA or complementary DNA by polymerase chain reaction (PCR), as previously described.[3] IGHV unmutated (UM) was defined as ≥98% identity to the germline; mutated (M) <98%. DNA samples were quantified via agarose gel electrophoresis and NanoDrop2000 (Thermo Fisher Scientific). Genomic DNA was fragmented to 200-250 bp for library construction. 302 samples used Ion AmpliSeqTM Library Kit 2.0 on Ion ChefTM System (Thermo Fisher Scientific) for sequencing on Ion Torrent NGS platform. The remaining 381 used Agilent SureSelect Human All Exon Kit (Agilent Technologies) on Illumina HiSeq 2000 for 150 bp paired-end sequencing (Illumina). Average depth reached 2000x. Detailed analyses were described in a previous study.[4]

Reference

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4. Yi S, Yan Y, Jin M, et al. High incidence of MYD88 and KMT2D mutations in Chinese with chronic lymphocytic leukemia. Leukemia. 2021;35(8):2412-2415.

Supplementary Table Legends

Supplementary Table 1. The immunophenotype features of CLL patients in the cohort with elevated IgM and/or with MYD88 mutation (Due to the size of the dataset, the complete table is provided as a separate Excel file).

Supplementary Table 2. The clinical and biological characteristics of CLL patients with AIHA.

Supplementary Table 3. Follow-up Status and Causes of Death.

Supplementary Table 4. Comparison of clinical and biological characteristics at diagnosis between patients with CLL complicated by AIHA with and without V4-34 gene usage.

Supplementary Table 5. Characteristics of patients with DNMT3A mutations in the cohort.

Supplementary Table 6. Indications for Treatment in CLL Patients Without AIHA.

Supplementary Table 7. First-line treatment response in CLL patients with and without AIHA.

Supplementary Table 8. Incidence of AIHA re-occurrence in patients with relapsed CLL stratified by treatment line.

Supplementary Table 9. AIHA response according to different CLL treatment regimens.

Supplementary Table 1. The immunophenotype features of CLL patients in the cohort with elevated IgM and/or with MYD88 mutation(Due to the size of the dataset, the complete table is provided as a separate Excel file).

Supplementary Table 2. The clinical and biological characteristics of CLL patients with AIHA.

Characteristics, n (%)	wAIHA (n=101)	cAIHA (n=45)	P-value
Male/Female	72/29 (71.3%)	36/9 (80.0%)	0.268
Age at diagnosis, mean ± SD	59.4 ± 10.4	60.4 ± 7.8	0.503
Age >65 years	27/101 (26.7%)	12/45 (26.7%)	0.993
Splenomegaly	77/90 (85.6%)	32/42 (76.2%)	0.186
Region of lymph node enlargement ≥3	45/57 (78.9%)	21/26 (80.8%)	0.849
WBC ≥100x10^9/L	24/99 (24.2%)	2/45 (4.4%)	0.004
ALB <35 g/L	11/100 (11.0%)	10/45 (22.2%)	0.076
β2-microglobulin >3.5 mg/L	37/64 (57.8%)	15/27 (55.6%)	0.842
IgA >4 g/L	3/77 (3.9%)	1/36 (2.8%)	1.000
IgM >3.04 g/L	6/74 (8.1%)	6/32 (18.8%)	0.210
IgG >15.6 g/L	11/79 (13.9%)	4/38 (10.5%)	0.826
Positive Immunofixation Electrophoresis	14/48 (29.2%)	10/26 (38.5%)	0.415
FISH examination			
Del (17p)	11/92 (12.0%)	3/42 (7.1%)	0.589
Del (11q)	6/78 (7.7%)	2/37 (5.4%)	0.954
Del (13q)	18/75 (24.0%)	5/35 (14.3%)	0.243
+12	6/54 (11.1%)	4/32 (12.5%)	1.000
With mutated IGHV	36/64 (56.3%)	17/25 (68.0%)	0.310
Abnormal karyotype	37/88 (42.1%)	17/42 (40.5%)	0.865
Rai stage			0.335
0	2/94 (2.1%)	4/40 (10.0%)	
Ⅰ	10/94 (10.6%)	4/40 (10.0%)	
Ⅱ	17/94 (18.1%)	6/40 (15.0%)	
Ⅲ	40/94 (42.6%)	14/40 (35.0%)	
Ⅳ	25/94 (26.6%)	12/40 (30.0%)	
Binet stage			0.825
A	10/82 (12.2%)	5/31 (16.1%)	
B	20/82 (24.4%)	8/31 (25.8%)	
C	52/82 (63.4%)	18/31 (58.1%)	
CLL-IPI stage			0.014
Low	21/72 (29.2%)	9/25 (36.0%)	
Intermediate	21/72 (29.2%)	14/25 (56.0%)	
High	24/72 (33.3%)	1/25 (4.0%)	
Very high	6/72 (8.3%)	1/25 (4.0%)	

Abbreviation: CLL Chronic Lymphocytic Leukemia; wAIHA Warm Autoimmune hemolytic anemia; cAIHA Cold Autoimmune hemolytic anemia; CLL-IPI Chronic Lymphocytic Leukemia International Prognostic Index; WBC White Blood Cell count; ALB Albumin; FISH Fluorescence In Situ Hybridization; IGHV Immunoglobulin Heavy Variable.

Supplementary Table 3. Follow-up Status and Causes of Death.

Follow-up status	No. of patients (n)	Percentage (%)
Lost to follow-up	313	22.3
Disease Progression	518	36.9
Deaths	345	24.6
Cause of death		
CLL-related death	203	58.8
Unknown cause	53	15.4
Infection-related	39	11.3
Intracerebral hemorrhage	15	4.3
Other causes	35	10.1

Table note: Follow-up categories were not mutually exclusive. Patients with disease progression who subsequently died are counted in both the "disease progression" and "death" categories. Percentages are calculated against the total cohort of 1,404 patients.

Supplementary Table 4. Comparison of clinical and biological characteristics at diagnosis between patients with CLL complicated by AIHA with and without V4-34 gene usage.

Characteristics, n (%)	Without V4-34 (n=68)	With V4-34 (n=19)	P-value
Male/Female	47/21 (69.1%)	15/4 (78.9%)	0.403
Age at diagnosis, mean ± SD	61.0±9.1	59.5±6.0	0.394
Age >65 years	22/68 (32.4%)	4/19 (21.1%)	0.341
Splenomegaly	57/65 (87.7%)	12/16 (75.0%)	0.375
Lymphadenopathy	56/68 (82.4%)	9/17 (52.9%)	0.025
Region of lymph node enlargement≥3	37/46 (80.4%)	5/7 (71.4%)	0.962
WBC, range	34.5 (0.5, 598.0)	14.7 (2.4, 122.9)	0.009
ALB <35 g/L	9/68 (13.2%)	2/19 (10.5%)	1.000
β2-microglobulin >3.5 mg/L	29/52 (55.8%)	7/12 (58.3%)	0.872
IgA >4 g/L	2/60 (3.3%)	0/15 (0.0%)	1.000
IgM >3.04 g/L	4/60 (6.7%)	3/15 (20.0%)	0.275
IgG >15.6 g/L	9/62 (14.5%)	2/15 (13.3%)	1.000
Positive Immunofixation Electrophoresis	13/41 (31.7%)	5/11 (45.5%)	0.621
FISH examination			
Del (17p)	10/67 (14.9%)	0/18 (0.0%)	0.183
Del (11q)	6/63 (9.5%)	0/18 (0.0%)	0.395
Del (13q)	15/62 (24.2%)	2/13 (15.4%)	0.745
+12	5/48 (10.4%)	2/16 (12.5%)	1.000
With mutated IGHV	36/68 (52.9%)	16/19 (84.2%)	0.014
Abnormal karyotype	27/65 (41.5%)	9/17 (52.9%)	0.399
Rai stage			0.051
0	2/66 (3.0%)	2/16 (12.5%)	
Ⅰ	10/66 (15.2%)	3/16 (18.8%)	
Ⅱ	16/66 (24.2%)	0/16 (0.0%)	
Ⅲ	20/66 (30.3%)	8/16 (50.0%)	
Ⅳ	18/66 (27.3%)	3/16 (18.8%)	
Binet stage			0.359
A	10/59 (17.0%)	1/12 (8.3%)	
B	18/59 (30.5%)	2/12 (16.7%)	
C	31/59 (52.5%)	9/12 (75.0%)	
CLL-IPI stage			0.031
Low	16/62 (25.8%)	6/13 (46.2%)	
Intermediate	19/62 (30.6%)	7/13 (53.8%)	
High	20/62 (32.3%)	0/13 (0.0%)	
Very high	7/62 (11.3%)	0/13 (0.0%)	

Abbreviation: CLL Chronic Lymphocytic Leukemia; AIHA Autoimmune hemolytic anemia;CLL-IPI Chronic Lymphocytic Leukemia International Prognostic Index; WBC White Blood Cell count; ALB Albumin; FISH Fluorescence In Situ Hybridization; IGHV Immunoglobulin Heavy Variable.

Table note: This table includes only AIHA patients with available and evaluable IGHV gene-usage data. Of the 146 patients with AIHA in the overall cohort, detailed IGHV gene-usage analysis was available in 87 cases.

Supplementary Table 5. Characteristics of patients with *DNMT3A* mutations in the cohort.

CLL with AIHA or not	Gender	Age	Mutated gene	Exon	Nucleotide	Amino acid	VAF
wAIHA	Male	61	*DNMT3A*	exon19	c.2191T>G	p.F731V	40.60%
cAIHA	Male	65	*DNMT3A*	exon19	c.2311C>T	p.R771*	5.90%
cAIHA	Male	63	*DNMT3A*	exon19	c.2311C>T	p.R771*	4.30%
cAIHA	Male	71	*DNMT3A*	exon18	c.2108T>G	p.L703R	2.60%
wAIHA	Female	55	*DNMT3A*	exon23	c.2679G>A	p.W893*	1.40%
Without AIHA	Female	60	*DNMT3A*	exon14	c.1600delC	p.Q534Sfs*117	15.40%
Without AIHA	Male	80	*DNMT3A*	exon23	c.2644C>T	p.A882C	11.90%
Without AIHA	Female	53	*DNMT3A*	exon14	c.1597T>C	p.Y533H	10.80%
Without AIHA	Female	55	*DNMT3A*	exon23	c.2645G>A	p.R882H	8.30%
Without AIHA	Male	75	*DNMT3A*	exon11	c.1418_1419del	p.E473Afs*18	7.90%
Without AIHA	Male	59	*DNMT3A*	exon19	c.2204A>G	p.Y735C	6.30%
Without AIHA	Male	74	*DNMT3A*	exon19	c.2311C>T	p.R771*	4.50%
Without AIHA	Female	80	*DNMT3A*	exon18	c.2141C>G	p.S714C	4.50%
Without AIHA	Male	63	*DNMT3A*	exon23	c.2645G>A	p.R882H	3.60%
Without AIHA	Male	58	*DNMT3A*	exon23	c.2706delC	p.F902Lfs*4	3.40%
Without AIHA	Male	59	*DNMT3A*	exon8	c.941G>A	p.W314*	1.80%
Without AIHA	Male	52	*DNMT3A*	exon8	c.958C>T	p.R320*	1.80%
Without AIHA	Female	66	*DNMT3A*	exon11	c.1344C>A	p.Y448*	1.80%
Without AIHA	Male	63	*DNMT3A*	exon7	c.715delG	p.V239Wfs*77	1.70%
Without AIHA	Male	58	*DNMT3A*	exon9	c.1096C>A	p.R366S	1.70%
Without AIHA	Female	71	*DNMT3A*	exon23	c.2645G>A	p.R882H	1.40%
Without AIHA	Male	65	*DNMT3A*	exon8	c.932T>G	p.V311G	1.30%
Without AIHA	Male	66	*DNMT3A*	exon19	c.2204A>G	p.Y735C	1.20%
Without AIHA	Male	59	*DNMT3A*	exon17	c.2074C>T	p.Q692*	1.00%
Without AIHA	Female	50	*DNMT3A*	exon23	c.2644C>A	p.R882S	0.70%

Abbreviation: CLL Chronic Lymphocytic Leukemia; AIHA Autoimmune hemolytic anemia; wAIHA Warm Autoimmune hemolytic anemia; cAIHA Cold Autoimmune hemolytic anemia; VAF Variant allele frequency.

Supplementary Table 6. Indications for Treatment in CLL Patients Without AIHA.

Indication for treatment	No. of patients (n)	Percentage (%)
Progressive lymphocytosis	184	31.3
Bone marrow infiltration–related cytopenia	128	21.8
Massive or symptomatic splenomegaly	109	18.5
Progressive or symptomatic lymphadenopathy	94	16.0
B symptoms	54	9.2
Symptomatic organ dysfunction attributable to CLL	19	3.2

Table note: Although multiple indications for treatment could coexist in individual patients, each patient was categorized according to the primary indication that prompted initiation of CLL-directed therapy.

Supplementary Table 7. First-line treatment response in CLL patients with and without AIHA.

Treatment regimen	**CR, n (%)**	**PR, n (%)**	**SD, n (%)**	**PD, n (%)**
CLL patients with AIHA				
Targeted therapy	10 (35.7)	16 (57.1)	1 (3.6)	1 (3.6)
Immunochemotherapy	11 (26.2)	22 (52.4)	8 (19.0)	1 (2.4)
Chemotherapy	2 (4.4)	21 (46.7)	18 (40.0)	4 (8.9)
CLL patients without AIHA				
Targeted therapy	54 (32.7)	97 (58.8)	13 (7.9)	1 (0.6)
Immunochemotherapy	36 (26.1)	74 (53.6)	24 (17.4)	4 (2.9)
Chemotherapy	15 (7.6)	120 (60.6)	34 (17.2)	29 (14.6)
Unknown	0	2(66.7)	0	1 (33.3)

Abbreviation: CR Complete Response; PR Partial Response; SD Stable Disease; PD Progressive Disease.

Supplementary Table 8. Incidence of AIHA re-occurrence in patients with relapsed CLL stratified by treatment line.

Treatment line	**No.of patients treated**	**No.of relapsed CLL patients evaluated**	**AIHA (re-)occurrence, n (%)**
First-line therapy	902	324	25 (7.7)
Second-line therapy	307	76	9 (11.8)
Third-line therapy	69	18	3 (16.7)

Supplementary Table 9. AIHA response according to different CLL treatment regimens.

Treatment regimen	No. of patients	CR, n (%)	PR, n (%)	NR, n (%)
Targeted therapy–based regimens				
BTKi only	22	13 (59.1)	7 (31.8)	2 (9.1)
Zanubrutinib	10	8	2	0
Ibrutinib	11	4	5	2
Orelabrutinib	1	1	0	0
BTKi + anti-CD20 antibody	9	4 (44.4)	4 (44.4)	1 (11.1)
BTKi + R	3	2	1	0
BTKi + BR	2	1	0	1
BTKi + FCR	2	1	1	0
BTKi + RCHOP/RCHOP-like	2	0	2	0
BCL2i	1	0	1 (100)	0
Venetoclax	1	0	1	0
Chemotherapy and chemoimmunotherapy regimens				
Chemotherapy	34	12 (35.3)	10 (29.4)	12 (35.3)
Chlorambucil	19	6	6	7
CHOP/CHOP-like	9	3	3	3
FC	4	2	1	1
Bendamustine	2	1	0	1
Chemoimmunotherapy	43	19 (44.2)	15 (34.9)	9 (20.9)
RCHOP/RCHOP-like	29	16	9	4
FCR	8	3	3	2
R + Chlorambucil	5	0	2	3
BR	1	0	1	0

Abbreviation:R Rituximab; BR Bendamustine + Rituximab; FCR Fludarabine + Cyclophosphamide + Rituximab; RCHOP Rituximab + Cyclophosphamide + Hydroxydaunorubicin (Doxorubicin) + Vincristine (Oncovin) + Prednisone; FC Fludarabine + Cyclophosphamide; CHOP Cyclophosphamide + Hydroxydaunorubicin (Doxorubicin) + Vincristine (Oncovin) + Prednisone.

Supplementary Tables and Figure Legends

Supplementary Figure 1. The patient inclusion and exclusion diagram

Supplementary Figure 2. Clinical characteristics of IGHV mutation status in CLL patients and its relationship with AIHA. (A) Distribution of IGHV mutations in CLL patients. (B) Comparison of IGHV mutational status in CLL patients with and without AIHA. (C) Stratified comparison of IGHV mutation rates in CLL patients with and without AIHA.

Supplementary Figure 3. Comparison of Immunoglobulin Gene-related Characteristics in CLL Patients with and without AIHA. (A) Proportions of IGHD Segments in CLL Patients with and without AIHA. (B) Proportions of IGHJ Segments in CLL Patients with and without AIHA. (C) Comparison of CDR3 Length in CLL Patients with and without AIHA.

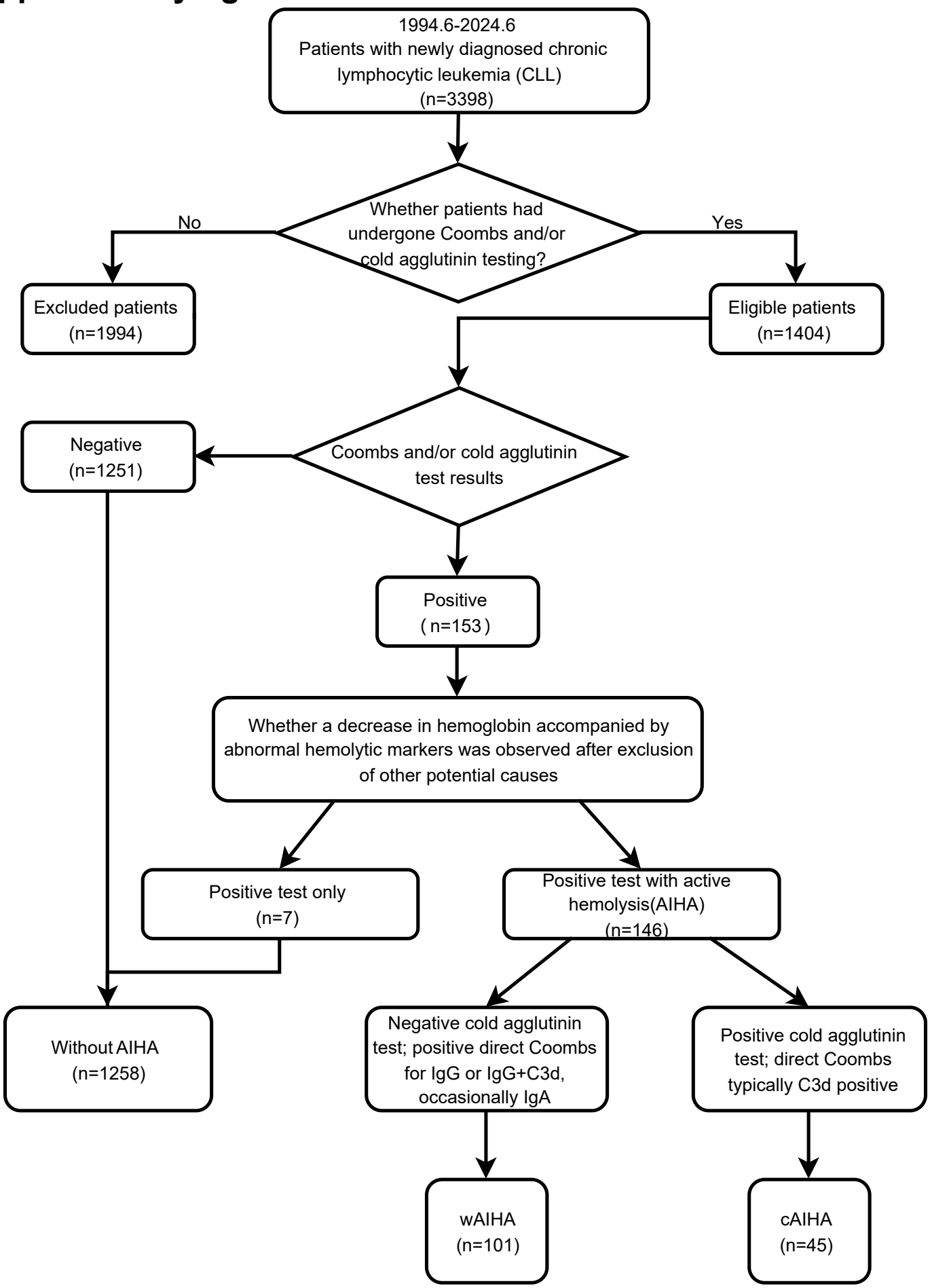
Supplementary Figure 4. Stratified survival analysis of the prognostic impact of IGHV mutation status on CLL patients. (A-C) Impact on Time to first treatment (TTFT), progression-free survival (PFS), and overall survival (OS) in all CLL patients. (D-F) Impact on TTFT, PFS, and OS in CLL patients with AIHA. (G-I) Impact on TTFT, PFS, and OS in CLL patients without AIHA.

Supplementary Figure 5. Analysis of the prognostic impact of IGHV4-34 gene status on CLL patients. (A) Impact on time to first treatment. (B) Impact on progression-free survival. (C) Impact on overall survival.

Supplementary Figure 6. Correlation between CLL treatment outcomes and concurrent AIHA treatment outcomes. (A) Distribution of disease response in all CLL patients. (B) Disease response after treatment in CLL patients with AIHA. (C) Response of hemolysis after treatment in CLL patients with AIHA.

Supplementary Figure 7. Correlation between the treatment outcomes of CLL and the treatment outcomes of concurrent AIHA.

Supplementary figure 1



Supplementary Figure 2

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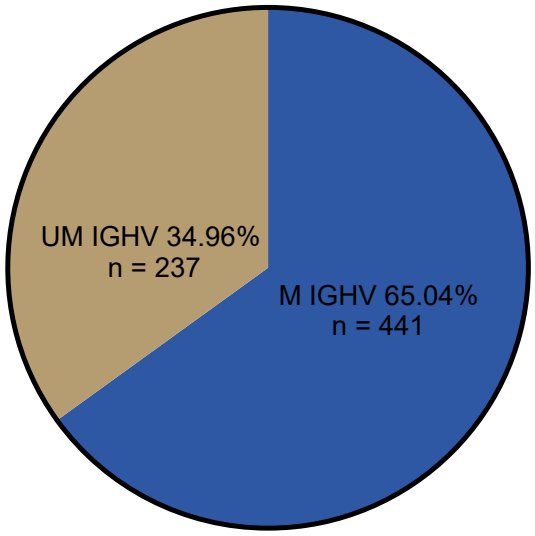

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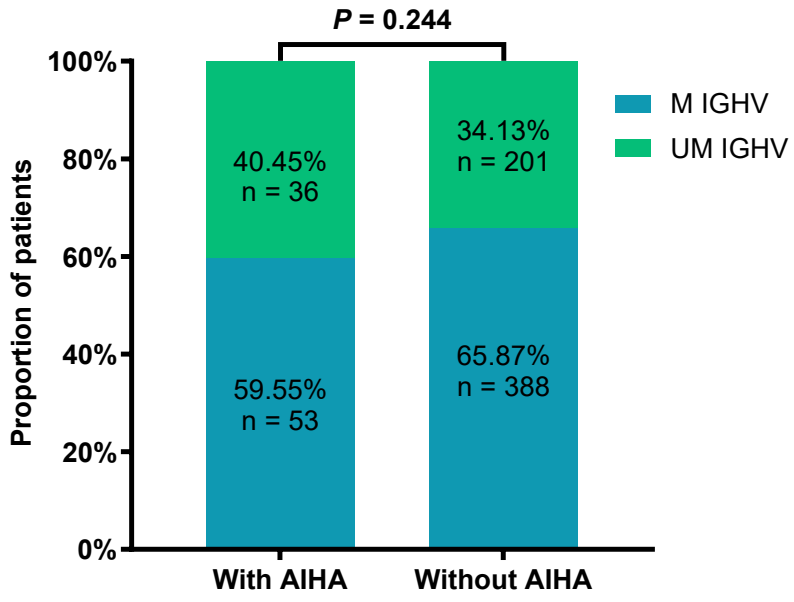

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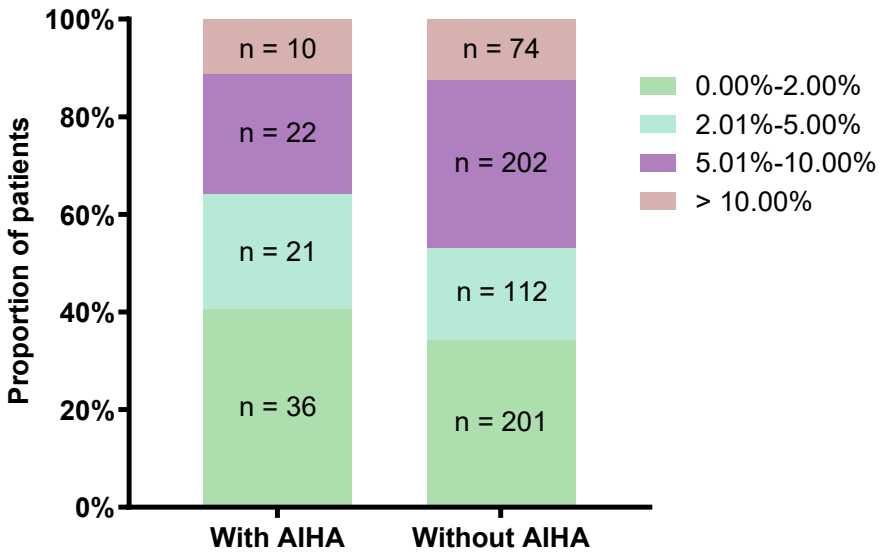
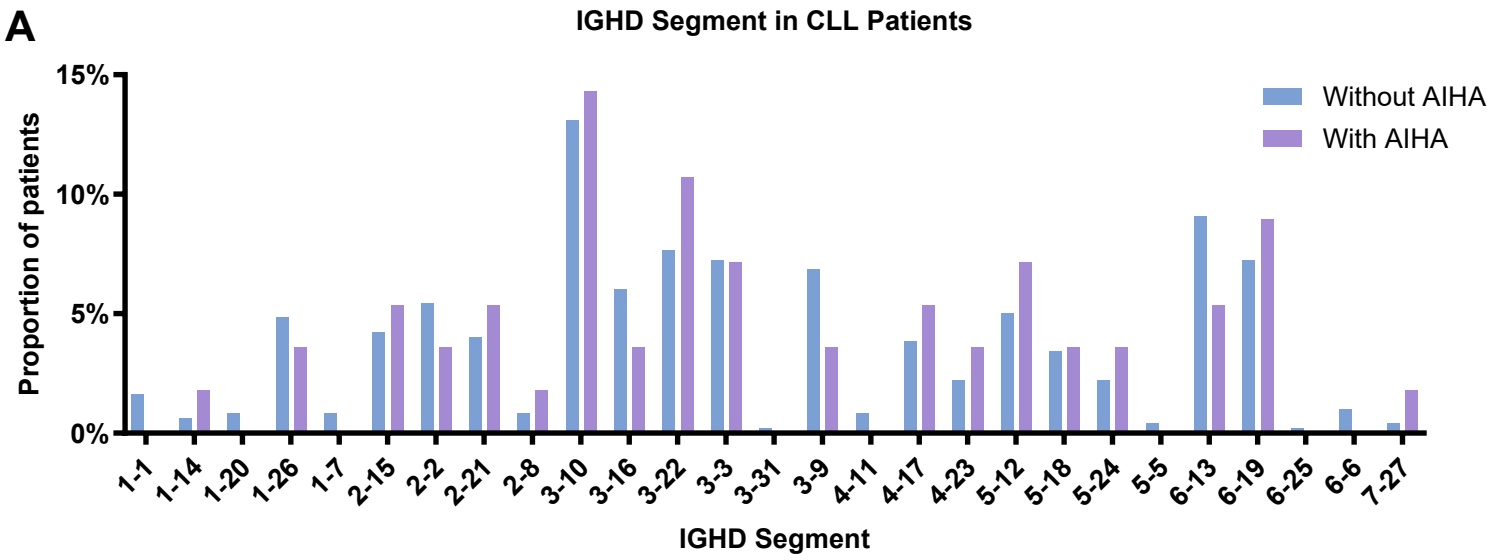
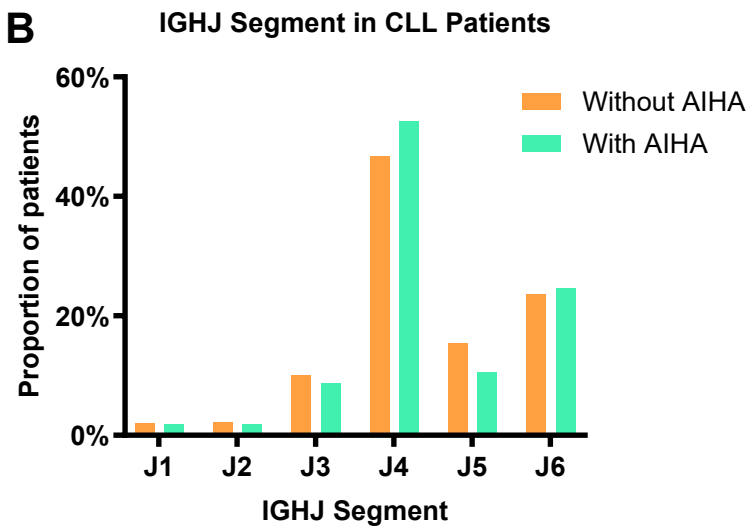
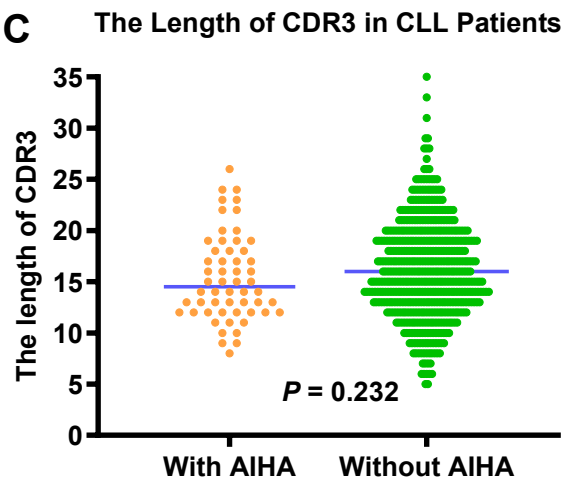


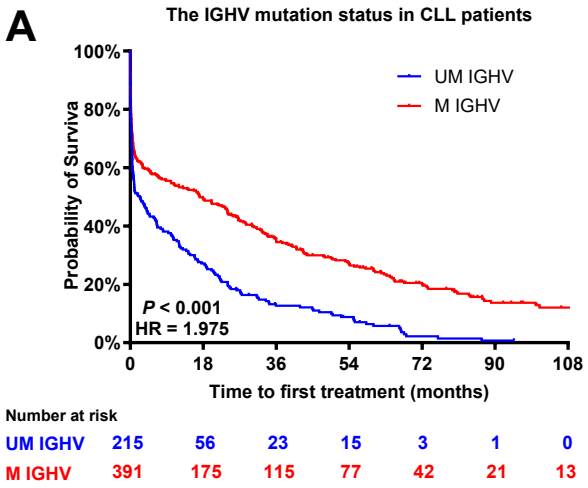
Supplementary figure 3

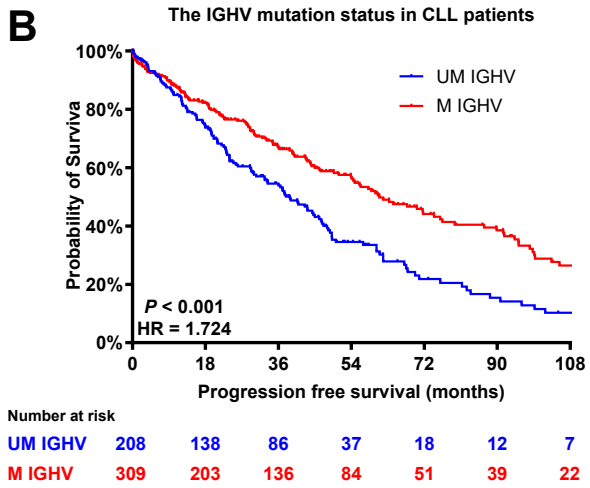


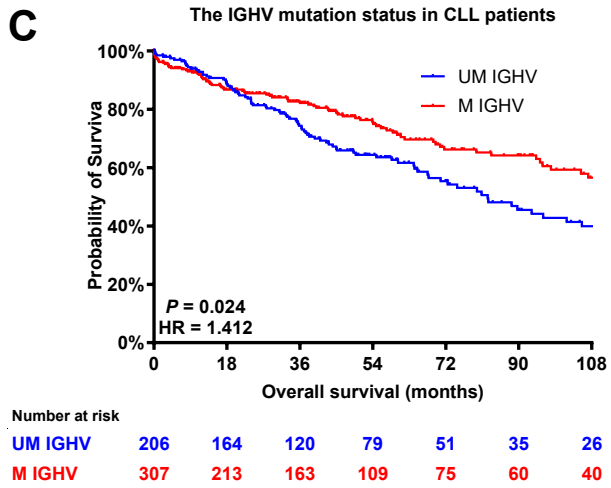


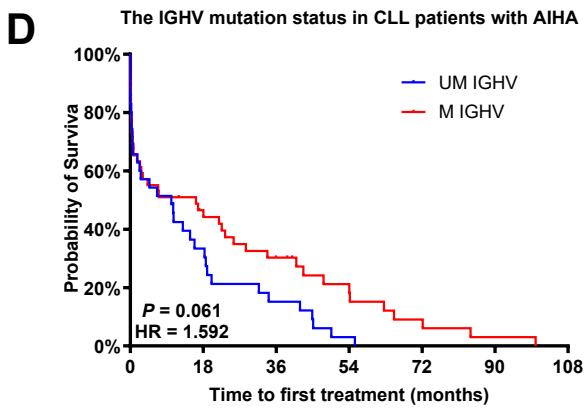


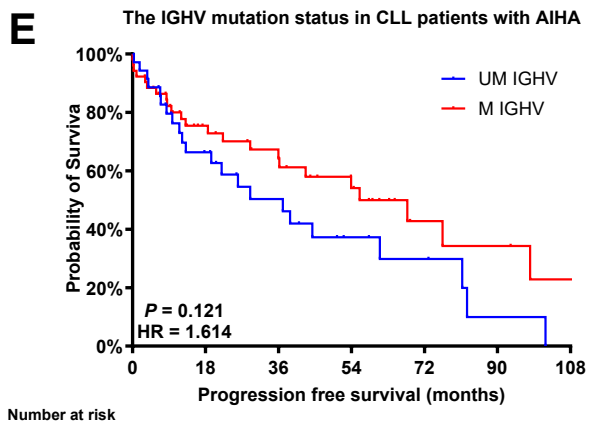
Supplementary figure 4

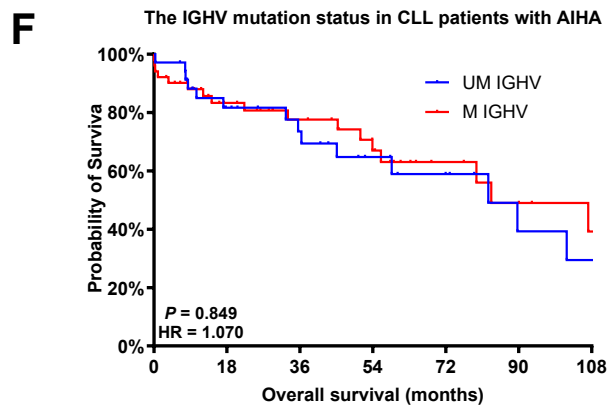


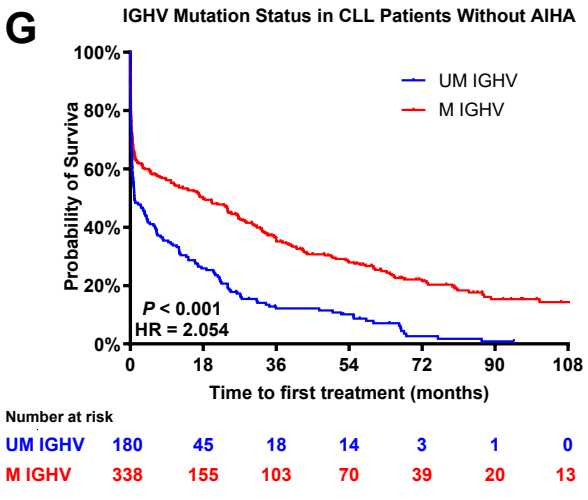


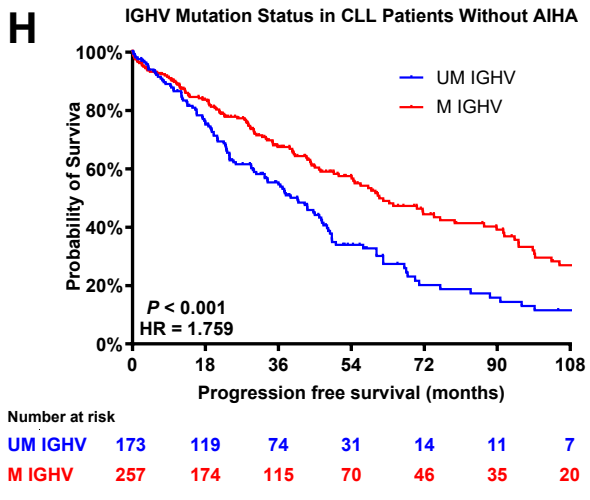


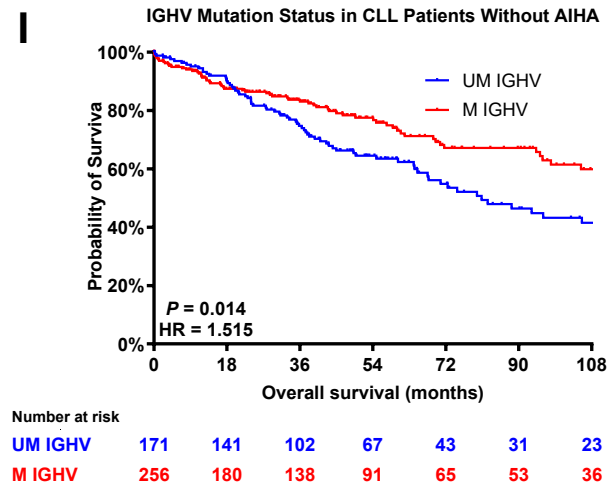












Supplementary figure 5

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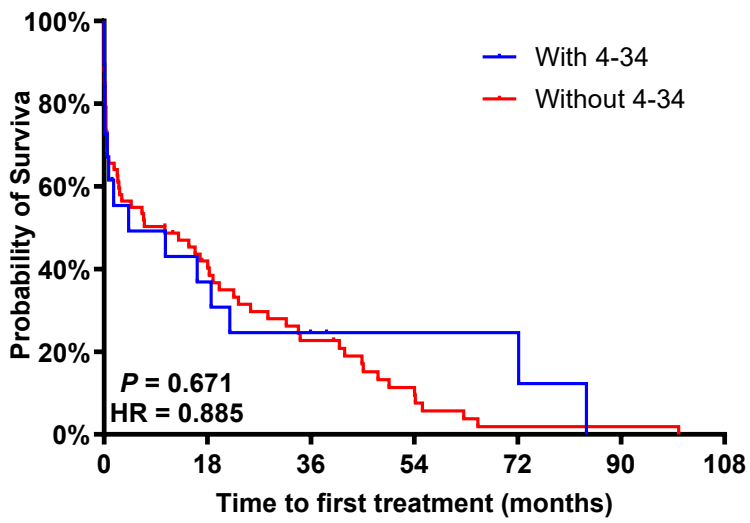
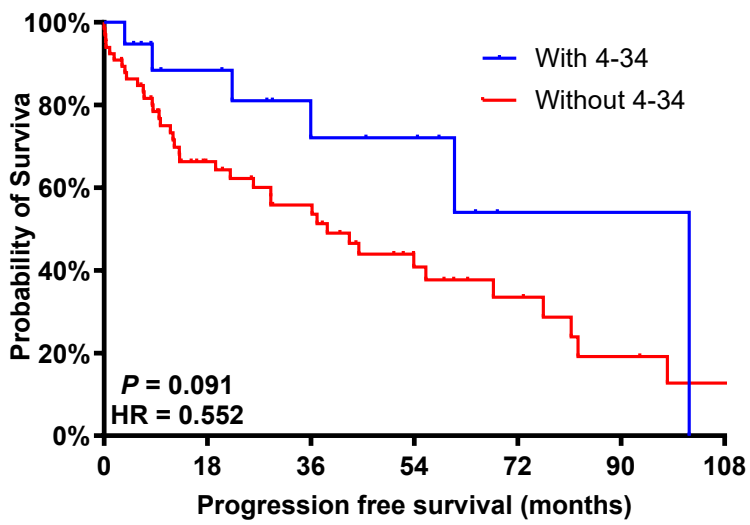


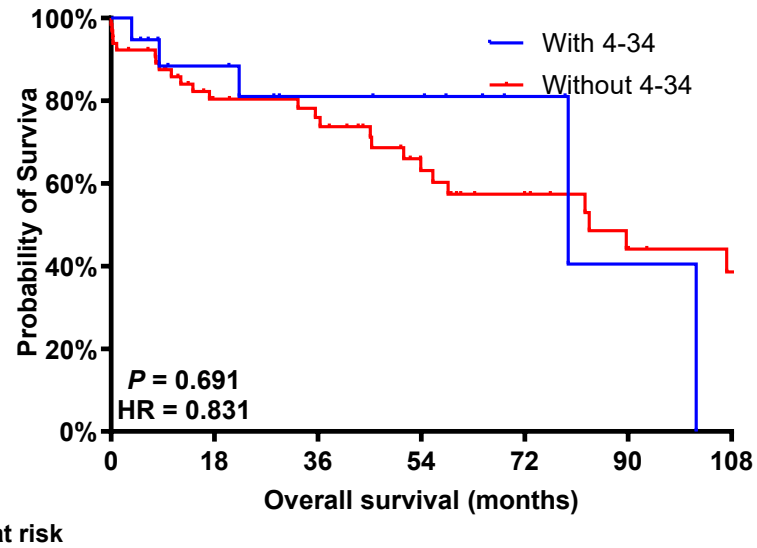
Number at risk							
With 4-34	19	6	3	2	2	0	0
Without 4-34	67	24	13	6	1	1	0

B



Number at risk							
With 4-34	19	13	8	7	1	1	0
Without 4-34	66	33	25	13	8	4	2

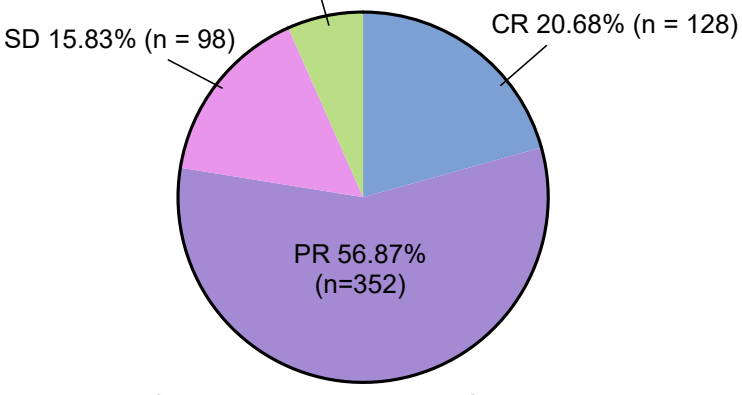
C



Number at risk							
With 4-34	19	13	9	8	3	1	0
Without 4-34	65	41	34	22	15	10	7

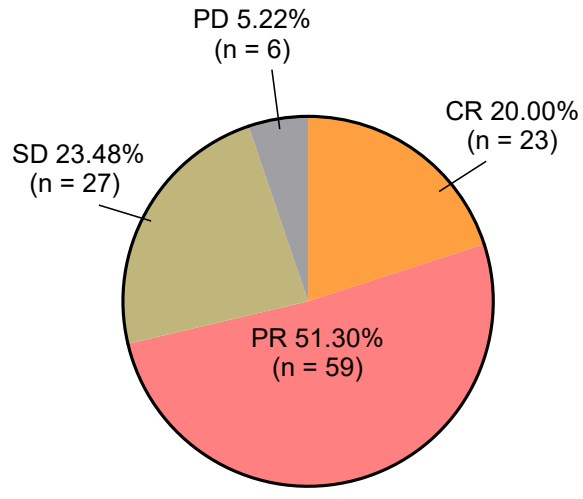
Supplementary figure 6

A



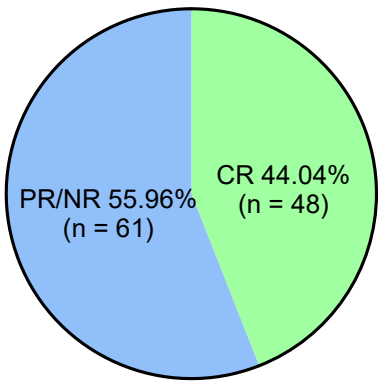
Distribution of disease response in all CLL patients (N = 619)

B



Disease response after treatment in CLL patients with AIHA (N = 115)

C



Response of hemolysis after treatment in CLL patients with AIHA (N = 109)

Supplementary Figure 7