LETTER

CHRONIC LYMPHOCYTIC LEUKEMIA

Clinicobiological characteristics and treatment efficacy of novel agents in chronic lymphocytic leukemia with IGLV3-21^{R110}

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TO THE EDITOR:

The molecular composition of the leukemia-specific B cell receptor (BCR) is of key importance in chronic lymphocytic leukemia (CLL) [1]. Firstly, using the burden of somatic hypermutation (SHM) present in the immunoglobulin (IG) heavy chain (IGH) variable region, a distinction can be made between patients with mutated IGHV (<98% germline homology), that generally have indolent disease, and those with unmutated IGHV (≥98% germline homology), with generally more aggressive disease. Secondly, up to 41% of CLL patients can be grouped in subsets based on recurrent amino acid (aa) sequence similarities in their IGH rearrangement [2]. Patients within these IGH stereotyped subsets often demonstrate consistent clinicobiological profiles [3, 4]. However, individually, these IGH stereotyped subsets are rare: the largest subset, #2, represents 2.5% of all CLL cases [2].

Recently, a novel immunogenetically defined CLL subset was described, based on recurrent sequence similarities within the IG light chain (IGL) [5, 6]. Patients within this subset, referred to as IGLV3-21^{R110}, are characterized by rearrangement of a specific IG lambda variable gene, IGLV3-21, with a distinctive somatic hypermutation (SHM) present in the linker region between the IGLJ and IGLC genes (G110R). This mutation, which is detectable in CLL patients years prior to diagnosis, allows for antigen-independent, light chain based auto-aggregation of BCRs on the cell surface [7, 8]. This auto-aggregation is additionally dependent on germline residues exclusively present in alleles IGLV3-21*01 or IGLV3-21*04, establishing these alleles as heritable risk factors for the development of IGLV3-21^{R110} CLL [5, 9]. Importantly, this newly defined IGL stereotyped subset accounts for approximately 20% of all CLL patients and is associated with shorter time-to-first treatment (TTFT) and inferior overall survival, irrespective of IGHV mutational status [5]. Although two previous studies have examined the molecular characteristics of IGLV3-21^{R110} CLL, the combined cytogenetic, immunogenetic and mutational landscape of IGLV3-21^{R110} CLL remains incompletely characterized [5, 6]. In addition, the clinical impact of this IGL genotype in the context of therapy is still largely unknown.

In order to address these knowledge gaps, we characterized the light chain genotype, clinicobiological features, and response to therapy of patients enrolled in the HOVON-139/GIVE trial and the Dutch sub-cohort of the HOVON-141/VIsion trial. The HOVON-139/GIVE trial is a phase-II trial, evaluating the efficacy of first-line MRD-guided duration of treatment with obinutuzumab and venetoclax in unfit CLL patients [10]. The HOVON-141/VIsion trial is a phase-II trial in relapsed or refractory (R/R) CLL patients, evaluating the efficacy of MRD-guided ibrutinib and venetoclax combination treatment [11, 12]. To determine the IG light chain rearrangement, we performed PCR amplification and Sanger sequencing on pre-treatment cDNA. In the absence of cDNA, the IGLV3-21^{R110} light chain genotype was determined by PCR amplification and Sanger sequencing on gDNA, using novel, custom-designed primer sequences that target the IGLV3-21^{R110} leader region and the 5′ end of the intron sequence between the IGLJ and IGLC gene.

For 65/70 patients from the HOVON-139/GIVE trial and 129/133 patients from the Dutch cohort of the HOVON-141/VIsion trial, samples were available for IGL sequencing. The IGLV3-21^{R110} genotype was present in 16/65 patients (25%) in the first-line cohort, and at a similar frequency, in 32/129 patients (25%) in the R/R cohort. For an overview of clinical characteristics, stratified by IG light chain genotype, see Supplementary Table 1.

Copy number alterations (CNAs) were successfully determined through genomic array analysis for 191/194 patients (Fig. 1A and Supplementary Table 2), as previously described [13]. Loss of 13q14 and 11q22 were significantly enriched in IGLV3-21^{R110} patients, compared to all other patients (del13q14: 79% vs. 57%, $P = 0.009$; del11q22: 34% vs. 18%, $P = 0.03$). In contrast, the IGLV3-21^{R110} genotype and trisomy 12 or loss of 17p13 were mutually exclusive (trisomy 12: 0% vs. 12%, $P = 0.008$; del17p13: 0% vs. 12%, $P = 0.01$). There was no difference regarding the presence of genomic complexity (≥3 CNAs) or high genomic

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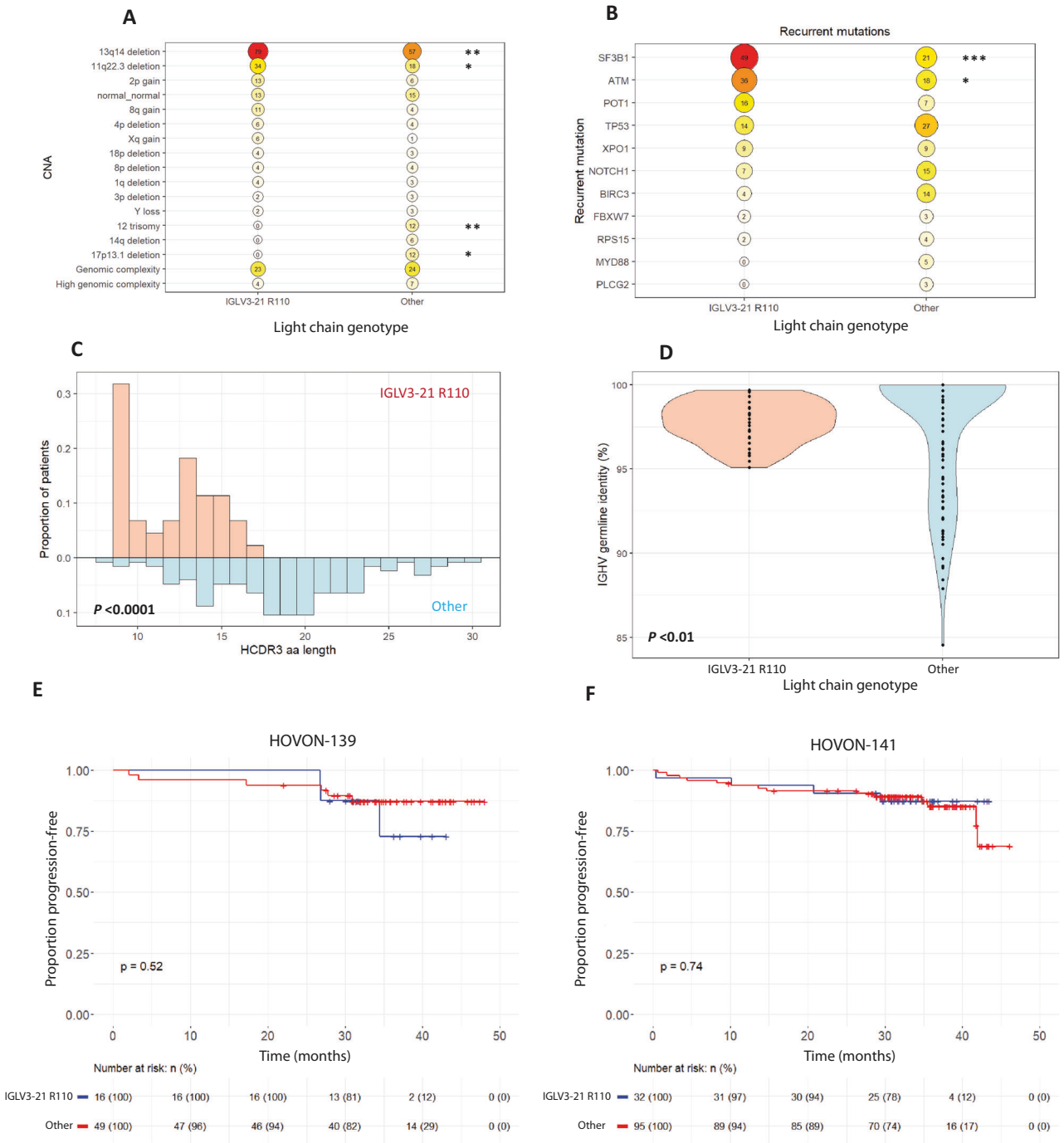

Fig. 1 Clinicobiological profile of IGLV3-21^{R110} CLL. A, **B** Balloon plots indicating the prevalence of specific copy number alterations (**A**) or somatic mutations (**B**), stratified by IG light chain genotype. The increasing size and darkening colors of the balloons indicate a higher frequency. The numbers in the balloons indicate the frequency of specific aberrations (%). The asterisks indicate the level of significance, as determined by a Fisher's exact test: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. **C** Mirrored histogram, indicating frequencies of HCDR3 aa length (**F**). Red bars indicate frequencies in IGLV3-21^{R110} patients, blue bars indicate frequencies in all other patients. *P*-values were obtained by Mann–Whitney Wilcoxon test. **D** Violin plot indicating the burden of somatic hypermutation present in the IGHV gene of the leukemia-specific rearrangement, stratified by light chain genotype. Violin width indicates density and can be interpreted as mirrored histograms. *P*-values were obtained by a Mann–Whitney Wilcoxon test. **E**, **F** Kaplan–Meier plots and risk tables estimating progression-free survival in the HOVON-139 (**C**) or HOVON-141/VIsion trial (**D**), stratified by light chain genotype. *P*-values were obtained by log-rank tests. aa amino acid, CNA copy number alteration, HCDR3 heavy-chain complementarity determining region 3.

complexity (≥5 CNAs) in both groups (≥3 CNAs, 23% vs. 24%, $P = 1$; ≥5 CNAs, 4% vs. 7%, $P = 0.7$). Targeted next-generation sequencing (NGS) for genes recurrently mutated in CLL was performed in 162/194 patients (Fig. 1B, Supplementary Table 3). Mutations in the *SF3B1* and *ATM* genes were significantly more common in IGLV3-21^{R110} patients, compared to all other patients (*SF3B1*: 49% vs. 21%, $P = 0.0008$; *ATM*: 36% vs. 18%, $P = 0.02$). No significant difference regarding the prevalence of *TP53* mutations was found comparing IGLV3-21^{R110} patients to all other patients (14% vs. 27%).

The leukemia-specific heavy-chain rearrangement could successfully be determined in 170/194 patients (Supplementary Table 4). IGHV and IGHD gene usage of IGLV3-21^{R110} patients was markedly skewed, compared to all other patients (IGHV, $P < 0.001$; IGHD, $P < 0.001$) (Supplementary Fig. 1A, B). Strikingly, the five most commonly used IGHV genes within IGLV3-21^{R110} patients (IGHV3-21, IGHV3-23, IGHV3-48, IGHV3-15, and IGHV3-11) are phylogenetically closely related [14]. IGHD3-3, the most commonly used IGHD gene in our entire cohort, was mutually exclusive with the IGLV3-21^{R110} genotype. Conversely, excessive junctional trimming, resulting in near complete loss of the IGHD gene, was exclusively present in IGLV3-21^{R110} patients (Supplementary Fig. 1B). IGHJ gene usage was not markedly different between the two groups ($P = 0.3$) (Supplementary Fig. 1C). Preferential use of IGHV and IGHD genes could lead to structural differences in the IG heavy-chain complementarity determining region 3 (HCDR3). Indeed, the HCDR3 was markedly shorter in IGLV3-21^{R110} patients, compared to all other patients (median HCDR3 aa length 13 [range: 9–17] vs. 19 [range 8–30], $P < 0.0001$) (Fig. 1C). In fact, in our cohort, no patient with the IGLV3-21^{R110} genotype had a HCDR3 length >17 aa. Lastly, the pattern of SHM in the IGHV gene was distinct between the two groups (Fig. 1D). Whereas the IGHV SHM imprint of IGLV3-21^{R110} patients was centered around the 98% cutoff, the range was much wider in all other patients (range 95.1–99.7% vs. 85–100%, $P = 0.01$). Notably, neither a completely unmutated IGHV (i.e., 100% germline homology), nor an excessively mutated IGHV (<95% homology) was present in IGLV3-21^{R110} patients.

No predictive impact of the IGLV3-21^{R110} genotype was observed regarding clinical response, minimal residual disease (MRD) status and progression-free survival (PFS) in either trial (Fig. 1 and Supplementary Fig. 1). Specifically, no significant differences were observed between patients with and without the IGLV3-21^{R110} genotype in clinical response rates and MRD detectability (MRD < 10^{-4}) after first-line induction with two cycles of obinutuzumab, followed by six cycles of venetoclax and obinutuzumab, followed by six cycles of venetoclax alone (%CR: 56% vs. 52%, $P = 0.9$; %uMRD < 10^{-4} in peripheral blood: 87% vs. 98%, $P = 0.15$) (Supplementary Fig. 1D, F). Also, no significant differences could be found between patients with and without the IGLV3-21^{R110} genotype in clinical response rate and MRD detectability (MRD < 10^{-4}) in the R/R setting following treatment with 15 cycles of combination treatment with ibrutinib and venetoclax (%CR: 64% vs. 72%, $P = 0.9$; %uMRD<10^{-4} in peripheral blood: 52% vs. 61%, $P = 0.11$) (Supplementary Fig. 1D, F). Finally, no significant differences in PFS were observed between IGLV3-21^{R110} patients and patients with any other light chain in either trial (HOVON-139/GIVE: 100% vs. 94%, $P = 0.52$; HOVON-141/VIsion: 91% vs. 92%, $P = 0.74$) (Fig. 1E, F).

Taken together, in our study, we have characterized the clinicobiological features of the largest cohort of IGLV3-21^{R110} patients ($n = 48$) reported thus far. The prevalence of the IGLV3-21^{R110} genotype in our cohorts was 25%, which is higher compared to the prevalence of 7–17% identified in previously studied populations [5, 6]. This discrepancy could be explained by enrichment of IGLV3-21^{R110} in clinical trial populations, as IGLV3-21^{R110} CLL is associated with a shorter TTFT, or due to geographical differences in the frequency of the IGLV3-21*01 and IGLV3-21*04 alleles [5].

We demonstrate that CLL with the IGLV3-21^{R110} is typified by a distinct molecular profile. In these patients, lesions targeting *SF3B1* and *ATM* are enriched. This is in line with the data reported by Nadeu et al., who characterized the genetic landscape of a cohort of 28 IGLV3-21^{R110} patients using whole-genome sequencing [6]. Moreover, genomic array analysis revealed enrichment of del13q14 in IGLV3-21^{R110} patients, but mutual exclusivity with trisomy 12 and del17p13 in our cohort. These patterns suggest that CLL with IGLV3-21^{R110} genotype may rely on distinct intracellular signaling pathways, including aberrant splicing and dysfunctional DNA damage repair mediated through loss of *ATM*, but not *TP53*. This hypothesis warrants additional functional validation. In addition, IGLV3-21^{R110} patients were characterized by distinctive IGH rearrangements, using phylogenetically related IGHV genes with markedly shorter HCDR3s and a borderline IGHV SHM imprint. These findings may reflect additional (patho) physiological selection pressure involving not only the IG light chain, but also the IG heavy chain. Lastly, despite its distinct clinicobiological profile, there was no evidence for a predictive impact of the IGLV3-21^{R110} genotype on the efficacy of the novel therapies employed in the HOVON-139/GIVE and HOVON-141/VIsion trials. This is supported by the stable prevalence of the IGLV3-21^{R110} genotype in our first-line and R/R cohort. Our results suggest that the evaluated regimens of novel targeted therapies may mitigate the adverse risk profile of IGLV3-21^{R110} CLL. These observations require further validation in a larger series, as the number of IGLV3-21^{R110} patients that we evaluated was limited, especially in the HOVON-139/GIVE trial. Furthermore, to determine whether these patients should preferentially receive novel therapies, characterization of the predictive impact of IGLV3-21^{R110} in the setting of chemoimmunotherapy is warranted.

METHODS

For an extended version of the methods, please refer to the Supplementary Information.

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AUTHOR CONTRIBUTIONS

PJH, MDL, and AWL conceived of the study. PJH and YEE performed the light chain sequencing. JMND, SK, CUN, APK, and MDL were involved in designing, operating and biobanking the HOVON-139/GIVE and/or HOVON-141/VIsion trials and facilitated the availability of the samples. CHMM, AMvdKK, ORFM, and MMM performed and analyzed the genomic array and targeted NGS analysis. LME performed the heavy-chain sequencing. KH assisted in primer design. PMK contributed to the data analysis. PJH, MDL, AWL, PMK, and PEW interpreted and reviewed the data. PJH, MDL, and AWL wrote the manuscript. All authors critically read and approved the final version of the manuscript.

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ADDITIONAL INFORMATION

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