IGLV3-21^{R110} mutation has prognostic value in patients with treatment-naive chronic lymphocytic leukemia

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Abstract:

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IGLV3-21^{R110} mutation has prognostic value in patients with treatment-naive chronic lymphocytic leukemia

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Running head: Prognostic value of IGLV3-21^{R110}

To the Editor:

Chronic lymphocytic leukemia (CLL) displays high biological and clinical heterogeneity.[1,2] Few prognostic factors are used in clinical practice including immunoglobulin heavy-chain variable (IGHV) gene somatic hypermutation (SHM) status, chromosome aberrations and gene mutations, which remain insufficient for personalizing patient management.[3,4] Recent studies have shown that expression of the immunoglobulin lambda light chain IGLV3-21 gene carrying a SHM-derived G>C mutation changing the glycine at position 110 to an arginine (IGLV3-21^{R110}) defines a subset of CLL with an intermediate epigenetic profile and an aggressive clinical course.[5,6] When occurring on the IGLV3-21*01 or *04 alleles, the R110 mutation allows homotypic B cell receptor (BCR) interactions triggering cell-autonomous BCR signaling[5,7] and/or facilitates T-cell-independent engagement with superantigen.[8] IGLV3-21^{R110} has been found in up to 6.5% of patients with CLL at diagnosis and in up to 25% of patients enrolled in clinical trials.[5,6,9] We[6] and others[5] have shown that all CLL cases belonging to the aggressive stereotyped subset #2 carried the IGLV3-21^{R110}. Nonetheless, approximately half of IGLV3-21^{R110} CLL are not classified as stereotyped subset #2 but seemed to have a similar clinical outcome,[5,6] suggesting that the conventional stereotyped subset #2 classification might not completely recognize this clinically-aggressive subgroup of CLL. In addition, the IGLV3-21^{R110} seems to have prognostic value independently of the IGHV gene SHM status and methylation-based epigenetic subtypes.[5,6] However, further studies on independent cohorts are needed to support its application in clinical practice.[1,2,10–12] The aim of this study was to assess the prognostic value of the IGLV3-21^{R110} in large and independent population-based cohorts of patients with CLL.

We designed a multiplex IGLV3-21^{R110}-specific polymerase chain reaction (msPCR) integrating two forward primers aligning to distinct regions of the IGLV3-21 gene and two R110-specific reverse primers matching the IGLJ1 and IGLJ2/3 genes, respectively. We used a third pair of primers targeting *FBXW7* as an internal control. To make the results comparable among samples, the intensity of each IGLV3-21^{R110} product was normalized by the intensity of the *FBXW7* band (Figure 1A, Supplemental Table 1, Supplemental Methods). PCR conditions were set up in a cohort of 12 patients (including 6 IGLV3-21^{R110} mutated) and validated in 165 CLL (including 7 IGLV3-21^{R110} mutated) from our previous study in which IGLV3-21^{R110} status was determined by whole-genome/exome sequencing and RNA-seq.[6] The concordance was 100% (Supplemental Table 2). The msPCR assay was then used to characterize two independent CLL cohorts of 622 (cohort 1) and 376 (cohort 2) patients, respectively. To further validate the msPCR results, all but one IGLV3-21^{R110} positive samples of cohort 1 were subjected to next-generation sequencing and analyzed using IgCaller,[13] while the results obtained in 78 patients from cohort 2 were compared to the IGLV3-21^{R110} status previously reported by whole-genome sequencing/RNA-

seq.[6] Fully concordant results were obtained in both validations (Figure 1B, Supplemental Figure 1-2, Supplemental Table 3-9, Supplemental Methods). Cohort 1 included 110 patients with high-count monoclonal B-cell lymphocytosis (MBL) and 512 with overt CLL, while cohort 2 included only patients with overt CLL. Although CLL patients in cohort 1 showed a shorter time to first treatment (TTFT) compared to cohort 2, no differences were observed in terms of disease stages, IGHV gene SHM status and epigenetic subtypes (Supplemental Figure 3, Supplemental Table 3). The primary end-point of the study was TTFT measured from time of diagnosis considering only patients diagnosed as CLL (Supplemental Methods). The study was approved by the Hospital Clínic of Barcelona Ethics Committee. Informed consent was obtained for all patients.

In cohort 1, the IGLV3-21^{R110} mutation was found in 21/622 (3.4%) tumors, similarly distributed in MBL (3/110, 2.7%) and CLL (18/512, 3.5%) as well as in CLL with mutated (M-CLL) and unmutated (U-CLL) IGHV genes. Similar results were observed in cohort 2, although with a higher incidence of the IGLV3-21^{R110} mutation (29/376, 7.7%) (Figure 1C, Supplemental Table 10). Concordant with previous observations,[6] IGLV3-21^{R110} was remarkably enriched in the epigenetic intermediate CLL (i-CLL) subtype, representing 25.8% and 41.5% of i-CLL patients in cohort 1 and 2, respectively (Figure 1C). IGLV3-21^{R110} CLL carried borderline IGHV identity, whereas most i-CLL lacking the IGLV3-21^{R110} had an IGHV identity below 97% (Figure 1D, Supplemental Table 11). Moreover, 13 CLL with IGLV3-21^{R110} carried stereotyped IGH genes, 12/13 were classified as subset #2 (8 in cohort 1; 4 in cohort 2) and one as subset #14. The remaining IGLV3-21^{R110} CLL carried non-stereotyped immunoglobulin genes (19 major stereotyped subsets analyzed, Supplemental Methods). Genomic data regarding CLL driver alterations were available for patients belonging to cohort 2. As previously reported,[6] mutations in *SF3B1* and *ATM* were more frequently found in IGLV3-21^{R110} CLL compared to non-IGLV3-21^{R110} (*SF3B1:* 41.4% *vs* 10.3%, P<0.0001; *ATM*: 25.0% *vs* 6.3%, P=0.003). In this cohort, *NOTCH1* mutations were also enriched in IGLV3-21^{R110} CLL (33.3% *vs* 10.0%, P=0.003). Contrarily, the IGLV3-21^{R110} mutation was mutually exclusive with the presence of trisomy 12 and del(17p) (trisomy 12: 0% vs 14.3%, P=0.03; del(17p): 0% vs 13.1%, P=0.056), as recently suggested.[9] No differences were found regarding the distribution of *TP53, BIRC3* and *NFKBIE* mutations, del(13q) and del(11q) (Supplemental Figure 4). Clinical analyses performed in each cohort separately showed that M-CLL carrying the IGLV3-21^{R110} had a shorter TTFT compared to M-CLL lacking this mutation (P<0.02) and similar to U-CLL (P>0.12) (Supplemental Figure 5). Regarding epigenetic subtypes, i-CLL patients carrying the IGLV3-21^{R110} had a shorter TTFT compared i-CLL lacking this mutation (P<0.01) and similar to naïve-like CLL (n-CLL; P>0.1). Contrarily, i-CLL lacking the IGLV3-21^{R110} had a TTFT similar to memory-like CLL (m-CLL; P>0.5) **(**Figure 1E).

We then combined these novel data together with the previously published results of 489 independent patients from our International Cancer Genome Consortium (ICGC)-CLL cohort (54 MBL and 435 CLL)[6] and performed the analyses on a merged cohort of 1487 patients with IGLV3-21^{R110} status available (1323 CLL and 164 MBL) (Figure 1B, Supplemental Figure 6). The IGLV3-21^{R110} mutation was present in 78/1487 (5.2%) of patients, similarly distributed in CLL (73/1323, 5.5%) and MBL (5/164, 3.05%) (P=0.26). IGLV3-21^{R110} CLL encompassed both M-CLL (41/72, 56.9%) and U-CLL (31/72, 43.1%) [note that IGHV status was not available in one patient], while 58/68 (85.2%) were classified as i-CLL [epigenetic subtype was not available in 5 IGLV3-21^{R110} CLL]. All stereotyped subset #2 tumors (N=23; 22 CLL, 1 MBL) carried the IGLV3-21^{R110} but 42/66 (65.1%) IGLV3-21^{R110} CLL were not subset #2 [stereotypy was not available in 12 patients] (Figure 2A). No statistical differences were found regarding the distribution of genomic alterations in IGLV3-21^{R110} CLL classified as subset #2 compared to non-stereotyped IGLV3-21^{R110} cases (Supplemental Figure 7).

Compared to previous studies in smaller cohorts[5,6], clinical analyses in this integrated cohort provided a higher resolution to study the prognostic value of the IGLV3-21^{R110} within CLL subtypes. Regarding the epigenetic classification of CLL, we confirmed[6] that the presence of the IGLV3-21^{R110} stratifies i-CLL patients in two subgroups with significant differences in their TTFT (P<0.001), with i-CLL carrying the IGLV3-21^{R110} following a disease course similar to n-CLL (P=0.11) while i-CLL lacking the IGLV3-21^{R110} similar to m-CLL (P=0.23) (Supplemental Figure 8). Of note, i-CLL lacking the IGLV3-21^{R110} and classified as U-CLL had a shorter TTFT compared to i-CLL lacking the IGLV3-21^{R110} belonging to M-CLL (P=0.04) and m-CLL (P=0.007). Contrarily, i-CLL lacking the IGLV3-21^{R110} and classified as M-CLL had a TTFT virtually identical to m-CLL (P=0.7) (Figure 2B). In a multivariable model, IGLV3-21^{R110} retained prognostic value for TTFT independently of the disease stage and n-CLL subtype, whereas the i-CLL subtype did not retain independent prognostic value (Figure 2C). Regarding IGHV subtypes, we found that the IGLV3-21^{R110} has prognostic impact in M-CLL and U-CLL (Figure 2D). M-CLL carrying the IGLV3-21^{R110} had a significantly shorter TTFT compared to M-CLL lacking the mutation (P<0.001) but a trend towards a longer TTFT that U-CLL (P=0.054). Contrarily, U-CLL harboring the IGLV3-21^{R110} had a shorter TTFT than U-CLL lacking the mutation (P=0.04) (Figure 2D). Considering that 22/61 (36%) CLL carrying the IGLV3-21^{R110} belonged to the stereotyped subset #2, we performed an analysis stratifying IGLV3-21^{R110} CLL based on their stereotypy. All IGLV3-21^{R110} CLL had a similar TTFT independently of belonging to the stereotyped subset #2 (Figure 2E, Supplemental Figure 9). In line with these results, IGLV3-21^{R110} had prognostic value in a multivariable analysis independently of the disease stage and IGHV gene SHM status, while the stereotyped subset #2 lost its independent prognostic value (Figure 2F, Supplemental Figure 10). IGLV3-21^{R110} also retained

prognostic value independently of *TP53* mutations/deletions, trisomy 12, and deletions of 11q and 13q (Figure 2G).

Overall, we have developed a reliable msPCR assay suitable for IGLV3-21^{R110} screening in large cohorts of patients, allowing us to study the IGLV3-21^{R110} mutation in 1487 patients. We have found new associations of the IGLV3-21^{R110} with other CLL driver alterations while refined the prognostic value of this mutation within CLL subtypes. Our results confirm that the IGLV3-21^{R110} recognizes a subgroup of CLL with an aggressive clinical behavior independently of the epigenetic subtypes, IGHV gene SHM status, and stereotyped subset #2, being stereotyped subset #2 prognostically irrelevant for TTFT once considered the IGLV3-21^{R110}. Of note, IGLV3-21^{R110} CLL patients might benefit from targeted therapies[9] in detrimental of chemoimmunotherapy regimens.[5,6] The msPCR assay developed here, as well as similar PCR-based methods recently described,[9,14] might help to characterize the IGLV3-21^{R110}. Nonetheless, including IGLV3-21^{R110} and IGHV gene SHM testing in gene panels may result in a one-test, integrative next-generation sequencing solution that would indeed facilitate the routine (immuno)genomic characterization of CLL. Overall, this study confirms that the IGLV3-21^{R110}, but not stereotyped subset #2, fully recognizes a clinically-aggressive subgroup of CLL.

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Authorship

Contribution: C.S. contributed to the design of the study, performed experiments, analyzed and interpreted data, and wrote the manuscript. B.P.-B. performed experiments, and analyzed and interpreted data. N.R. contributed to sample preparation, and performed experiments. H.P.-A. performed calcium flux analyses. T.B., M.D.-F., M.K., A.C.-M., P.M., M.A., M.G., A.N.-B., E.Co., A.R.P., M.A., M.J.T., J.L., B.A.K., C.K.H., S.R.-G., A.E., C.J.W., G.G., T.Z., A.L.-G., J.I.M.-S., D.C., and J.D. provided samples, provided data, contributed to experiments and/or analyzed data. E.Ca. contributed to the design of the study and preparation of the manuscript. F.N. designed and supervised the study, performed experiments, analyzed and interpreted data, and wrote the manuscript. All authors reviewed and approved the manuscript.

Conflict-of-interest disclosure: C.J.W., G.G., B.A.K. and C.K.H. are inventors on a patent "Compositions, panels, and methods for characterizing chronic lymphocytic leukemia" (PCT/US21/45144). E.Ca. has been a consultant for Takeda, NanoString, AbbVie and Illumina; has received honoraria from Janssen, EUSPharma and Roche for speaking at educational activities and research funding from AstraZeneca and is an inventor on 2 patents filed by the National Institutes of Health, National Cancer Institute: "Methods for selecting and treating lymphoma types," licensed to NanoString Technologies, and "Evaluation of mantle cell lymphoma and methods related thereof", not related to this project. F.N. has received honoraria from Janssen, AbbVie, and SOPHiA GENETICS for speaking at educational activities. E.Ca. and F.N. have licensed the use of the protected IgCaller algorithm to Diagnóstica Longwood. The remaining authors declare no competing financial interests.

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Figure legends

Figure 1. IGLV3-21^{R110} detection through a mutation-specific polymerase chain reaction assay and its clinical value in two independent CLL cohorts. A. Primer design combining two forward primers aligning to distinct regions of the IGLV3-21 gene (green arrows), two R110-specific reverse primers matching the IGLJ1 and IGLJ2/3 genes, respectively (red and orange arrows). A third pair of primers targeting exon 9 of *FBXW7* (black arrows) was used as an internal control (top). QIAxcel Advanced System (QIAGEN) capillary electrophoresis image of the msPCR analysis. The black arrow indicates amplification of the internal control (*FBXW7* exon 9). The middle and bottom arrows (in green) indicate the two bands amplifying in IGLV3-21^{R110} mutated tumors. C+, positive control; C-, negative control; R110, sample positive for the IGLV3-21^{R110}; WT, wild-type (i.e., sample negative for the IGLV3-21^{R110}). **B.** Schema of the cohorts studied. UHH, University Hospital Heidelberg [cohort described in reference [15]; no IGLV3-21^{R110} analysis performed in this previous publication]; DFCI, Dana-Farber Cancer Institute; *120 CLL from DFCI have been previously published in reference [16]; Nadeu et al Blood 2021, reference [6]. **C.** Frequency of IGLV3-21^{R110} mutations in CLL subtypes in cohort 1 (top) and cohort 2 (bottom). n-CLL, naive-like CLL. **D.** Distribution of IGHV mutational load (% IGHV identity) and epigenetic subtypes within M-CLL, IGLV3-21^{R110} CLL, and U-CLL. **E.** Comparison of TTFT among patients with CLL stratified according to epigenetic subtypes and IGLV3-21^{R110} in cohort 1 (left) and cohort 2 (right).

Figure 2. IGLV3-21^{R110} status and prognostic value in an integrative cohort of 1487 patients with CLL (n=1323) or MBL (n=164). A. Oncoprint representation showing the disease stage, IGHV gene SHM status, epigenetic subtypes, and stereotyped subset of IGLV3-21^{R110} positive and negative CLL (left) and MBL (right). Bar plots on the right show the frequency of each variable in IGLV3-21^{R110} positive and negative CLL/MBL. **B.** TTFT curves of CLL patients stratified based on the epigenetic subtype. Patients classified as i-CLL were divided based on the presence or absence of the IGLV3-21^{R110}. i-CLL without (w/o) IGLV3-21^{R110} were further stratified based on their IGHV gene SHM status as M-CLL or U-CLL. **C.** Multivariable analysis of TTFT integrating disease stage (Binet stage), epigenetic subtypes, and IGLV3-21^{R110} in patients with CLL. **D.** TTFT curves of CLL patients stratified by IGHV gene SHM status and presence/absence of IGLV3-21^{R110}. **E.** TTFT curves of CLL patients stratified by IGHV gene SHM status, presence/absence of IGLV3-21^{R110}, and stereotyped subset. Patients were first stratified based on the IGLV3-21^{R110}. CLL without (w/o) the IGLV3-21^{R110} were divided based on their IGHV gene SHM status. CLL carrying the IGLV3-21^{R110} were stratified based on their stereotyped subset as stereotyped subset #2 (#2), no stereotyped subset #2 (no #2), or unknown subset (unknown). **F.** Multivariable analysis of TTFT integrating disease stage (Binet stage), IGHV gene SHM status, stereotyped subset #2, and IGLV3-21^{R110} in patients with CLL. **G.** Multivariable analysis of TTFT integrating Binet stage, IGHV gene SHM status, stereotyped subset #2, IGLV3-21^{R110}, *TP53* mutation/deletion, trisomy 12, and deletions of 11q and 13q in patients with CLL.

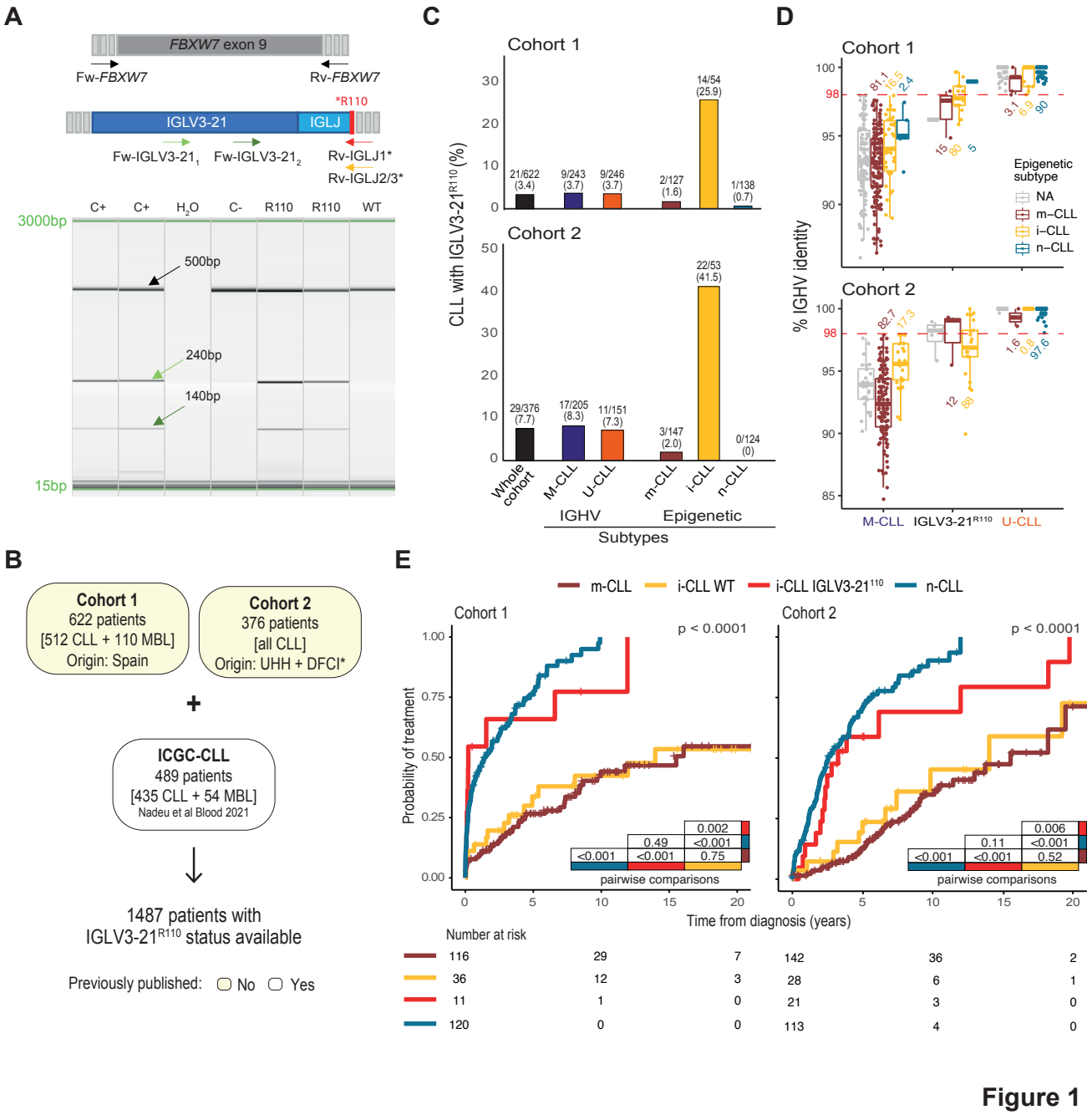

Figure 1

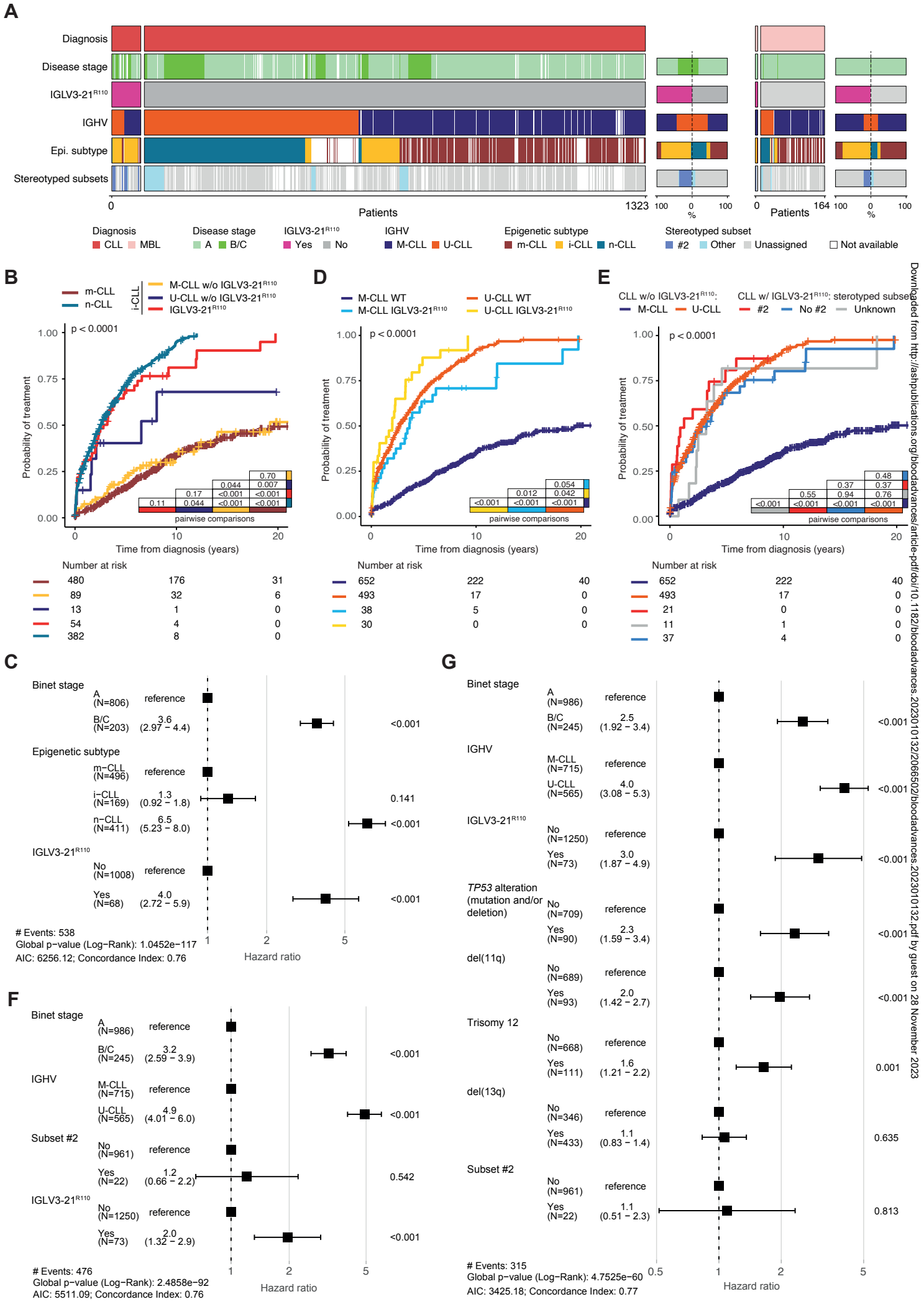

Figure 2