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LETTER

Clinical impact of immunoglobulin heavy chain repertoire in mantle cell lymphoma: A study from the Fondazione Italiana Linfomi (FIL) Phase III MCL0208 trial

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Mantle cell lymphoma (MCL) is an aggressive B-cell non-Hodgkin lymphoma (NHL) characterized by a distinct biological and clinical profile.[1] It is generally associated with poor long-term outcomes, with a median overall survival (OS) of approximately 5 years.[2] Immunologic mechanisms in the pathogenesis of lymphoproliferative disorders are increasingly recognized,[3–5] and increasing evidence suggests that antigenic selection may play a key role in the pathogenesis of MCL.[6,7] However, while IGHV mutation status and stereotyped B-cell receptors (BCRs) are well-established prognostic markers in chronic lymphocytic leukemia (CLL),[8] their role in MCL remains unclear. Previous studies described a biased VDJ gene usage rearrangement in MCL, with a preferential usage of IGHV3-21, IGHV4-34, IGHV1-8, and IGHV3-23 genes[9] and suggested that the 97% IGHV gene identity cutoff was able to predict MCL survival.[10] In addition, similarly to CLL,[11] stereotyped receptors have been identified in MCL patients[9] demonstrating the biological and clinical relevance of clustering according to BCR in these patients. However, the available data are derived from retrospective and heterogeneous cohorts, making it difficult to draw definitive conclusions. In this study, we investigate the role of the IGH repertoire and antigenic selection in the large, homogeneously treated and prospective cohort of newly diagnosed MCL patients from the Fondazione Italiana Linfomi (FIL) MCL0208 trial. The availability of long-term follow-up and MRD data provided a unique opportunity for this analysis.

The FIL MCL0208 protocol (NCT02354313) is a Phase III, multicenter, open-label, randomized trial, designed to evaluate the efficacy and safety of 24-month lenalidomide (LEN) maintenance versus observation (OBS) in MCL patients (18–65 years old) in complete or partial remission after first-line high-dose chemo-immunotherapy followed by autologous stem cell transplantation.[12,13]

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Minimal residual disease (MRD) monitoring in bone marrow (BM) and peripheral blood (PB) was included in this study as a secondary endpoint to establish the molecular response. The clinical trial and the biological study were approved by the ethics committees of all the enrolling centers, and all patients provided written informed consent for research purposes in accordance with institutional review board requirements and the Declaration of Helsinki. Collection and storage of biological samples for MRD monitoring and DNA extraction were performed as described in Supplementary Methods. Immunoglobulin heavy chain (IGH) rearrangements were screened at baseline in all enrolled patients using classical polymerase chain reaction and Sanger sequencing as previously published by Voena et al.[14] (see Supplementary Methods for more information). Focusing on the Sanger sequencing results, 211 out of 300 untreated young MCL patients (70%) enrolled in the FIL MCL0208 clinical trial had an available sequenced IGH rearrangement: all 211 sequenced cases showed IGH-VH clonality and a productive CDR3 amino acid sequence. In contrast, the JH regions in two patients showed a polyclonal background, resulting in 209/300 (70%) interpretable sequences for IGH usage analysis. In this series, we found 33 IGHV genes (Figure 1A). The most prevalent were IGHV3-21 ($n = 45$, 22%) and IGHV4-34 ($n = 33$, 16%). Furthermore, among the 26 IGHD and 6 IGHJ genes identified, IGHD3-3 ($n = 20$, 10%) and IGHJ4 ($n = 83$, 40%) were the most representative groups, respectively (Figure 1B,C). Interestingly, IGHV3-21 rearrangements were strongly associated with the IGHD3-3 ($n = 10/45$, 22%) and IGHJ6 (66%) genes, whereas IGHV4-34 recombined preferentially with IGHD2-2 (24%) and IGHJ4 (33%) genes (Figure 1D,E). Although restricted VH CDR3 regions were identified in 4/209 (2%) patients (Table S1), none of the restricted CRD3 sequences were assigned to a described stereotyped CLL subset by the ARResT/AssignSubsets application. Overall, the preferential pairing of IGHV3-21 and IGHV4-34 with specific DH and JH segments indicated a biased VDJ recombination pattern.

A comparison of the selected patients for IGH repertoire analysis ($n = 209$, Table S2) and the unselected ones ($n = 91$) revealed several

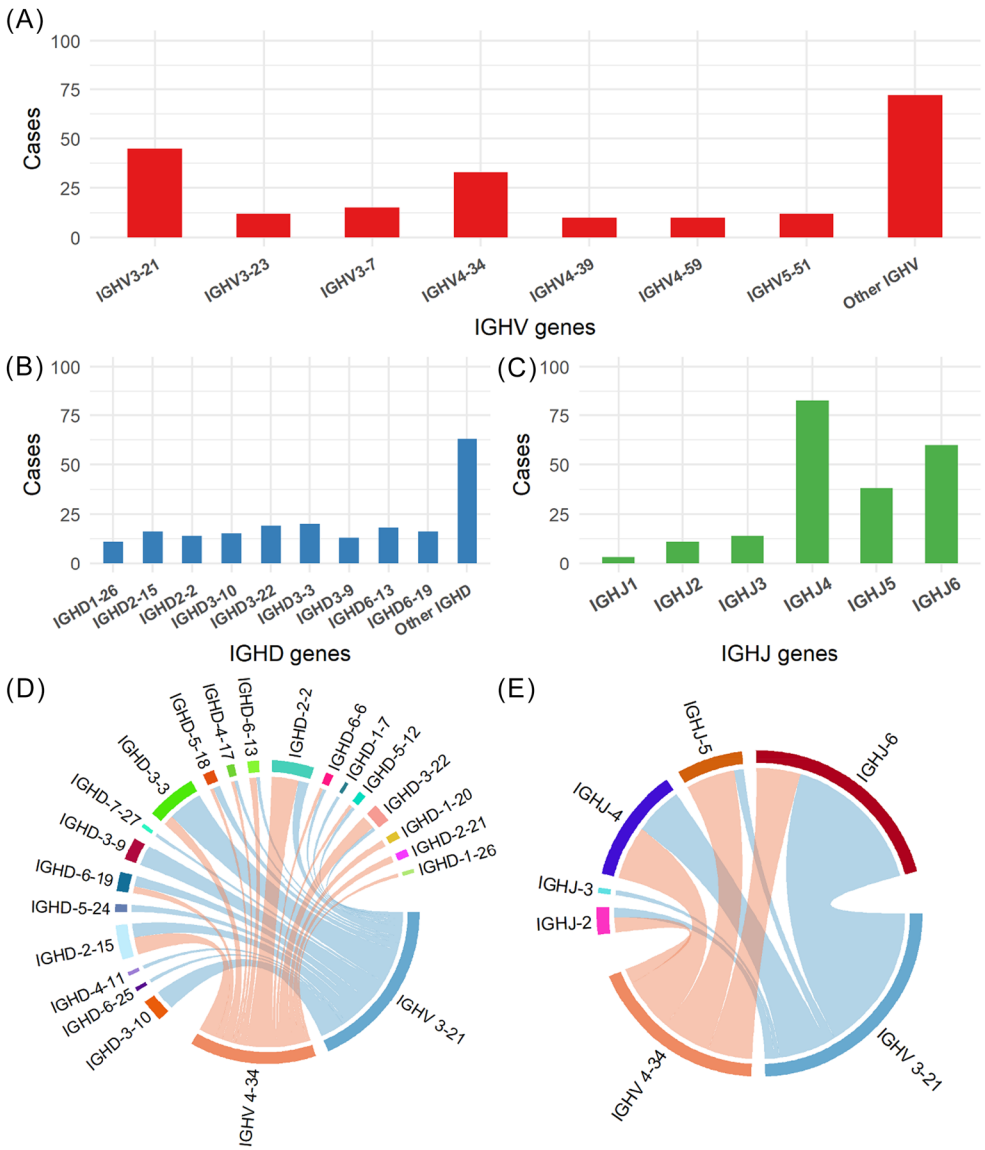

FIGURE 1 **Overview of IGH repertoire in MCL0208.** Landscape of IGHV **(A)**, IGHD **(B)**, and IGHJ **(C)** genes identified in MCL0208 cohort. Associations between IGHV and IGHD genes **(D)** and IGHV and IGHJ genes **(E)** in MCL0208 cohort. In (D) and (E), each arc represents a specific immunoglobulin gene, and ribbons connecting arcs indicate observed associations between genes; the thickness of each ribbon is proportional to the frequency of that specific pairing in the cohort.

notable differences. The median follow-up for patients analyzed for IGH repertoire analysis was 84 months from enrollment. The selected patients were older (58 vs. 55 years, P = 0.025) and exhibited more frequently intermediate or high Mantle Cell Lymphoma International Prognostic Index (MIPI) scores (51% vs. 15%, P = 0.001) and *KMT2D* mutation (15% vs. 2%, P = 0.025). This selection likely reflects the focus on MRD-evaluable cases with higher MCL BM or PB infiltration,[15] which may account for their slightly worse outcome both in terms of progression-free survival (PFS, P = 0.0025, Figure S1A) and OS, (P = 0.072, Figure S1B). Interestingly, patients with IGHV3-21 were often younger than the ones with other IGHV genes (median age 54 vs. 58 years, respectively, P = 0.003) and were characterized by lower Ki67 index (86% vs. 64% cases, respectively, P = 0.007) and lower MIPI score (73% vs. 43% cases, respectively, P < 0.001). Furthermore, IGHV4-34 patients showed no cases of blastoid morphology (0% vs. 11%, respectively, P = 0.049) and a nonsignificant trend toward a lower frequency of *TP53* disruption compared to the rest of the cohort (3.8% vs. 17%, P = 0.084, Table S3). Notably, IGHV4-34 patients showed longer PFS (5-year PFS 60% [45%–80%] vs. 46% [39%–54%], respectively; P = 0.034, Figure 2A) and longer OS (5-year OS 91% [81%–100%] vs. 71% [65%–79%], respectively, P = 0.022; Figure 2B) compared to all other IGHV cases. Interestingly, although long-term PFS and OS in IGHV3-21 patients were not significantly different when compared to the other VH genes (5-year PFS 51% [37%–68%] and 47% [40%–55%], respectively, P = 0.53, Figure S2A; 5-year OS 81% [70%–94%] and 72% [66%–80%], respectively, P = 0.51, Figure S2B), a time-dependent effect on PFS was observed in these patients, suggesting that the hazard ratio may change over time. Lastly, lenalidomide did not appear to have a significant effect on PFS according to the IGHV4-34 and IGHV3-21 genes (Figure S3). Notably, these findings are consistent with those of Khouja et al.,[16] who previously reported improved PFS and OS in IGHV4-34 patients. Although biased selection of IGHV4-34 and IGHV3-21 genes is not specifically associated with MCL—having been previously reported in other lymphoproliferative neoplasms[17–19]—and skewed IGHV4-34 usage has also been reported in autoimmune disease,[20] our findings further support the hypothesis of reactive lymphomagenesis in at least a subset of MCL cases. In our cohort, the analysis of IGHV sequence showed a median IGHV germline homology of 99.19% (89.93%–100%). Modeling IGHV germline identity using restricted cubic splines suggested 97% as a potential cutoff to discriminate different risks of progression (Figure S4). Overall, 184/209 cases (88%) showed IGHV homology ≥ 97% (FR1-unmutated) and 25/209 (12%) had IGHV homology < 97% (FR1-mutated). Interestingly, although no statistically significant differences in baseline clinical and molecular prognostic factors were identified between IGHV FR1-mutated and FR1-unmutated patients (Table S4), a significant better PFS was observed in FR1-mutated patients compared to FR1-unmutated cases (5-year PFS 64% [45%–80%] and 46% [39%–54%], respectively, P = 0.045, Figure 2C), while OS was similar between the two groups (5-year OS 80% [65%–97%] and 74% [67%–81%], respectively, P = 0.50, Figure 2D). A preferential usage of IGHV4-59 (5/25; 20%) and

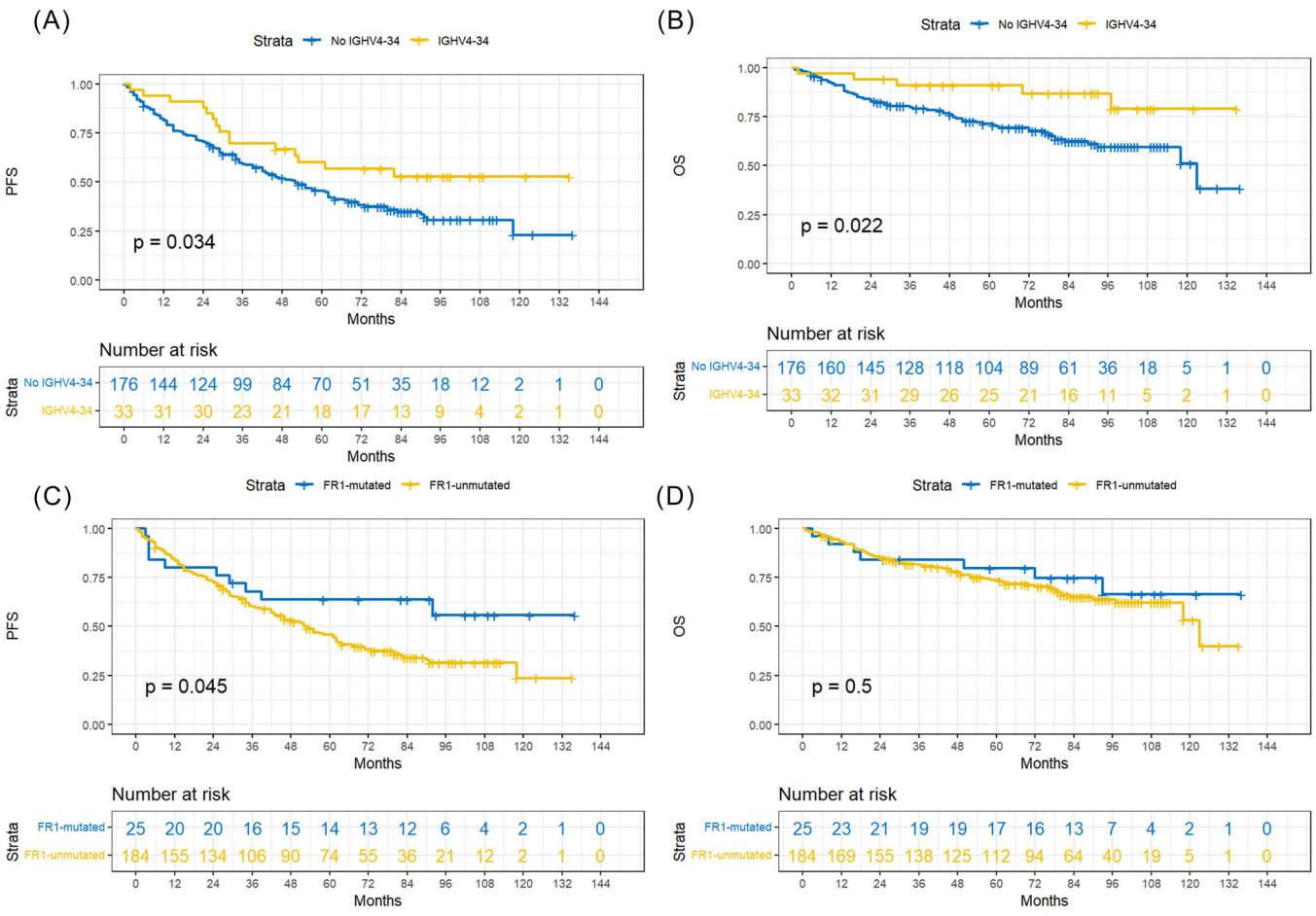

FIGURE 2 **Survival analysis according to IGHV4-34 gene and 97% IGHV homology cutoff.** Kaplan–Meier curves for progression-free (PFS) and overall survival (OS): **(A)** and **(B)** compare IGHV4-34 patients to other IGHV cases; **(C)** and **(D)** compare FR1-mutated (IGHV homology < 97%) and FR1-unmutated (IGHV homology ≥ 97%) patients.

IGHV3-74 (3/25; 12%) emerged in the FR1-mutated group, while in the FR1-unmutated series, IGHV3-21 and IGHV4-34 were the most representative IGHV genes ($n = 45/184$, 24% and $n = 31/184$, 17%, respectively, Figure S5A). In addition, when splitting patients in FR1-truly unmutated (IGHV homology = 100%), FR1-minimally mutated (IGHV homology 97%–99.99%), and FR1-mutated (IGHV homology < 97%), no patient carrying IGHV3-21 resulted FR1-mutated, whereas 2/33 (6%) patients carrying IGHV4-34 belonged to the FR1-mutated group (Figure S5B,C). Interestingly, patients with FR1-minimally mutated status exhibited intermediate PFS outcomes (5-year PFS 51% [43%–61%]), falling between those observed in FR1-mutated (5-year PFS 64% [47%–86%]) and FR1-truly unmutated groups (5-year PFS 35% [25%–49%], P = 0.028, Figure S6A). However, no significant differences in OS were observed among the three groups (P = 0.31, Figure S6B).

Navarro et al.[10] showed that highly mutated IGHV cases (<97% identity) correlated with lower MCL genomic complexity, enrichment for non-nodal subtypes, a memory B-cell transcriptional signature, and better OS. In our study, the prognostic relevance of IGHV mutation status was confirmed in a multivariable PFS model including IGHV gene usage, age, *TP53* disruption, *KMT2D* mutation, Ki67 ≥ 30%, blastoid histology, and high MIPI score (HR PFS for FR1-unmutated = 2.12, [1.10–4.10], P = 0.025, Table S5). This suggests that the 97% identity cutoff may help to delineate a biologically distinct subset of classical MCL with features that, at least in part, may speculatively overlap with those of non-nodal disease. Lastly, in our series, differently from BTK inhibitors,[21] lenalidomide did not impact on PFS according to IGHV mutation status (Figure S7). Although IGH sequencing was initially planned for MRD markers screening purposes, and therefore FR1 primers and Sanger sequencing were not optimized for IGHV mutation analysis, this study highlights the clinical significance and consistent prognostic relevance of IGHV usage and mutation status in MCL. Notably, Thurner et al. identified anti-LRPAP1 autoantibodies in 13% of MCL patients, suggesting a possible antigenic role for LRPAP1, especially given their clonal light-chain restriction and association with better outcomes.[6,7]

Together with prior evidence, the present findings tentatively suggest the hypothesis of a speculative antigen-driven and potentially autoreactive mechanism in the pathogenesis of MCL. Further research is necessary to ascertain whether a skewed IG repertoire and stereotyped BCRs may indicate an autoreactive component of MCL pathogenesis. Finally, collaborative efforts analyzing larger cohorts of MCL patients from multiple clinical trials, along with the development of dedicated bioinformatic tools for immunoglobulin data analysis, may clarify whether IGHV genes, IGHV mutational status, and potential stereotyped BCRs could have a prognostic role and inform the use of immunomodulatory agents and BTK inhibitors in MCL patients.

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AUTHOR CONTRIBUTIONS

Simone Ragaini: Conceptualization; methodology; data curation; formal analysis; writing—original draft; writing—review and editing. **Elisa Genuardi**: Conceptualization; methodology; data curation; formal analysis; writing—original draft; writing—review and editing. **Beatrice Alessandria**: Conceptualization; methodology; data curation; formal analysis; writing—original draft; writing—review and editing. **Aurora Maria Civita**: Investigation; data curation; writing—review and editing. **Andrea Evangelista**: Methodology; formal analysis; writing—review and editing. **Daniela Drandi**: Investigation; writing—review and editing. **Carlotta Montana**: Investigation; writing—review and editing. **Sofia Russo**: Investigation; writing—review and editing. **Chiara Consoli**: Investigation; writing—review and editing. **Mariapia Pironti**: Investigation; writing—review and editing. **Stefan Hohaus**: Investigation; writing—review and editing. **Gerardo Musuraca**: Investigation; writing—review and editing. **Nicola Cascavilla**: Investigation; writing—review and editing. **Chiara Ghiggi**: Investigation; writing—review and editing. **Monica Tani**: Investigation; writing—review and editing. **Gianluca Gaidano**: Investigation; writing—review and editing; supervision. **Jacopo Olivieri**: Investigation; writing—review and editing. **Sara V. Usai**: Investigation; writing—review and editing. **Nicola Di Renzo**: Investigation; writing—review and editing. **Mario Luppi**: Investigation; writing—review and editing. **Vittorio Stefoni**: Investigation; writing—review and editing. **Federica Cavallo**: Investigation; writing—review and editing. **Marco Ladetto**: Conceptualization; methodology; writing—review and editing; supervision; funding acquisition. **Simone Ferrero**: Conceptualization; methodology; writing—original draft; writing—review and editing; supervision; project administration; funding acquisition.

CONFLICT OF INTEREST STATEMENT

S.R. received speaker's honoraria from Roche, Beigene, Pierre Fabre, and Novartis; received travel grants from Kyte-Gilead. **S.F.** is a consultant for Janssen, EUSA Pharma, AbbVie, and Sandoz; is on the advisory board of Janssen, EUSA Pharma, Recordati, Incyte, Roche, AstraZeneca, CSL Behring, and Italfarmaco; received speaker's honoraria from Janssen, EUSA Pharma, Recordati, Lilly, Beigene, Gilead, and Gentili; and received research funding from Gilead, Beigene, and Morphosys. **M.L.** has relationship in terms of consultancy, participation to advisory boards, invitation to scientific meetings, institutional research support, and contracts with: AbbVie, Acerta, Amgen, ADC Therapeutics, BeiGene, Celgene/BMS, Eusapharma, GSKI, Gentili, Gilead/Kite, Novartis, Incyte J&J, Jazz, Lilly, Regeneron, Roche, and Sandoz; he has non-financial interests as PI or strategic investigator in studies supported by: Celgene, J&J, BeiGene, and ADC Therapeutics. **G.G.** has been consulting in Advisory Boards for AbbVie, AstraZeneca, Beigene, Incyte, Johnson & Johnson, and Lilly, and received speaker's honoraria from AbbVie, AstraZeneca, Beigene, Hikma, Incyte, Johnson & Johnson, and Lilly. The remaining authors declare no competing financial interests.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information can be found in the online version of this article.

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