Poster #: LB05

XXI iwCLL International Workshop on CLL

Integrated immunogenetic and cytogenetic profiling reveals distinct biological patterns among stereotyped BCR subsets in CLL

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OBJECTIVES

- To characterize IGHV-IGHD-IGHJ rearrangements and somatic hypermutation status (SHM) in **a large cohort of 4,257 CLL patients** from 11 centers in Poland
- To investigate the **distribution of stereotyped BCR subsets** and their association with disease-related genetic aberrations
- To explore whether **integrated immunogenetic and cytogenetic profiling** can identify biologically distinct clusters of CLL cases based on IGHV SHM, cytogenetic aberrations, and stereotyped BCR subset assignment

CONCLUSIONS

- The study demonstrates **a broad diversity of immunogenetic configurations in CLL**, with stereotyped BCR subsets showing distinct IGHV gene usage patterns and non-random associations with SHM status and cytogenetic alterations.
- Subset assignment appears to reflect **shared structural and functional features of BCRs**, rather than strict IGHV gene identity, suggesting a convergent, potentially antigen-driven selection process shaping clonal architecture.
- Integration of immunogenetic and cytogenetic profiles revealed several biologically distinct clusters, which differed in terms of cytogenetic composition and subset distribution, though the clinical relevance of these groups requires further investigation.

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INTRODUCTION

- Antigen-driven stimulation via the B-cell receptor (BCR) immunoglobulin **is critical for CLL cell survival and proliferation**, with IGHV SHM status serving as a key prognostic marker.
- Approximately 30-40% of CLL cases express stereotyped BCR immunoglobulins, which delineate **subsets with shared biological features**, genomic alterations, and clinical outcomes, including therapy response and risk of Richter transformation.
- Comprehensive analysis of stereotyped subsets, IGHV gene usage, and SHM status in a large patient cohort provides insights into CLL immunogenetic architecture and **supports improved prognostic assessment and treatment guidance**.

METHODS

- **Patient cohort:** 4,257 newly diagnosed and previously untreated CLL patients
- **IGHV assessment:** analysis performed with accordance to the ERIC guidelines using Sanger (n = 2,808), NGS (n = 1,418) or both methods (n = 31)
- **IGHV classification**: U-CLL (germline identity, GI ≥ 98%), M-CLL (GI < 98%, borderline (GI 97-97.99%)
- **Statistical analysis**: categorical variables summarized as counts and percentages; associations were evaluated with χ^2 or Fisher's exact test
- **Dimensionality reduction and clustering**: UMAP applied to IGHV SHM status and cytogenetic features; Kmeans clustering used to identify dominant clusters mapping to stereotyped subsets

RESULTS

- Overall, **4,335 IGHV-IGHD-IGHJ** rearrangements were evaluated, with double productive sequences observed in 76 cases (1.8%).
- Specific IGHV segments showed **preffered** IGHD/IGHJ **pairing patterns**.
- Approximately 10% of IGHV seq.uences were assigned to major stereotyped subsets, with **high-hisk subsets predominantly associated with U-CLL**.
- Most subsets exhibited **predominant or exclusive usage of a single IGHV gene**, with some subsets (e. g. #1, #99) showing heterogenous IGHV usage yet consistent association with U-CLL or M-CLL.
- *TP53* mutations were enriched in aggressive stereotyped subsets (#1, #2, #5, #6, #64B, #99), and del(17p), del(11q), and trisomy 12 were more frequent in U-CLL, indicating **a link between immunogenetic features and high-risk genetic lesions**.
- Dimensionality reduction and clustering revealed **enrichment of specific subsets in groups defined by distinct cytogenetic and immunogenetic features**, suggesting underlying biological diversity among CLL cases. However, prognostic implications **require further outcome-based validation**.

REFERENCES

1. Agathangelidis A, Chatzidimitriou A, Chatzikonstantinou T, et al. Immunoglobulin gene sequence analysis in chronic lymphocytic leukemia: the 2022 update of the recommendations by ERIC, the European Research Initiative on CLL. *Leukemia*. 2022;36(8):1961-1968.
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Table 1. Baseline characteristics of CLL patients with IGHV-IGHD-IGHJ rearrangements.

Single productive rearrangements				
	U-CLL	**M-CLL**	**Borderline**	**All cases**
No. of patients	2,115 (53.4%)	1,658 (41.9%)	186 (4.7%)	3,959 (93%)
Age (median, range) [years]	69, 29-94	70, 27-97	70, 36-91	70, 27-97
Male	1,327 (62.7%)	853 (51.5%)	110 (59.1%)	2,290 (57.8%)
Del(17p)	105/786 (13.6%)	44/592 (7.43%)	4/60 (6.7%)	153/1,438 (10.6%)
Del(11q)	173/672 (25.7%)	26/520 (5%)	15/55 (27.3%)	214/1,247 (17.2%)
Trisomy 12	103/528 (19.5%)	42/430 (9.8%)	7/50 (14%)	152/1,008 (15.1%)
Del(13q)*	136/252 (54%)	238/332 (71.7%)	22/29 (75.9%)	396/613 (64.6%)
Normal FISH	116/522 (22.2%)	94/426 (20.1%)	9/50 (18%)	219/998 (21.9%)
***TP53* mutation**	78/487 (16%)	30/356 (8.4%)	2/40 (5%)	110/883 (12.5%)
Subset assigned	297	95	19	411
Single productive + single unproductive rearrangement				
	U-CLL	**M-CLL**	**Borderline**	**All cases**
No. of patients	149	59	13	221 (5.2%)
Age (median, range) [years]	67, 36-95	71, 45-94	72 ,63-92	68, 36-95
Male	91	24	9	124
Del(17p)	4/37 (10.8%)	0/6 (0%)	0/2 (0%)	4/45 (8.9%)
Del(11q)	10/36 (27.8%)	0/6 (0%)	1/2 (50%)	11/44 (25%)
Trisomy 12	4/30 (13.3%)	0/6 (0%)	0/2 (0%)	4/38 (10.5%)
Del(13q)*	12/15 (80%)	5/6 (83.3%)	0/1 (0%)	17/22 (77.7%)
Normal FISH	3/30 (10%)	1/6 (16.7%)	1/2 (50%)	5/38 (13.1%)
***TP53* mutation**	4/31 (12.9%)	1/9 (11,1%)	0/3 (0%)	5/43 (11.6%)
Subset assigned	25	3	0	28
Double productive rearrangements				
	U-CLL	**M-CLL****	**Disordant SHM**	**All cases**
No. of patients	25	30	22	77 (1,8%)
Age (median, range) [years]	71, 50-83	68, 49-85	73, 42-95	71, 42-95
Male	14 (56%)	12 (40%)	8 (36.4 %)	34 (44.1%)
Del(17p)	1/9 (11.1%)	0/12 (0%)	1/11 (9.1%)	2/32 (6.2%)
Del(11q)	1/7 (14.3%)	1/12 (8.3%)	2/11 (18.2%)	4/30 (13.3%)
Trisomy 12	0/7 (0%)	1/12 (8.3%)	1/10 (10%)	2/29 (6.9%)
Del(13q)*	4/5 (80%)	8/10 (80%)	6/7 (85.7%)	18/22 (81.8%)
Normal FISH	1/7 (14.3%)	2/12 (16.7%)	1/10 (10%)	4/29 (13.8%)
***TP53* mutation**	2/5 (40%)	3/8 (37.5%)	1/3 (33.3%)	6/16 (37.5%)
Subset assigned	6	3	1	10

*del(13q) as a sole aberration, **including two borderline cases

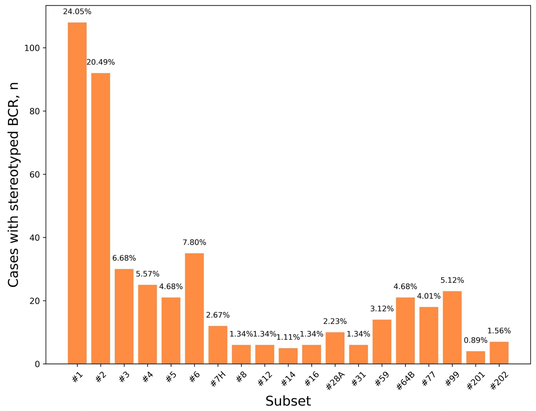

Figure 1. Distribution (in number and percentage) of cases with stereotyped BCR across different subsets.

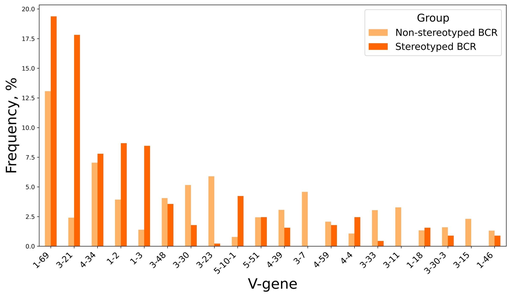

Figure 2. Frequency (%) of the top 20 V-genes used in stereotyped versus non-stereotyped BCR groups.

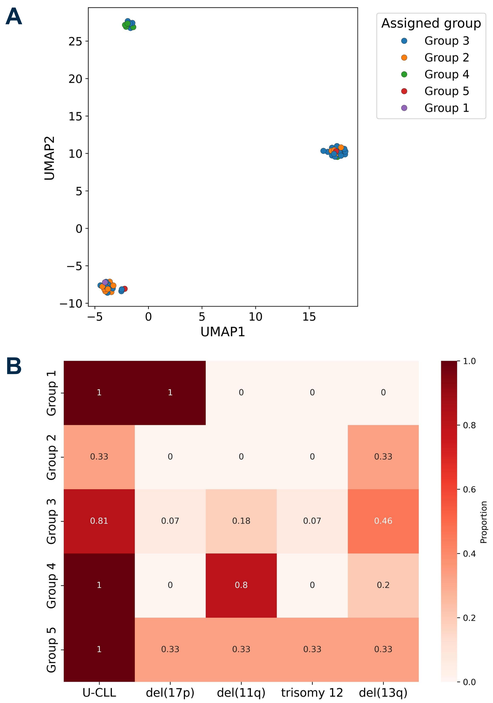

Figure 3. Integration of cytogenetic alterations and IGHV SHM status defines five distinct groups (clusters).
(A) UMAP visualization of combined cytogenetic features and IGHV SHM status identifies five distinct groups (clusters) by K-means clustering (k=5). Group numbering is arbitrary. Notably, **Group 3** comprised the largest proportion of cases (57.1%). **Group 1** contained subset #59; **Group 2** included subsets #202, #4, #5, #64B, and #77; **Group 3** encompassed subsets #1, #2, #28A, #3, and #7H; **Group 4** consisted of subsets #12, #31, and #99; while **Group 5** was represented by subset #6. (B) Heatmap showing the frequency of selected genetic features across five groups. Color intensity corresponds to the relative frequency of each feature within a given cluster.