


Katayoon Shirneshan⁷, Annika Gutermuth⁷, Christian Thiede⁵,
Ulrich Germing⁸, Thomas Schroeder⁸ , Guido Kobbe⁸,
Sabrina Klesse¹, Christian Koenecke¹, Brigitte Schlegelberger⁶,
Nicolaus Kröger⁹, Detlef Haase⁷, Konstanze Döhner⁴,
Wolfgang R. Sperr^{10,11} , Peter Valent^{10,11}, Arnold Ganser¹,
Felicitas Thol¹, Torsten Haferlach², Uwe Platzbecker³,
Michael Heuser¹

¹Department of Hematology, Hemostasis, Oncology and Stem Cell Transplantation, Hannover Medical School, Hannover, Germany

²MLL Munich Leukemia Laboratory, Munich, Germany

³Department of Hematology and Cell Therapy, Medical Clinic and Policlinic I, Leipzig University Hospital, Leipzig, Germany

⁴Department of Internal Medicine III, University Hospital Medical Center, Ulm, Germany

⁵Department of Internal Medicine I, University Hospital Carl Gustav Carus, Technical University Dresden, Dresden, Germany

⁶Department of Human Genetics, Hannover Medical School, Hannover, Germany

⁷Department of Hematology and Oncology, Georg-August-Universität-Göttingen, Göttingen, Germany

⁸Department of Hematology, Oncology and Clinical Immunology, Medical Faculty, University of Duesseldorf, Duesseldorf, Germany

⁹Department of Stem Cell Transplantation, University Medical Center Hamburg-Eppendorf, Hamburg, Germany

¹⁰Department of Internal Medicine I, Division of Hematology & Hemostaseology, Medical University of Vienna, Vienna, Austria

¹¹Ludwig Boltzmann Institute for Hematology and Oncology, Medical University of Vienna, Vienna, Austria

Correspondence

Michael Heuser, Department of Hematology, Hemostasis, Oncology and Stem Cell Transplantation, Hannover Medical School, Carl-Neuberg Strasse 1, 30625 Hannover, Germany.
Email: heuser.michael@mh-hannover.de

ORCID

Victoria Panagiota  <https://orcid.org/0000-0001-9715-6391>

Thomas Schroeder  <https://orcid.org/0000-0002-1653-7959>

Wolfgang R. Sperr  <https://orcid.org/0000-0003-3288-8027>

REFERENCES

- Haase D, Stevenson KE, Neuberg D, et al. TP53 mutation status divides myelodysplastic syndromes with complex karyotypes into distinct prognostic subgroups. *Leukemia*. 2019;33(7):1747-1758.
- Germing U, Lauseker M, Hildebrandt B, et al. Survival, prognostic factors and rates of leukemic transformation in 381 untreated patients with MDS and del(5q): a multicenter study. *Leukemia*. 2012;26(6):1286-1292.
- Bernard E, Nannya Y, Hasserjian RP, et al. Implications of TP53 allelic state for genome stability, clinical presentation and outcomes in myelodysplastic syndromes. *Nat Med*. 2020;26(10):1549-1556.
- Lindsley RC, Saber W, Mar BG, et al. Prognostic mutations in myelodysplastic syndrome after stem-cell transplantation. *N Engl J Med*. 2017;376(6):536-547.
- Steensma DP, Bejar R, Jaiswal S, et al. Clonal hematopoiesis of indeterminate potential and its distinction from myelodysplastic syndromes. *Blood*. 2015;126(1):9-16.
- Kleiblova P, Shaltiel IA, Benada J, et al. Gain-of-function mutations of PPM1D/Wip1 impair the p53-dependent G1 checkpoint. *J Cell Biol*. 2013;201(4):511-521.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

Received: 11 March 2021 | Accepted: 15 March 2021

DOI: 10.1002/ajh.26167

Mutational and immunogenetic landscape of HCV-associated B-cell lymphoproliferative disorders

To The Editor:

Besides robust epidemiological evidences, the direct link between HCV and B-cell lymphoproliferative disorders (LPDs) has been sustained by clinical studies that showed lymphoma regression after HCV eradication.^{1,2} However, data regarding molecular characteristics of HCV-associated LPDs are still limited so far. The main purpose of our study was to explore the mutational profile of 27 patients with previously untreated HCV-associated low-grade LPDs by means of an extensive NGS genes panel.

Seven and twenty patients were diagnosed and managed at the Division of Hematology, Fondazione IRCCS Policlinico San Matteo, Pavia, Italy and at the Reference Center for Mixed Cryoglobulinemia, University "La Sapienza", Rome, Italy, respectively. For all patients, either peripheral blood (PB) (n = 19) or bone marrow (BM) (n = 6) samples or formalin-fixed paraffin-embedded (FFPE) tissue (n = 2) obtained at the time of LPD diagnosis were available (online supplemental methods). Clinical and virological data were retrospectively collected. The study was approved by the Ethics Committees of the Fondazione IRCCS Policlinico San Matteo, Pavia, Italy and of Sapienza University, Rome, Italy.

Immunoglobulin heavy variable (IGHV) and light variable chain (IGLV) genes rearrangements were assessed using the IGH Somatic Hypermutation Assay v2.0 kit (Invivoscribe, San Diego, California) or according to the BIOMED-2 guidelines. All IGH, IGK (κ light chain) and IGL (λ light chain) rearrangements were analyzed using the IMGT databases and the IMGT/V-QUEST tool to identify CDR3 AA sequences. Heavy chain CDR3 (HCDR3) and light chain CDR3 (LCDR3) stereotypy and homology to anti-HCV E2 antibodies and rheumatoid factors (RF) were searched, as previously described in

13 patients.³ Targeted NGS analysis (144 genes panel) was performed by a probe-capture based strategy, as we previously described⁴ (Table S1). The average depth of coverage was 1700x. Bioinformatics analysis was performed by an ad hoc pipeline, as already reported.⁵

Quantitative variables were summarized as median and range. Qualitative variables were described as counts and relative frequencies of each category. Association between two categorical variables was evaluated by Fisher's exact test. Mann-Whitney test was used to compare a quantitative variable among two independent groups of patients. Overall Survival (OS) was calculated as the time between diagnosis and death for any cause or last follow-up. Progression-Free Survival (PFS) was defined as the time between diagnosis and the date of progression, or death or last follow-up. OS and PFS were estimated by Kaplan-Meier product-limit method. Note, p values < .05 were considered significant. Statistical analyses were performed by Stata 16 (StataCorp. 2019. Stata Statistical Software: Release 16, College Station, TX: StataCorp LLC).

Clinical and virological characteristics of patients with indolent B-cell non-Hodgkin's lymphomas (B-NHLs) ($n = 22$) and type II mixed cryoglobulinemia (MC) ($n = 5$) are listed in Table S2. After a median follow-up of 4.3 years (range 0.3–25.2), the 5-year OS was 71% (95% CI 41.6%–87.5%) and 5-year PFS was 66.9% (95% CI 40%–83.8%).

Data on productive IGHV rearrangements were available for 24 patients (Figure S1 and Table S3). So, IGHV1-69 was the most used rearrangement (6, 25%), especially in type II MC (4/5, 80%). IGLV rearrangement was studied in 15 cases, revealing strong gene usage bias, with *Vk3D-20* as the most frequent (6/15, 40%) and preferential pairing with IGHV1-69 in all cases but one. The KCD3 sequences were stereotyped in 10 out of 14 cases, all treated with direct-acting antiviral agents (DAA) (Table S3). Patients with stereotyped KCD3s showed a higher number of hematological responses to AT compared with non-stereotyped KCD3 group (60% vs 0%, respectively; difference between risks 60%, 95% CI: 30%–90%; $p = .044$).

The NGS analysis revealed 85 somatic mutations in 42 genes with at least one mutation (median three, range 1–8) in 25 cases (Figure 1, Panel A). Two patients with type II MC did not show mutations. Overall, the most frequently mutated genes were *TNFAIP3* (7, 28%), *KMT2C* (5, 20%), *FAT4* (5, 20%), *TBL1XR1* (5, 20%), *FAT1* (4, 16%), *CARD11* (3, 12%), *KLF2* (3, 12%), *KLHL6* (3, 12%), *PTPRD* (3, 12%). Of note, patients with type II MC did not harbor recurrent mutations of *KMT2C*, *FAT4*, *TBL1XR1*, *CARD11* genes. Full annotation and variant allele frequencies for each mutation are reported in Table S4.

Overall, genes regulating chromatin organization were the most mutated (48.2%), followed by genes belonging to NF- κ B signaling (44.4%), cell communication (40.7%), NOTCH (22.2%) and BCR (18.5%) signaling pathways (Figure S2).

Remarkably, while IGHV status did not significantly differ according to involved pathway (Figure 1, Panel B), the *Vk3D-20* and

Vk3-15 subsets of stereotyped KCD3 were enriched in mutations involving chromatin organization, NF- κ B, BCR and NOTCH signaling pathways compared to the patients with non-stereotyped KCD3s ($p = .002$). Conversely, the latter group was highly enriched ($p < .001$) in sporadically mutated genes involving transcriptional regulation, MAPK, and protein-binding signaling pathways (Table S5). The relative frequencies of pathways and IGLV rearrangements according to LCD3s are illustrated in Figure 1 (Panel C).

In this study we performed an extended NGS analysis to characterize 27 HCV-positive LPDs.

Interestingly, we found that pathways regulating epigenetics were the most involved, in line with the fact that HCV might modulate epigenetics in hepatocellular carcinoma (HCC) tumorigenesis.⁶ It is also noteworthy that the aforementioned pathways, together with NF- κ B, BCR and NOTCH signaling pathways, were more frequently mutated in patients harboring stereotyped LCD3s, who also showed a higher number of hematological responses to AT. These findings suggest that a subset of HCV-related LPDs may harbor distinct molecular features as well as specific immunogenetic signature, as recently reported,³ further supporting the concept that specific stereotyped B-cell receptors (BCRs) may promote or select oncogenic mutations in LPDs, as already proposed in chronic lymphocytic leukemia.

We recognized that our data suffer from some limitations: we could not confirm the somatic origin of mutations because of the lack of control samples; moreover, the retrospective nature of the study, together with the relatively small size of the series, the heterogeneity of LPDs and the diverse follow-up, limited us to provide significant associations between clinical and molecular features.

In summary, to the best of our knowledge, this was the first study that explored by means of an extensive targeted NGS panel the mutational profile of HCV-associated B-cell LPDs, identifying recurrently mutated genes and pathways. Our results demonstrate also that a subset of HCV-related LPDs may harbor distinct molecular and immunogenetic features and bring out the potential correlation between specific BCR configurations, genetic lesions and regression of the lymphoproliferation after HCV eradication. A clinical trial with DAAs in indolent HCV-positive lymphomas is ongoing with ancillary biological studies and will provide further data in this setting (NCT02836925).

ACKNOWLEDGEMENTS

This work was supported by grants of AIRC (under IG 2017- ID. 20 767 project – P.I. Luca Arcaini) and of “Ricerca Corrente Fondazione IRCCS Policlinico San Matteo” (P.I. Luca Arcaini), by the Italian Ministry of Education, University and Research (MIUR) to the Departments of Molecular Medicine (DMM) of the University of Pavia under the initiative “Dipartimenti di Eccellenza (2018–2022)” and by grants of the Fondazione Regionale Ricerca Biomedica (FRRB), Milan, Italy (FRRB project no. 2015–0042, genomic profiling of rare hematologic malignancies, development of personalized

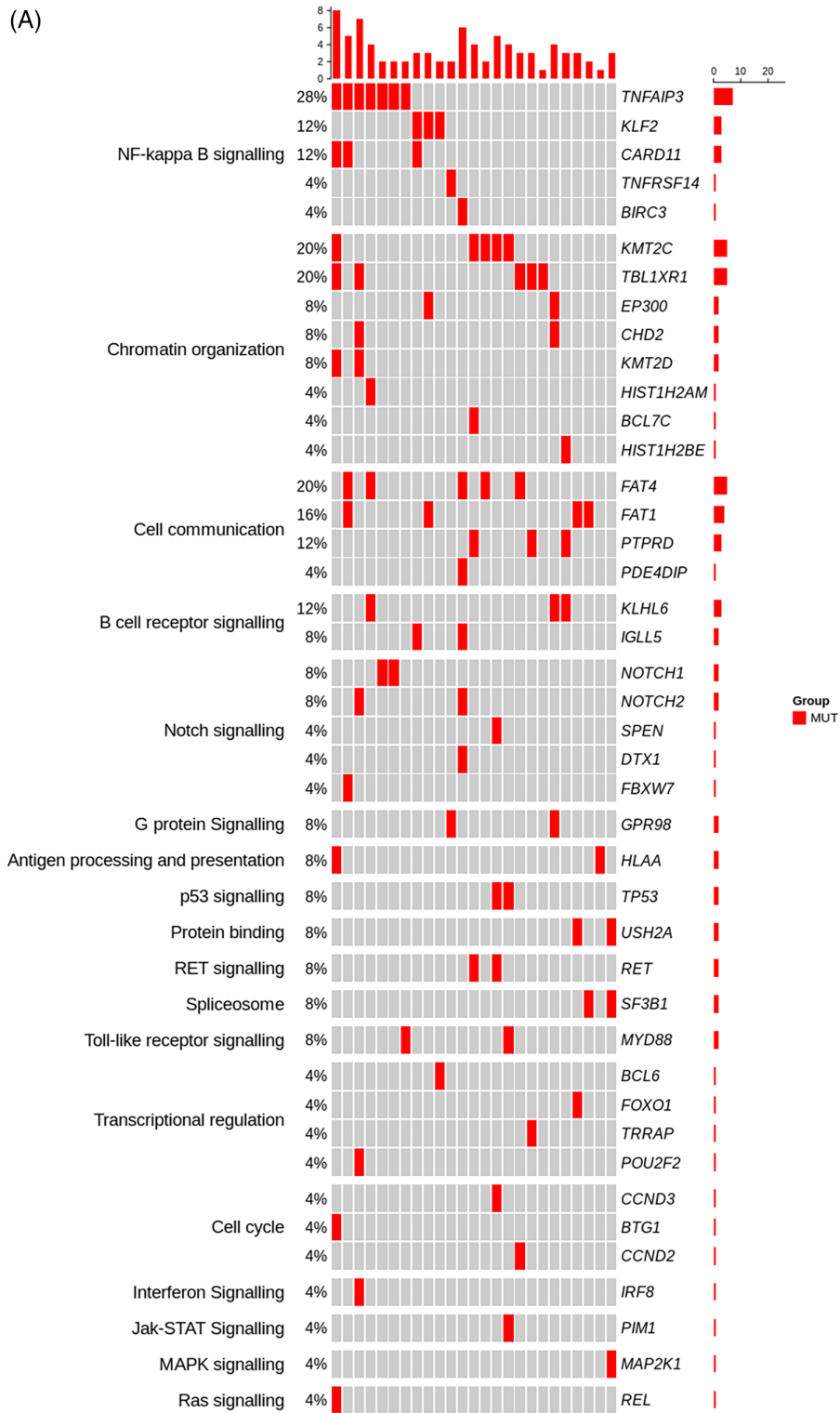


FIGURE 1 Pattern of somatic mutations in 27 patients with B cell lymphoproliferative disorders associated to hepatitis C virus. (A), Prevalence of driver genetic lesions according to the involved pathway; (B), Circos plots representing the relative frequencies and pairwise co-occurrence of the involved pathways according to IGHV usage; (C), Circos plots representing the relative frequencies and pairwise co-occurrence of the involved pathways and IGLV rearrangements according to light chains CDR3 sequences

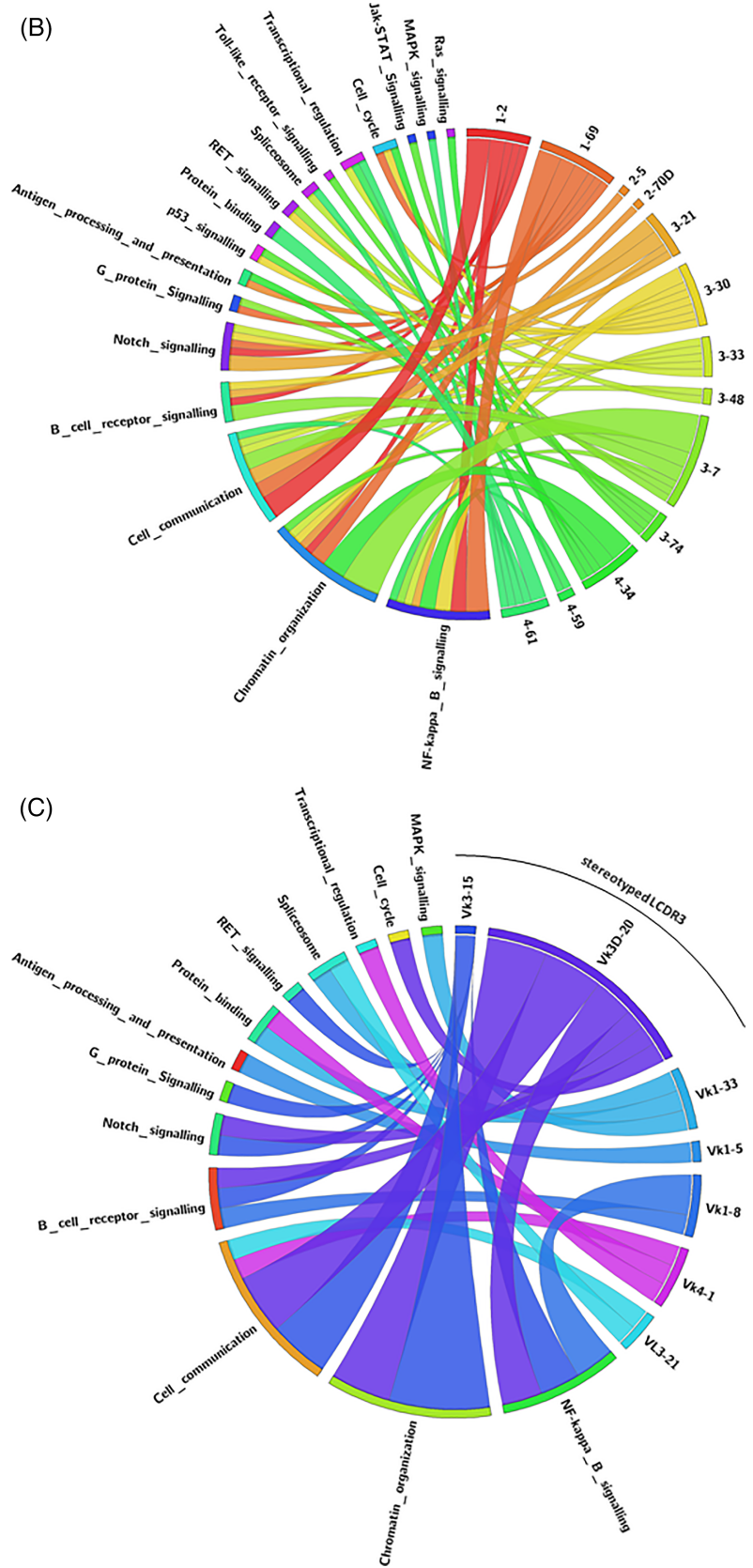


FIGURE 1 (Continued)

medicine strategies, and their implementation into the Rete Ematologica Lombarda clinical network) and by grants from the Istituto Pasteur Italia-Fondazione Cenci Bolognetti 2017–76 (to Marcella Visentini).

CONFLICT OF INTEREST

None of the authors has relevant conflicts of interest related to the content of this work.

L.A. received advisory honoraria from Roche, Celgene, Janssen-Cilag, Verastem, Eusa Pharma, and Incyte, research support from Gilead, and travel expenses from Roche, Celgene, Janssen-Cilag, and Eusa Pharma. E.R. has shares of enGenome srl, an Italian bioinformatics company. A.P. was an advisory board member for Roche, Merck, Pfizer, Sandoz, and Takeda and a speaker for Roche, Gilead, and Bristol Myers Squibb. M.V. received advisory honoraria from Janssen, Roche, Astra Zeneca and travel expenses from AbbVie. R.B. was an advisory board member and a speaker for AbbVie, Gilead Sciences and Merck Sharp & Dohme.

AUTHOR CONTRIBUTIONS

L.A. conceived, designed, and supervised the study; S.Z., C.C., M.F., E. R., performed IGHV and NGS analyses in Pavia; M.Vi. and Y.A.M. performed IGLV and IGHV analyses in Rome; M.Vi., A.P., M.C., S.C., I.D., S.R., M.R., C.Z., F.B., C.C., N.F. M.V., M.M., V.Z., R.B., M.P. acquired clinical data; E.R. performed bioinformatics analysis; L.A., I.D., V.V.F., S.Z. and M.Vi. analyzed and interpreted data and wrote the manuscript; and all authors revised and approved the final version of the manuscript.

DATA AVAILABILITY STATEMENT

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

Irene Defrancesco^{1,2}, Marcella Visentini^{3,4}, Silvia Zibellini², Ylenia Aura Minafo^{3,5,12}, Sara Rattotti² , Virginia Valeria Ferretti⁶, Ettore Rizzo⁷, Marzia Varettoni² , Marco Frigeni^{2,13} , Alessandro Pulsoni³, Milvia Casato³, Stefania Colantuono³, Marianna Rossi², Chiara Candido², Caterina Zerbi⁸, Fabio Bergamini⁸, Caterina Cristinelli⁸, Nicole Fabbri⁸, Michele Merli⁹ , Valentina Zuccaro¹⁰, Raffaele Bruno^{1,10}, Marco Paulli^{8,11}, Luca Arcaini^{2,8} 

¹Department of Medical, Surgical, Diagnostic and Pediatric Sciences, University of Pavia, Pavia, Italy

²Division of Hematology, Fondazione IRCCS Policlinico San Matteo, Pavia, Italy

³Department of Translational and Precision Medicine, Sapienza University of Rome, Rome, Italy

⁴Laboratory affiliated to Istituto Pasteur Italia - Fondazione Cenci Bolognetti, Sapienza University of Rome, Rome, Italy

⁵Department of Molecular Medicine, Sapienza University of Rome, Rome, Italy

⁶Clinical Epidemiology and Biometrics Unit, Fondazione IRCCS Policlinico San Matteo, Pavia, Italy

⁷enGenome srl, Pavia, Italy

⁸Department of Molecular Medicine, University of Pavia, Pavia, Italy

⁹Division of Hematology, University Hospital "Ospedale di Circolo e Fondazione Macchi"-ASST Sette Laghi, University of Insubria, Varese, Italy

¹⁰Division of Infectious Diseases, Fondazione IRCCS Policlinico San Matteo, Pavia, Italy

¹¹Division of Anatomic Pathology, Fondazione IRCCS Policlinico San Matteo, Pavia, Italy

¹²Now at Istituto Dermatologico dell'Immacolata, IDI-IRCCS, Rome, Italy

¹³Now at Division of Hematology, Azienda Socio-Sanitaria Territoriale Papa Giovanni XXIII, Bergamo, Italy

Correspondence

Luca Arcaini, Division of Hematology, Fondazione IRCCS Policlinico San Matteo, Viale Golgi 19, 27100 Pavia, Italy.

Email: luca.arcaini@unipv.it

Irene Defrancesco and Marcella Visentini are these two authors equally contributed

ORCID

Sara Rattotti  <https://orcid.org/0000-0002-2682-3414>

Marzia Varettoni  <https://orcid.org/0000-0001-7304-1629>

Marco Frigeni  <https://orcid.org/0000-0003-2493-7749>

Michele Merli  <https://orcid.org/0000-0002-0905-5927>

Luca Arcaini  <https://orcid.org/0000-0002-9504-991X>

REFERENCES

1. Arcaini L, Besson C, Frigeni M, et al. Interferon-free antiviral treatment in B-cell lymphoproliferative disorders associated with hepatitis C virus infection. *Blood*. 2016;128(21):2527-2532.
2. Frigeni M, Besson C, Visco C, et al. Interferon-free compared to interferon-based antiviral regimens as first-line therapy for B-cell lymphoproliferative disorders associated with hepatitis C virus infection. *Leukemia*. 2020;34(5):1462-1466.
3. Minafo YA, Del Padre M, Cristofolletti C, et al. A stereotyped light chain may shape virus-specific B-cell receptors in HCV-dependent lymphoproliferative disorders. *Genes Immun*. 2020;21(2):131-135.
4. Defrancesco I, Zibellini S, Boveri E, et al. Targeted next-generation sequencing reveals molecular heterogeneity in non-chronic lymphocytic leukemia clonal B-cell lymphocytosis. *Hematol Oncol*. 2020;38:689-697.
5. Varettoni M, Zibellini S, Defrancesco I, et al. Pattern of somatic mutations in patients with Waldenström macroglobulinemia or IgM monoclonal gammopathy of undetermined significance. *Haematologica*. 2017;102(12):2077-2085.
6. Hamdane N, Jühling F, Crouch E, et al. HCV-induced epigenetic changes associated with liver cancer risk persist after sustained virologic response. *Gastroenterology*. 2019;156(8):2313-2329.e7.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.