haematologica
Journal of the Ferrata Storti Foundation

Rapid identification of the high-risk IGLV3-21^{R110} subgroup of chronic lymphocytic leukemia by flow cytometry

by Mischa Y. L. Vervoordeldonk, Maud A. J. I. van den Oetelaar, Marielle J. P. Maas, Mark-David Levin, Vincent H. J. van der Velden and Anton W. Langerak

Received: August 4, 2026.
Accepted: November 4, 2026.

Citation: Mischa Y. L. Vervoordeldonk, Maud A. J. I. van den Oetelaar, Marielle J. P. Maas, Mark-David Levin, Vincent H. J. van der Velden and Anton W. Langerak. Rapid identification of the high-risk IGLV3-21^{R110} subgroup of chronic lymphocytic leukemia by flow cytometry.
Haematologica. 2026 Nov 12. doi: 10.3324/haematol.2026.301762 [Epub ahead of print]

Publisher's Disclaimer.
E-publishing ahead of print is increasingly important for the rapid dissemination of science. Haematologica is, therefore, E-publishing PDF files of an early version of manuscripts that have completed a regular peer review and have been accepted for publication.
E-publishing of this PDF file has been approved by the authors.
After having E-published Ahead of Print, manuscripts will then undergo technical and English editing, typesetting, proof correction and be presented for the authors' final approval, the final version of the manuscript will then appear in a regular issue of the journal.
All legal disclaimers that apply to the journal also pertain to this production process.

Rapid identification of the high-risk IGLV3-21^{R110} subgroup of chronic lymphocytic leukemia by flow cytometry

Mischa Y. L. Vervoordeldonk[1*], Maud A. J. I. van den Oetelaar[1*], Mariëlle J. P. Maas[1], Mark-David Levin[2], Vincent H. J. van der Velden[1], Anton W. Langerak[1]

[*]These authors contributed equally to this work

Affiliations

[1]Dept. of Immunology, Erasmus MC, Rotterdam, the Netherlands

[2]Dept. of Internal Medicine, Albert Schweitzer Hospital, Dordrecht, the Netherlands

Corresponding Author: Professor Anton W. Langerak, PhD

Department of Immunology, Erasmus MC, Dr. Molewaterplein 40, 3015 GD Rotterdam, The Netherlands

E-mail: a.langerak@erasmusmc.nl

Data sharing statement

Data will be shared upon reasonable request to the corresponding author.

Authorship Contributions

MYLV, VHJvdV and AWL conceived of the study. MAJIO and MYLV performed flow cytometric staining. MAJIO performed the IGL and IGH sequencing. MAJIO and MJPM interpreted the flow cytometric assay test results. MYLV performed the data analysis. MYLV and AWL contributed to data interpretation. MYLV, MAJIO, MDL, VHJvdV and AWL wrote the manuscript. All authors read and approved the final version of the manuscript.

Disclosure of Conflicts of Interest

The IGLV3-21^{R110} antibody was provided as a gift by SinABiomedics without any restrictions. In addition, SinABiomedics had no role in the drafting of this manuscript.

Acknowledgements

We would like to thank all patients who provided the samples to perform this study.

Artificial Intelligence

Artificial intelligence has not been used for the design, data analysis, preparation of text, or any other part of this study.

Chronic lymphocytic leukemia (CLL) is a lymphoproliferative disorder characterized by accumulation of mature, monoclonal $CD5^+$ B-cells. In CLL, clinicians initiate treatment only after observing disease-related symptoms [1]. The required active surveillance can be a source of anxiety for patients [2]. Improved prognosis at diagnosis could relieve this burden, as patients can be better counseled. Additionally, clinicians can adjust the frequency of medical examinations based on the prognosis.

Prognosis strongly relies on the composition of the B-cell receptor (BCR) of the CLL clone. The BCR is composed of an immunoglobulin heavy chain (IgH) paired with either an immunoglobulin kappa light chain (IgK) or immunoglobulin lambda light chain (IgL). Every IgH, IgK, and IgL chain is generated through the recombination of different genes (V, J, and for IGH locus also D), resulting in BCR sequences that vary between B-cells. Sequencing of the IGHV gene repertoire in CLL revealed that its somatic hypermutation (SHM) status classifies CLL into unmutated (U-CLL; ≥98% germline homology) or mutated (M-CLL; <98% germline homology), with U-CLL patients requiring treatment more often and earlier than M-CLL patients. Sequencing of the IGLV gene repertoire revealed that the IGLV3-21 gene was of prognostic value in combination with a G110R substitution (IGLV3-21^{R110}), which enables ligand-independent BCR signaling [3,4]. Although IGLV3-21^{R110} patients often show a borderline IGHV SHM status [3,5,6], they consistently exhibit poor clinical outcomes [3,6-8]. With IGLV3-21^{R110} occurring in 10–20% of unselected CLL cohorts and over 20% of to-be-treated populations, it represents a clinically relevant biomarker beyond the U-CLL/M-CLL distinction [3,5,9]. In addition, it may guide future therapeutic strategies, as CAR-T cell therapies and bispecific antibodies targeting G110R are currently under development [10,11], and IGLV3-21^{R110} might hold predictive value in the case of BTK monotherapy [12].

Taken together, these findings highlight the value of incorporating routine detection of IGLV3-21^{R110} into the diagnostic work-up of CLL. While molecular testing is the gold standard, it requires expertise and specialized infrastructure [8,13]. Alternatively, monoclonal antibodies specific to IGLV3-21^{R110} have been developed for flow cytometry, a widely available and established technique in clinical hematology [3,9]. While these antibodies offer an accessible approach, they have yet to undergo validation or broad implementation in clinical practice. To this end, we evaluated the concordance of a monoclonal antibody specific for IGLV3-21^{R110}, developed by SinABiomedics, with that of the gold-standard molecular tests available, in a real-life setting of treatment-requiring CLL patients at the Laboratory Medical Immunology of Erasmus MC. Here, we show that detection of IGLV3-21^{R110} by flow cytometry is a rapid and easily implemented diagnostic tool, which may provide additional information not detected by genotyping alone. In addition, we report on the molecular characteristics of the BCR in the largest IGLV3-21^{R110} cohort published to date. Importantly, we identified a yet unknown pairing of IGLV3-21^{R110} with heavy chain rearrangements using IGHV3-53, a gene that was observed exclusively in IGLV3-21^{R110}.

A total of 1,084 CLL patients were included in this study. These patients were eligible for treatment and had molecular testing requested between August 2021 and April 2025. Samples were prospectively included in a consecutive manner when they entered our diagnostic lab, and tests were performed on fresh material. No patient was included more than once. Most patients were treatment-naïve at the time of sampling. Ethics approval was provided by the Erasmus MC ethical committee (MEC-2023-0691, MEC-2021-0652). To detect IGLV3-21^{R110} using flow cytometry, bone marrow or peripheral blood samples from patients were washed and taken up in 100 uL PBS+BSA+Azide. Cells were subsequently stained with 1 uL CD20-PB (BioLegend, Cat. 302320), 5 uL IGLV3-21^{R110}-PE (SinABiomedics, Cat. SIN-A-1001), 15 uL CD5-PercP-Cy5.5 (BD Biosciences, Cat. 341109), 5 uL CD19-PE-Cy7 (Beckman Coulter, Cat. IM3628), and 2.5 uL IgK-APC (Agilent Technologies, Cat. C0222), and incubated for 30 minutes in the dark at room temperature. After incubation, cells were washed and acquired within 60 minutes on a BD FACSLyric Flow Cytometer (BD Biosciences). The acquired data was analyzed using BD Infinicyt Software (BD Biosciences).

Doublets were excluded using FSC-H/FSC-A and debris was excluded using SSC-A/FSC-A. CLL cells were gated as $CD5^{+}/CD19^{+}/CD20^{dim}$. Using our flow cytometric assay we could subdivide CLL clones as either being IgK+, IGLV3-21^{R110}+, or Ig-/IgL+ not expressing IGLV3-21^{R110} (Figure 1B-C). Samples were interpreted as being positive for IGLV3-21^{R110} when the median PE fluorescence of cells in the CLL gate was above 200 using the EuroFlow standardized settings [14]. This threshold was determined based on the fluorescence PE background in the T-cell gate ($CD19^{-}/CD5^{+}$)(Supplementary Figure 1A). Some IGLV3-21^{R110} cases were considered to be "dim" (Supplementary Figure 1B). The results of the flow cytometry test and molecular test were interpreted blinded from each other, and inter-observer reproducibility of the flow cytometric assay in a 10% sub-cohort analysis showed 100% concordance using 20 Ig-/IgL+, 20 IGLV3-21^{R110}, and 6 dim IGLV3-21^{R110} cases. Molecular testing for IGLV3-21^{R110} and IGH were performed as described previously [5].

We evaluated the concordance between the flow cytometric assay and the molecular assay using 461 CLL cases of our cohort, thereby excluding all patients with IgK+ CLL without any concurrent IGLV3-21^{R110} or Ig-/IgL+ CLL population (Figure 1A). These 461 patients included IGLV3-21^{R110} (n=173) and Ig-/IgL+ (n=270) cases and cases with multiple clones (n=18). Of these, 273/461 (59.2%) cases were negative and 180/461 (39.0%) were positive for IGLV3-21^{R110} with both assays. For cases that were discordant, 7/461 (1.5%) showed a negative result with the flow cytometric assay but a positive result with the molecular assay, whereas only 1/461 (0.2%) case showed a positive result with the flow cytometric assay but a negative result with the molecular assay. The IGLV3-21^{R110} stainings for discordant patients are presented in Supplementary Figure 1C. Taken together, our data resulted in a concordance of 98.3% between the flow cytometric and molecular assay, with a sensitivity of 96.3% (CI 95%; 92.4 – 98.5; p-value < 0.001) and a specificity of 99.6% (CI 95%; 97.9 – 99.9; p-value < 0.001)(Table 1).

For three discordant cases with enough remaining material, intracellular staining for IGLV3-21^{R110}, as well as IgK or IgL, was performed, but all stayed negative, questioning whether there is any considerable BCR expression (Supplementary Figure 1D). The clinical relevance of a positive molecular result in the absence of clear surface expression remains uncertain in the case of prognosis or G110R targeting therapies. Thus, flow cytometry may provide complementary functional information beyond genotyping, although further clinical correlation is needed.

One limitation of our current flow cytometric assay is the need for prior knowledge of IgK/IgL isotype, as the assay lacks an IgL antibody. However, when we tested IGLV3-21^{R110}, IgK, and IgL antibodies together in the same mix, IGLV3-21^{R110} staining did not seem to be hampered, warranting further evaluation of this combination as an initial screening tube. Early identification of IGLV3-21^{R110} positivity at diagnosis may facilitate patient counseling given its strong prognostic value. However, including this antibody in an initial screening tube would add costs, and so addition of the IGLV3-21^{R110} antibody should be evaluated by individual institutions, as it also depends on the available resources. A strength of this study was that it included a large and representative cohort of treatment-requiring CLL patients, with sufficient numbers of both IGLV3-21^{R110}-positive and negative cases. Importantly, the molecular assay used as the reference standard is well-established and widely accepted for IGLV3-21^{R110} detection [5]. Finally, the testing was conducted in a routine diagnostic setting, supporting the generalizability of the findings to real-world clinical practice.

Following validation of the IGLV3-21^{R110} flow cytometric assay in our large real-world CLL cohort, we determined whether there was biased usage for IGHV genes within IGLV3-21^{R110} compared to Ig-/IgL+ cases. We observed enrichment of IGHV genes previously associated with IGLV3-21^{R110}, such as IGHV3-21 and IGHV3-48. Additionally, IGHV3-53 was observed exclusively in IGLV3-21^{R110} cases (Figure 2A;

Supplementary Table 1). Examining the HCDR3, excluding subset #2 (which uniformly exhibits an HCDR3 length of 9 amino-acids), the HCDR3 amino-acid length in IGLV3-21^{R110} cases was shorter than Ig-/IgL+ cases (median 13 [IQR 11–14.75] vs. 17 [14–22]; P < 0.001)(Figure 2B). Finally, consistent with previous reports, IGLV3-21^{R110} cases were often borderline mutated (median 97.6 [IQR 96.6–98.6] vs. 96.16 [93.8–100]; P = 0.037)(Figure 2C). Overall, IGLV3-21^{R110} presents distinct features within the IGHV genes which may reveal more about the biology of this subset. Unfortunately, our small series precluded a detailed analysis of potential stereotypic features for IGHV3-53, as described for other IGHV sequences in CLL. In regard to the length of the HCDR3, it has been previously hypothesized that subset #2 HCDR3 regions are shorter to enable the ligand-independent BCR signaling established by the IGLV3-21^{R110} mutation [4], but this need for a short HCDR3 region may thus be extended to IGLV3-21^{R110} group as a whole.

In summary, our cohort consisted of 1,084 CLL patients comprising 461 patients with an Ig-/IgL+ clone, and 173 of these had IGLV3-21^{R110} detectable by our flow cytometry test (16% of all 1,084 patients, 38% of all 461 Ig-/IgL+ patients). We demonstrate the feasibility of incorporating an IGLV3-21^{R110} monoclonal antibody into routine diagnostic flow cytometric testing as a practical, rapid, and scalable tool for improved CLL diagnostics, as well as research applications. Furthermore, our findings underline that flow cytometrically detected surface expression of IGLV3-21^{R110} may be clinically more relevant than genotyping alone.

Reference

[1] Hallek M, Cheson BD, Catovsky D, et al. iwCLL guidelines for diagnosis, indications for treatment, response assessment, and supportive management of CLL. Blood. 2018;131(25):2745-2760.

[2] Wright KB, Bylund CL, Bagautdinova D, et al. Caring for an individual with chronic lymphocytic leukemia (CLL): understanding family caregivers' perceptions of social support, caregiver burden, and unmet support needs. J Cancer Educ. 2024;39(2):180-185.

[3] Maity PC, Bilal M, Koning MT, et al. IGLV3-21*01 is an inherited risk factor for CLL through the acquisition of a single-point mutation enabling autonomous BCR signaling. Proc Natl Acad Sci U S A. 2020;117(8):4320-4327.

[4] Minici C, Gounari M, Ubelhart R, et al. Distinct homotypic B-cell receptor interactions shape the outcome of chronic lymphocytic leukaemia. Nat Commun. 2017;8:15746.

[5] Hengeveld PJ, Ertem YE, Dubois JMN, et al. Clinicobiological characteristics and treatment efficacy of novel agents in chronic lymphocytic leukemia with IGLV3-21(R110). Leukemia. 2022;36(7):1935-1938.

[6] Nadeu F, Royo R, Clot G, et al. IGLV3-21R110 identifies an aggressive biological subtype of chronic lymphocytic leukemia with intermediate epigenetics. Blood. 2021;137(21):2935-2946.

[7] Stamatopoulos B, Smith T, Crompot E, et al. The light chain IgLV3-21 defines a new poor prognostic subgroup in chronic lymphocytic leukemia: results of a multicenter study. Clin Cancer Res. 2018;24(20):5048-5057.

[8] Syrykh C, Pons-Brun B, Russinol N, et al. IGLV3-21R110 mutation has prognostic value in patients with treatment-naive chronic lymphocytic leukemia. Blood Adv. 2023;7(23):7384-7391.

[9] Paschold L, Simnica D, Brito RB, et al. Subclonal heterogeneity sheds light on the transformation trajectory in IGLV3-21(R110) chronic lymphocytic leukemia. Blood Cancer J. 2022;12(3):49.

[10] Markl F, Schultheiss C, Ali M, et al. Mutation-specific CAR T cells as precision therapy for IGLV3-21(R110) expressing high-risk chronic lymphocytic leukemia. Nat Commun. 2024;15(1):993.

[11] Fischer C, Chen SS, Nimmerfroh J, et al. IGLV3-21(R110)-directed bispecific antibodies activate T cells and promote killing in a high-risk subset of chronic lymphocytic leukemia. Haematologica. 2026;111(2):572-582.

[12] Yosifov DY, Robrecht S, Giza A, et al. IGLV3-21(R110) and ibrutinib treatment: Results from the double-blind, randomized, placebo-controlled GCLLSG CLL12 trial in early-stage CLL. Hemasphere. 2026;10(6):e70385.

[13] Lopez-Oreja I, Lopez-Guerra M, Correa J, et al. All-CLL: a capture-based next-generation sequencing panel for the molecular characterization of chronic lymphocytic leukemia. Hemasphere. 2023;7(10):e962.

[14] Kalina T, Flores-Montero J, van der Velden VH, et al. EuroFlow standardization of flow cytometer instrument settings and immunophenotyping protocols. Leukemia. 2012;26(9):1986-2010.

Table 1: *Flow cytometric assay diagnostic performance compared to gold-standard molecular assay. Exact 95% confidence intervals for the sensitivity and specificity of the flow cytometric assay were calculated assuming a binomial distribution using the Clopper-Pearson test.*

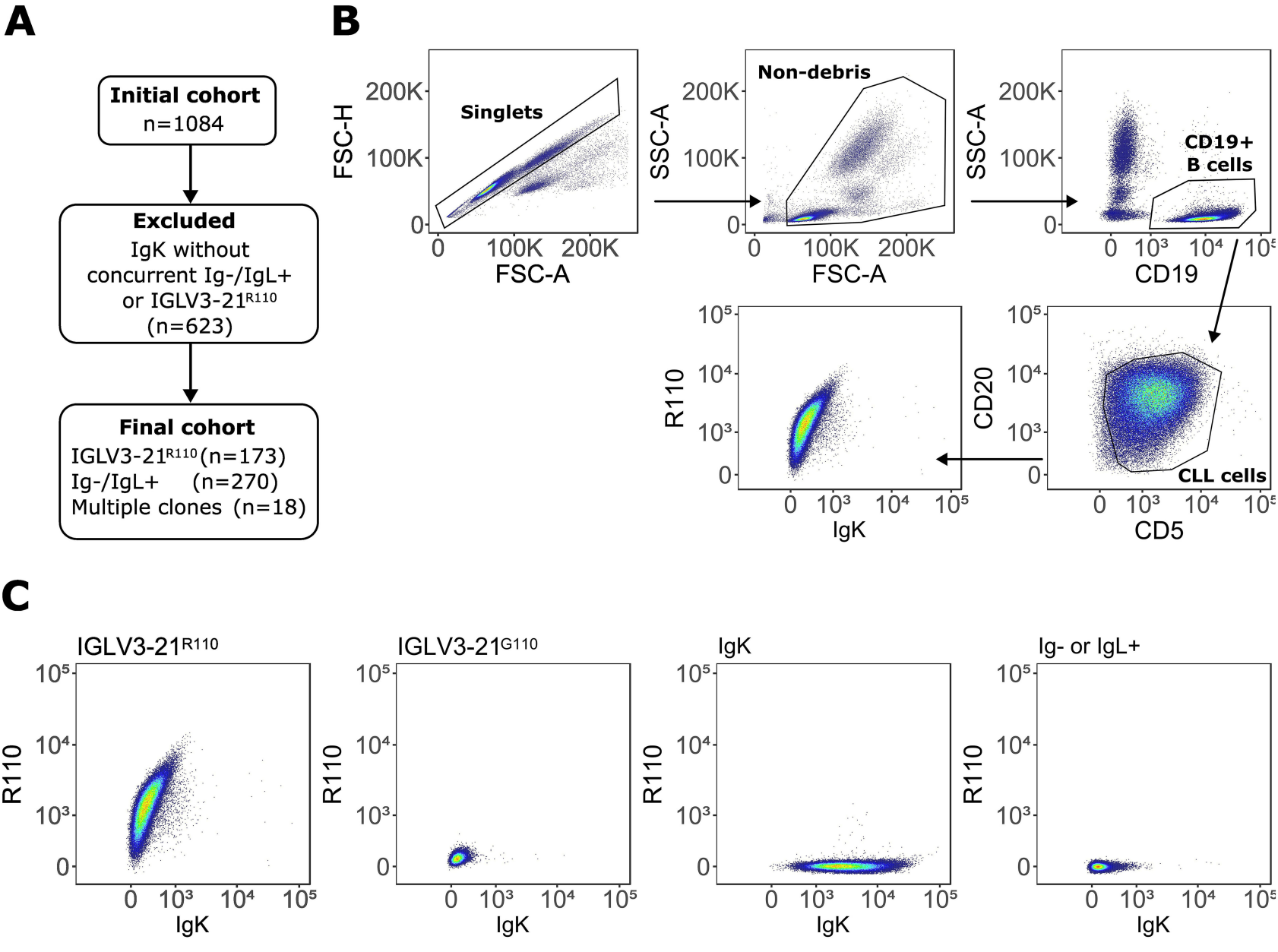
	Molecular Assay Positive	Molecular Assay Negative	
Flow Cytometric Assay Positive	TP = 180 (39.0%)	FP = 1 (0.2%)	PPV = 99.4 (95% CI 97 – 100%)
Flow Cytometric Assay Negative	FN = 7 (1.5%)	TN = 273 (59.2%)	NPV = 97.5% (95% CI 94.9 – 99%)
	Sensitivity = 96.3% (95% CI 92.4 – 98.5%)	Specificity = 99.6% (95% CI 98 – 100%)	

Figure 1: *Flow cytometric assay gating strategy and staining examples.*

(A) Flowchart of sample selection for diagnostic accuracy analysis.
(B) Gating strategy of representative sample. Singlets were selected based on FSC-A/FSC-H, debris was excluded using FSC-A/SSC-A, B-cells were identified using CD19/SSC-A and CLL cells were identified using CD5/CD20.
(C) Representative dot plots of IGLV3-21^{R110}+, IGLV3-21^{G110}+, IgK+, and Ig-/IgL+ cases; gated on CLL cells.

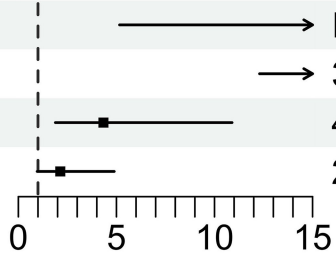
Figure 2: *Immunoglobulin heavy chain analysis in IGLV3-21^{R110} compared to Ig-/IgL+.*

(A) Immunoglobulin heavy chain variable (IGHV) gene usage in IGLV3-21^{R110} cases compared with Ig-/IgL+ cases. The allelic variation of the IGHV gene was ignored in our analysis (IGHV3-53*01 → IGHV3-53). Only IGHV genes with a frequency greater than 5% in the IGLV3-21^{R110} group were included for comparison. Dots represent odds ratio estimates; whiskers denote 95% confidence intervals. Fisher's exact test with Benjamini–Hochberg correction for multiple comparisons was applied.
(B) HCDR3 length in IGLV3-21^{R110} cases compared with Ig-/IgL+ cases. Differences in HCDR3 length were assessed using Mann-Whitney U test after exclusion of subset #2 cases.
(C) IGHV SHM load in IGLV3-21^{R110} cases compared with Ig-/IgL+. Differences in IGHV SHM load were assessed using Mann-Whitney U test.

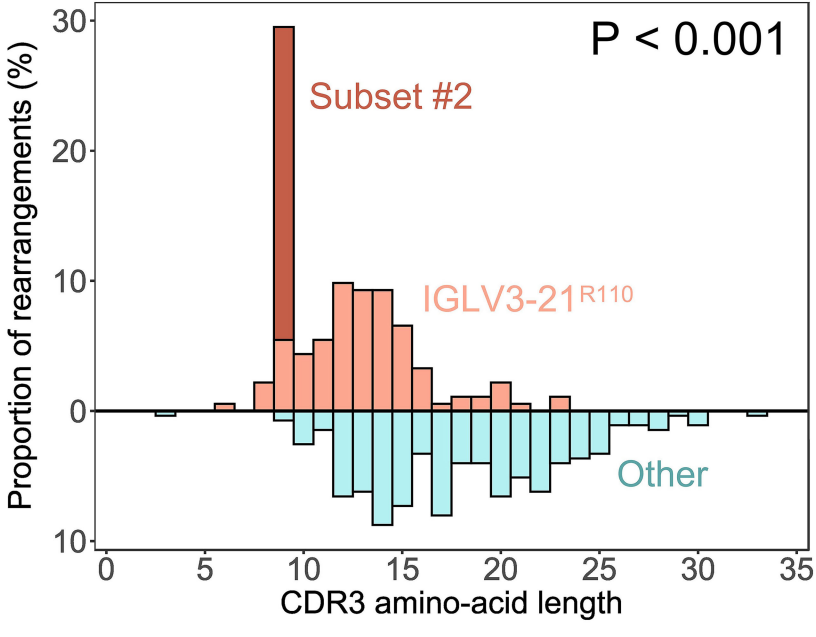

A
Initial cohort
n=1084
Excluded
IgK without concurrent Ig-/IgL+ or IGLV3-21R110 (n=623)
Final cohort
IGLV3-21R110 (n=173)
Ig-/IgL+ (n=270)
Multiple clones (n=18)
B
Singlets
FSC-H
FSC-A
Non-debris
SSC-A
CD19+ B cells
CD19
CD20
CD5
CLL cells
R110
IgK
C
IGLV3-21R110
IGLV3-21G110
IgK
Ig- or IgL+

A

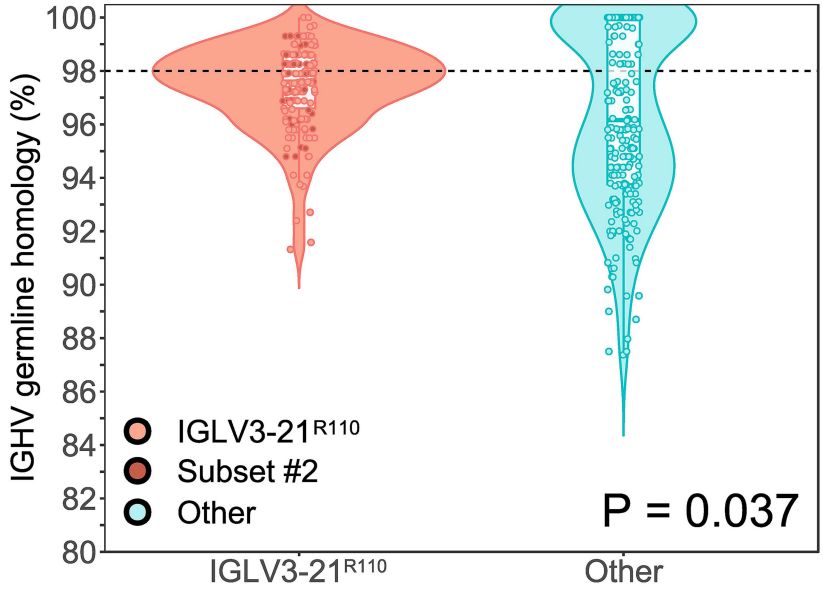
Variable	Other (N)	R110 (N)	OR 95% CI	P-value
3-53	0 / 270	14 / 179	Inf (5.16 - Inf)	0.000
3-21	3 / 270	56 / 179	39.02 (12.30 - 198.60)	0.000
3-48	9 / 270	24 / 179	4.34 (1.89 - 10.89)	0.000
3-23	13 / 270	18 / 179	2.14 (0.96 - 4.89)	0.056



C



B



Supplemental Figure 1: Flow cytometric assay concordance and examples
(A) Boxplot of median fluorescence intensity of PE within the CLL gate (CD5+/CD19+/CD20Dim) and T-cell gate (CD5+/CD19-). Proposed 200 MFI cutoff is shown using a dotted line.
(B) Dotplots as examples for IGLV3-21R110 staining in cases which were deemed “dim” positive. Such dimly positive cases should be considered for molecular confirmation testing.
(C) Dotplots of IGLV3-21R110 stainings of all discordant cases. Case 1 was positive with flow cytometry and negative with molecular testing. Cases 2 – 8 were negative with flow cytometry but positive with molecular testing.
(D) Dotplots of intracellular IGLV3-21R110 staining in discordant cases which were negative by surface IGLV3-21R110 staining. Additional lambda staining showed no considerable surface expression either compared to the positive control

Supplemental Table 1: counts of IGHV genes in IGLV3-21R110 and Ig-/IgL+

R110	IGHV	Count
IGLV3-21R110	1-18	8
Other	1-18	5
IGLV3-21R110	1-2	5
Other	1-2	12
IGLV3-21R110	1-24	3
Other	1-24	1
IGLV3-21R110	1-3	0
Other	1-3	3
IGLV3-21R110	1-45	0
Other	1-45	1
IGLV3-21R110	1-46	0
Other	1-46	2
IGLV3-21R110	1-69	0
Other	1-69	33
IGLV3-21R110	1-8	0
Other	1-8	5
IGLV3-21R110	2-26	0
Other	2-26	3
IGLV3-21R110	2-5	0
Other	2-5	9
IGLV3-21R110	2-70	0
Other	2-70	1
IGLV3-21R110	3-11	5
Other	3-11	7
IGLV3-21R110	3-13	3
Other	3-13	2
IGLV3-21R110	3-15	7
Other	3-15	4
IGLV3-21R110	3-20	0
Other	3-20	1
IGLV3-21R110	3-21	56
Other	3-21	3
IGLV3-21R110	3-23	18
Other	3-23	13
IGLV3-21R110	3-30	1
Other	3-30	23
IGLV3-21R110	3-30-3	1
Other	3-30-3	8
IGLV3-21R110	3-30-5	0
Other	3-30-5	1
IGLV3-21R110	3-33	1
Other	3-33	9
IGLV3-21R110	3-43	1
Other	3-43	1
IGLV3-21R110	3-48	24
Other	3-48	9
IGLV3-21R110	3-49	0
Other	3-49	2
IGLV3-21R110	3-53	14
Other	3-53	0
IGLV3-21R110	3-64	1
Other	3-64	0
IGLV3-21R110	3-64D	0
Other	3-64D	1
IGLV3-21R110	3-66	3
Other	3-66	3
IGLV3-21R110	3-7	1
Other	3-7	18
IGLV3-21R110	3-72	1
Other	3-72	1
IGLV3-21R110	3-74	0
Other	3-74	4
IGLV3-21R110	3-9	0
Other	3-9	7
IGLV3-21R110	4-10-1	0
Other	4-10-1	1
IGLV3-21R110	4-30-2	0
Other	4-30-2	1
IGLV3-21R110	4-30-4	0
Other	4-30-4	3
IGLV3-21R110	4-31	0
Other	4-31	2
IGLV3-21R110	4-34	4
Other	4-34	25
IGLV3-21R110	4-38-2	0
Other	4-38-2	1
IGLV3-21R110	4-39	4
Other	4-39	9
IGLV3-21R110	4-4	2
Other	4-4	1
IGLV3-21R110	4-43	0
Other	4-43	1
IGLV3-21R110	4-59	6
Other	4-59	8
IGLV3-21R110	4-61	5
Other	4-61	6
IGLV3-21R110	5-10-1	0
Other	5-10-1	1
IGLV3-21R110	5-51	0
Other	5-51	4
IGLV3-21R110	6-1	2
Other	6-1	1
IGLV3-21R110	7-4-1	1
Other	7-4-1	0
IGLV3-21R110	Inconclusive	2
Other	Inconclusive	14