Prognostic impact of genetic abnormalities in 536 first-line chronic lymphocytic leukaemia patients without 17p deletion treated with chemoimmunotherapy in two prospective trials: focus on IGHV mutated subgroups (a FILO study)

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Abstract

The potential prognostic influence of genetic aberrations in chronic lymphocytic leukaemia (CLL) can vary based on various factors, such as the immunoglobulin heavy variable (IGHV) status. We conducted an integrative analysis on genetic abnormalities identified through cytogenetics and targeted NGS in 536 CLL patients receiving first-line chemo(immuno)therapies (CIT) as part of two prospective trials. We evaluated the prognostic implications of main abnormalities, with specific attention to their relative impact according to IGHV status. In the entire cohort, unmutated (UM)-IGHV, complex karyotype, del(11q), and *ATM* mutations correlated significantly with shorter PFS. Focusing on the subset of M-IGHV patients, univariate analysis showed that complex karyotype, del(11q), *SF3B1*, and *SAMHD1* mutations were associated with significant lower PFS. The prognostic influence varied based on the patient's IGHV status, as these abnormalities did not affect outcomes in the UM-IGHV subgroup. *TP53* mutations had no significant impact on outcomes in M-IGHV subgroup. Our findings highlight the diverse prognostic influence of genetic aberrations depending on the IGHV status in symptomatic CLL patients receiving first-line CIT. The prognosis of gene mutations and cytogenetic abnormalities needs to be investigated with a compartmentalized methodology, taking into account the IGVH status of patients receiving first-line BTK and/or BCL2 inhibitors.

Introduction

Chronic lymphocytic leukaemia (CLL) is a genetically and clinically heterogeneous disease. Cytogenetic abnormalities, IGHV mutational status and more recently gene mutations have led to a better understanding of CLL heterogeneity and a better characterization of patient prognosis(Lazarian *et al*, 2017). Mutations of specific genes including *TP53*, *ATM*, *BIRC3*, *SF3B1*, *NOTCH1*, *EGR2, BRAF* or *KRAS* have been associated with poorer outcome, either shorter time to first treatment (TFT), progression-free survival (PFS) and/or overall survival (OS)(Nadeu *et al*, 2016; Blakemore *et al*, 2020; Knisbacher *et al*, 2022; Mansouri *et al*, 2023). However, the frequency and clinical impact of these mutations differed among the analyzed cohorts. Several factors, such as age, variant allele frequency (VAF), co-existing driver mutations and/or chromosomal alterations, could influence the clinical significance of a driver mutation in an individual patient. Therefore, integrative analyses of cytogenetics, IGHV status, and gene mutations are essential to accurately evaluate patient prognosis.

Recent years have seen a paradigm change in CLL therapies with the advent of BTK inhibitors (BTKi), the BCL2 inhibitor (BCL2i) venetoclax and the novel anti-CD20 monoclonal antibody obinutuzumab which have been transformative in CLL management(Shadman, 2023). These targeted therapies have demonstrated improved PFS and OS over chemoimmunotherapy (CIT) regimens, mainly bendamustine-rituximab (BR) and chlorambucil-obinutuzumab (CO) in randomized phase III studies in relapsed/refractory or first-line (1L) CLL. However, some questions remain, including whether CIT may still an acceptable option in certain CLL subpopulations, i.e IGHV mutated patients receiving 1L fludarabine-cyclophosphamide-rituximab (FCR). Recently, the fixed-duration combination of BTKi and CIT for 1L treatment of CLL patients was shown to achieve a high rate of undetectable minimal residual disease (uMRD) and sustained long-term remission even after discontinuing BTKi(Davids *et al*, 2019; Jain *et al*, 2021; Michallet *et al*, 2023).

The aim of this study was to evaluate, in prospective trials using 1L CIT, frequencies of gene mutations, their association with IGHV status and cytogenetic abnormalities and their impact on outcome. To address these questions, we undertook an integrative study including karyotype, fluorescence in situ hybridization (FISH), IGHV status and targeted next generation sequencing (NGS) in two French prospective clinical trials using 1L CIT.

Material and methods

We studied 536 CLL patient samples taken at randomization of two prospective phase 3 trials using 1L fludarabine-based CIT (CLL 2007 FMP [NCT00564512] and CLL 2007 SA [NCT00645606]) (Lepretre *et al*, 2012; Dartigeas *et al*, 2018). The CLL FMP 2007 was a phase 3 multicenter trial that involved untreated CLL patients, who were randomly assigned to receive six courses of oral fludarabine and cyclophosphamide (FC) in combination with either rituximab (FCR) or alemtuzumab (FCCam). The CLL FA 2007 was a randomised, multicentre phase 3 trial that included treatment-naive and fit CLL patients aged 65 years or older. Patients received four cycles of FCR and were randomly assigned, in case of response, to either receive rituximab maintenance every 8 weeks for up to 2 years or undergo observation. Patients were diagnosed using the iwCLL guidelines (Hallek *et al*, 2018), with informed consent obtained in accordance with the declaration of Helsinki. All CLL patients were symptomatic Binet stage B or C and devoid of 17p deletion. Demographic, clinical and biological characteristics of both cohorts are summarized in **Supplementary Table S1**. Our study included patients from these two prospective trials whom DNA material was available for NGS and who received fludarabine-based regimens. These patients (n=536) did not significantly differ from those

who were excluded due to insufficient DNA or not receiving protocol treatment (n=154) **(Supplementary Table S1).** We also did not observe significant differences when excluding patients that received alemtuzumab (n=70) in terms of prognostic factors associated or not with PFS and OS (data not shown). The average lymphocyte percentage of the total white cell count in pre-treatment blood samples was 85% (range, 60-100%; interquartile [IQR]25-75, 82-89%). Baseline assessments included conventional karyotype (K) and FISH analysis for del(13q), trisomy 12 (tri12), del(11q), del(17p) and IGHV mutational status. A karyotype was defined as complex (CK) if ≥ 3 clonal aberrations were present in chromosome banding analysis (CBA) (International Standing Committee on Human Cytogenetic Nomenclature, 2020). Highly CK (high-CK) and non-high-CK were defined if ≥ 5 and 3 or 4 clonal aberrations were identified respectively.

We investigated by targeted deep sequencing 33 genes and 2 non-coding regions including *TP53, SF3B1, NOTCH1, ATM, BIRC3, NFKBIE, EGR2, MYD88* and *POT1* (see **Supplementary Tables S2**). Briefly, DNA was extracted from total blood mononuclear cells. Targeted regions were PCR-amplified using an AmpliSeq (Life technologies) approach and PCR products sequenced in a MiSeq (Illumina) instrument with a mean depth of 1363X (range, 500-22460; IQR25-75, 700-1424) and a detection level of 1%. Targeted sequencing was analyzed according to previously described algorithms with minor modifications (Damm *et al*, 2014). Mutations were considered as clonal if the VAF was ≥40% and subclonal (high/low) if VAF was between 12.5 and 40% / ≤12.5%, respectively. This latter cutoff value represents the common detection threshold of mutations by Sanger sequencing (Rossi *et al*, 2014). Driver mutations were assigned to signaling pathways (DNA damage, chromatin modification, RNA processing, MAP kinase signaling, NOTCH signaling, NFKB signaling, BCR signaling; **Supplementary Table S3**)**.** A pathway was considered altered if at least one gene in the pathway was mutated.

Main clinical and biological characteristics were collected and included in analysis of prognostic factors (age, gender, Binet stage, response to therapy including minimal residual disease [MRD] assessment, FISH, chromosomal abnormalities and mutations). Only subgroups of five or more patients were included for prognostic analyses. Quantitative variables are presented as mean/median and range/IQR according to their distribution. Categorical variables are presented as numbers and related percentage. Analyses were performed as intent to treat. PFS was defined as the time between randomization and the first documented disease progression, death from any cause, or last follow up for surviving patients. PFS and OS were estimated by the non-parametric Kaplan-Meier method and then compared between groups by the log-rank test. The significance level of $p < 0.05$ was applied and statistical analyses were performed using the software SAS 9.3 (SAS Inc, Cary, NC) and R v3.4.4 (http://www.R-project.org).

Results

Main clinical and biological characteristics of CLL patients included in both prospective trials are summarized in **Supplementary Table S1**. Both cohorts harbored similar characteristics particularly regarding IGHV status, cytogenetic abnormalities and gene mutations, with the exception of older age at inclusion, related to specific inclusion criteria, and higher frequencies of *NOTCH1* and *KMT2D* mutations in the 2007 SA cohort. Considering the whole study population (n=536, **Table 1**), unmutated (UM) IGHV status was observed in 293/522 (56%) cases. The median number of cytogenetic abnormalities per sample was one (range, 0-11). Chromosomal abnormalities by K were observed in 319/470 (68%) of evaluable samples, including 17% CK, 4% high-CK, and 30% carrying

translocations. FISH analyses revealed 296/503 (59%) del(13q), 88/522 (17%) tri(12) and 114/534 (21%) del(11q). Due to specific inclusion criteria, no patient harbored del(17p). For a total of 1121 mutations, the median number of gene mutations was two per patient (range, 0-7) (see **Supplementary Table S4** for the complete list of variants). At least one mutation was observed in 443/536 (83%) patients, including 117 (22%) harboring one mutation, 127 (24%) two mutations and 199 (37%) three or more mutations. The most frequent mutations were identified in *SF3B1* (24%), *NOTCH1* (24%), *ATM* (18%), *NFKBIE* (10%), *KMT2D* (8%), *XPO1* (7%), *POT1* (7%), *EGR2* (7%), *PAX5* enhancer region (7%), *TP53* (6%), *BRAF* (6%), *IRF4* (4%), *FBXW7* (4%) and *BIRC3* (4%) (**Table 1** and **Figure 1A**). *MYD88* and *SAMHD1* mutations were mostly observed as clonal events whereas *BIRC3, KRAS* and *NRAS* mutations as subclonal ones (**Supplementary Table S4**). According to pathway assignation, one, two, three and more than three pathways were altered in 177 (33%), 109 (20%), 62 (12%), and 37 (7%) patients, respectively. The most frequently altered pathways were NOTCH signaling (26%), DNA damage (23%), and NFKB signaling (16%).

Significant associations between cytogenetic and molecular characteristics are detailed in **Supplementary Table S5**. Selected significant associations are presented in **Figure 1B**. In particular, *NOTCH1* mutations were associated with UM-IGHV (P<0.0001) and tri(12) (P=0.002); *FBXW7* mutations with tri(12) (P=0.001); *XPO1* and *NFKBIE* mutations with UM-IGHV (P=0.0007 and P=0.003 respectively); *ATM* mutations with del(11q) (P=0.0005); *TP53* with high-CK (P=0.02); and *MYD88* and *PAX5* enhancer mutations with M-IGHV (P=0.0002 and P=0.01 respectively). The frequencies of main cytogenetic and molecular characteristics were thus different in M- and UM-IGHV subpopulations, as represented in **Table 1** and **Figure 1C**.

The median follow-up for the whole study population was 61 months (IQR25-75, 40-70). Five-year PFS and OS were 54% (CI95%, 48-60) and 80% (CI95%, 75-83), respectively. Univariate analyses associated with PFS and OS for the whole study population are represented in **Supplementary Table S6 and Supplementary Figure S1**. Prognostic factors associated with shorter PFS included UM-IGHV (P<0.0001), CK (P=0.003), del(11q) (P=0.001), *ATM* mutations (P=0.01), DNA damage pathway (P<0.0001), the number of mutations ≥ 3 (P=0.01), while del(13q) was associated with longer PFS (P=0.002). A trend toward shorter PFS was observed in *TP53*-mutated patients (P=0.06). OS was pejoratively impacted by UM-IGHV (P=0.01), *ATM* (P=0.01), *TP53* (P=0.02), *FBXW7* (P=0.002) and *IRF4* (P=0.005) mutations. Del(13q) was associated with longer OS (P=0.03). By multivariate analyses, UM-IGHV retained prognostic significance for shorter PFS and OS (**Supplementary Table S6**).

Looking specifically to M-IGHV subgroup (n=229), 44% harbored normal karyotype, 60% isolated del(13q) and 9% del(11q) by FISH, which significantly differ from UM-IGHV subgroup (n=293; 24%, 28% and 32%, respectively; P<0.0001 for each comparison) (**Table 1** and **Figure 1C**). The distribution and frequencies of mutations and altered pathways were also different between M- and UM-IGHV. In the M-IGHV subgroup, the most frequent molecular abnormalities were *SF3B1* (24%), *ATM* (16%) and *NOTCH1* (11%) mutations along with *PAX5* enhancer (10%) variants, while mutations of *NFKBIE* (5%), *EGR2* (4%) and *XPO1* (3%) were uncommon. The mean number of mutations was lower in M-IGHV subgroup and the proportion of patients without any mutation significantly higher (22% vs. 13%, P=0.01). In this subgroup of M-IGHV patients receiving first-line fludarabine-based regimens, univariate analysis (**Table 2, Figure 2**) showed that CK, del(11q), *SF3B1* and *SAMHD1* mutations, and DNA damage pathway were associated with shorter PFS. The shortest PFS were observed in patients harboring CK and del(11q) with respective median PFS of 53.7 (95%CI, 46.6-not reached) and 52.0 (95%CI, 43.3-70) months. Nonetheless, no molecular or cytogenetic abnormalities had a significant

impact on OS, with the exception of *XPO1* mutations; although it should be taken with caution regarding the low number of *XPO1*-mutated patients (n=6). *TP53* mutations (n=12) did not impact significantly outcomes in M-IGHV patients. Too few samples (n=4) were mutated for *IRF4* and *FBXW7* in M-IGHV subgroup to assess reliably their specific prognostic impact**.** By multivariate analyses, only del(11q) retained prognostic significance for shorter PFS (*P*=0.005) **(Supplementary Table S7).**
Cytogenetic and molecular abnormalities that impact prognosis differed in the UM-IGHV subgroup (**Figure 2J** and **Supplementary Table S8**). Notably, CK, del(11q), *SF3B1* and *SAMHD1* mutations, which were associated with shorter PFS in M-IGHV, had no impact in the UM-IGHV subgroup. On the other hand, *ATM* mutations resulted in significantly shorter PFS/OS, while del(13q) resulted in longer PFS/OS in UM-IGHV, which was not observed in M-IGHV.

Discussion

Adverse genetic features in the context of CIT have been largely described and include, among the most significant, UM-IGHV, del(11q), CK/high-CK, *NOTCH1/SF3B1/BIRC3* mutations and *TP53* abnormalities(Rossi *et al*, 2011, 2013; Baliakas *et al*, 2015). However, clinical impact of genetic abnormalities varied between analyzed cohorts, depending in part on the type of CIT used (CO, BR or FCR)(Knisbacher *et al*, 2022; Rossi *et al*, 2011; Tausch *et al*, 2020a; Spunarova *et al*, 2019), the stage of the disease and associated genetic landscape.
Recent studies have indeed demonstrated that mutations may affect outcomes differently in UM- and M-IGHV, in Binet stage A (Mansouri *et al*, 2023; Baliakas *et al*, 2019) and in global CLL cohorts (including both asymptomatic and symptomatic patients, receiving either CIT or BTKi)(Knisbacher *et al*, 2022). Comprehensive multiomic analyzes have also recently strongly suggested that UM- and M-IGHV harbored unique and distinct mechanisms of leukemogenesis(Knisbacher *et al*, 2022).
Our work focused on a large CLL cohort of 536 patients treated with 1L fludarabine-based regimens as part of two prospective trials. We undertook an integrative analysis of both cytogenetic and molecular abnormalities identified through FISH, CBA, and targeted NGS on 33 genes and 2 non-coding regions, to evaluate the prognostic implications of principal abnormalities, with a particular focus on their relative impact in UM- and M-IGHV. Our study has some limitations. Specifically, the population received CIT, which is currently restricted in CLL. Additionally, del(17p) patients were excluded, potentially influencing the genetic landscape that has been identified; finally, data on stereotyped subsets, particularly subset #2, which has been linked to a worse prognosis in M-IGHV, were not available.
The main genetic features identified in our study were in line with those reported previously in symptomatic CLL cohorts, as were the noteworthy links between certain mutations and IGHV status, in particular *NOTCH1*/*NFKBIE/XPO1* mutations with UM-IGHV and *MYD88*/*PAX5* enhancer mutations with M-IGHV(Jeromin *et al*, 2014; Puente *et al*, 2015). The significantly lower number of mutations in M-IGHV was also consistent with previous large-scale genomic studies(Knisbacher *et al*, 2022). In the present study, we provide insights into the distinct genetic landscape and varying prognostic impact of genetic abnormalities based on IGHV status of symptomatic CLL patients. We also focused on the specific aspects of the M-IGHV subgroup, in this cohort of 1L CLL patients without del(17p) treated prospectively by fludarabine-based regimens.
In M-IGHV patients, we have shown in particular that CK, del(11q), *SF3B1* and *SAMHD1* mutations pejoratively impacted PFS, while *NOTCH1* and *TP53* mutations had no significant impact. These results regarding del(11q), *SF3B1* and *NOTCH1* mutations were concordant with those reported recently in M-IGHV by Knisbacher et al(Knisbacher *et al*, 2022) using large-scale genomic sequencing.

The specific prognostic value of *SF3B1* mutations could be clarified by taking into account subset #2, which is known to have a negative prognostic impact in M-IGHV and is associated with the presence of *SF3B1* mutations(Jaramillo *et al*, 2020). Regarding the lack of impact of *TP53* mutations in the M-IGHV group, the limited number of mutated patients (n=12) and the exclusion of del(17p) must be taken into account, as numerous studies have shown that prognosis can vary according to the presence of one or more *TP53* abnormalities (mutation and/or deletion)(Malcikova *et al*, 2009; Griffin *et al*, 2023). However, these data are consistent with the lack of impact of *TP53* mutations observed in M-IGHV patients with stage A CLL(Mansouri *et al*, 2023).

Whilst CIT is increasingly restricted in CLL, recent findings have revealed that frontline fixed-duration combinations with fludarabine-based CIT and BTKi could lead to remarkable response rates and PFS(Davids *et al*, 2019; Jain *et al*, 2021; Michallet *et al*, 2023). The potential impact of genetic abnormalities regarding IGHV status may be examined in this context, should such combinations be developed in the future. Unlike CIT, durable remissions are seen with 1L venetoclax combination and BTKi, regardless of IGHV status, del(11q) or mutations in *ATM, SF3B1, BIRC3*, and *NOTCH1*(Tausch *et al*, 2020b; Barr *et al*, 2022). Adverse prognostic markers still include del(17p), *TP53* mutations and/or high-CK with fixed-duration BCL2i, continuous BTKi, or fixed-duration combinations(Tausch *et al*, 2020b; Brieghel *et al*, 2021; Fürstenau *et al*, 2023). Further research is necessary to investigate the precise prognosis of gene mutations and cytogenetic abnormalities in the context of frontline BTKi and BCL2i therapies, which may be conducted with a compartmentalized approach based on IGVH status.

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References

Baliakas, P., Hadzidimitriou, A., Sutton, L.-A., Rossi, D., Minga, E., Villamor, N., Larrayoz, M., Kminkova, J., Agathangelidis, A., Davis, Z., Tausch, E., Stalika, E., Kantorova, B., Mansouri, L., Scarfò, L., Cortese, D., Navrkalova, V., Rose-Zerilli, M.J.J., Smedby, K.E., Juliusson, G., et al (2015) Recurrent mutations refine prognosis in chronic lymphocytic leukemia. *Leukemia*, **29,** 329–336.

Baliakas, P., Moysiadis, T., Hadzidimitriou, A., Xochelli, A., Jeromin, S., Agathangelidis, A., Mattsson, M., Sutton, L.-A., Minga, E., Scarfò, L., Rossi, D., Davis, Z., Villamor, N., Parker, H., Kotaskova, J., Stalika, E., Plevova, K., Mansouri, L., Cortese, D., Navarro, A., et al (2019) Tailored approaches grounded on immunogenetic features for refined prognostication in chronic lymphocytic leukemia. *Haematologica*, **104,** 360–369.

Barr, P.M., Owen, C., Robak, T., Tedeschi, A., Bairey, O., Burger, J.A., Hillmen, P., Coutre, S.E., Dearden, C., Grosicki, S., McCarthy, H., Li, J.-Y., Offner, F., Moreno, C., Zhou, C., Hsu, E., Szoke, A., Kipps, T.J. & Ghia, P. (2022) Up to 8-year follow-up from RESONATE-2: first-line ibrutinib treatment for patients with chronic lymphocytic leukemia. *Blood Advances*, **6,** 3440–3450.

Blakemore, S.J., Clifford, R., Parker, H., Antoniou, P., Stec-Dziedzic, E., Larrayoz, M., Davis, Z., Kadalyayil, L., Colins, A., Robbe, P., Vavoulis, D., Forster, J., Carr, L., Morilla, R., Else, M., Bryant, D., McCarthy, H., Walewska, R.J., Steele, A.J., Chan, J., et al (2020) Clinical significance of TP53, BIRC3, ATM and MAPK-ERK genes in chronic lymphocytic leukaemia: data from the randomised UK LRF CLL4 trial. *Leukemia*, **34,** 1760–1774.

Brieghel, C., Aarup, K., Torp, M.H., Andersen, M.A., Yde, C.W., Tian, X., Wiestner, A., Ahn, I.E. & Niemann, C.U. (2021) Clinical Outcomes in Patients with Multi-Hit TP53 Chronic Lymphocytic Leukemia Treated with Ibrutinib. *Clinical Cancer Research: An Official Journal of the American Association for Cancer Research*, **27,** 4531–4538.

Damm, F., Mylonas, E., Cosson, A., Yoshida, K., Della Valle, V., Mouly, E., Diop, M., Scourzic, L., Shiraishi, Y., Chiba, K., Tanaka, H., Miyano, S., Kikushige, Y., Davi, F., Lambert, J., Gautheret, D., Merle-Béral, H., Sutton, L., Dessen, P., Solary, E., et al (2014) Acquired initiating mutations in early hematopoietic cells of CLL patients. *Cancer Discovery*, **4,** 1088–1101.

Dartigeas, C., Van Den Neste, E., Léger, J., Maisonneuve, H., Berthou, C., Dilhuydy, M.-S., De Guibert, S., Leprêtre, S., Béné, M.C., Nguyen-Khac, F., Letestu, R., Cymbalista, F., Rodon, P., Aurran-Schleinitz, T., Vilque, J.-P., Tournilhac, O., Mahé, B., Laribi, K., Michallet, A.-S., Delmer, A., et al (2018) Rituximab maintenance versus observation following abbreviated induction with chemoimmunotherapy in elderly patients with previously untreated chronic lymphocytic leukaemia (CLL 2007 SA): an open-label, randomised phase 3 study. *The Lancet. Haematology*, **5,** e82–e94.

Davids, M.S., Brander, D.M., Kim, H.T., Tyekucheva, S., Bsat, J., Savell, A., Hellman, J.M., Bazemore, J., Francoeur, K., Alencar, A., Shune, L., Omaira, M., Jacobson, C.A., Armand, P., Ng, S., Crombie, J., LaCasce, A.S., Arnason, J., Hochberg, E.P., Takvorian, R.W., et al (2019) Ibrutinib plus fludarabine, cyclophosphamide, and rituximab as initial treatment for younger patients with chronic lymphocytic leukaemia: a single-arm, multicentre, phase 2 trial. *The Lancet. Haematology*, **6,** e419–e428.

Fürstenau, M., Thus, Y.J., Robrecht, S., Mellink, C.H.M., van der Kevie-Kersemaekers, A.-M., Dubois, J., von Tresckow, J., Patz, M., Gregor, M., Thornton, P., Staber, P.B., Tadmor, T., Levin, M.-D., da Cunha-Bang, C., Schneider, C., Poulsen, C.B., Illmer, T., Schöttker, B., Janssens, A., Christiansen, I., et al (2023) High karyotypic complexity is an independent prognostic factor in patients with CLL treated with venetoclax combinations. *Blood*, **142,** 446–459.

Griffin, R., Wiedmeier-Nutor, J.E., Parikh, S.A., McCabe, C.E., O'Brien, D.R., Boddicker, N.J., Kleinstern, G., Rabe, K.G., Bruins, L., Brown, S., Bonolo de Campos, C., Ding, W., Leis, J.F., Hampel, P.J., Call, T.G., Van Dyke, D.L., Kay, N.E., Cerhan, J.R., Yan, H., Slager, S.L., et al (2023) Differential prognosis of single and multiple TP53 abnormalities in high-count MBL and untreated CLL. *Blood Advances*, **7,** 3169–3179.

Hallek, M., Cheson, B.D., Catovsky, D., Caligaris-Cappio, F., Dighiero, G., Döhner, H., Hillmen, P., Keating, M., Montserrat, E., Chiorazzi, N., Stilgenbauer, S., Rai, K.R., Byrd, J.C., Eichhorst, B., O'Brien, S., Robak, T., Seymour, J.F. & Kipps, T.J. (2018) iwCLL guidelines for diagnosis, indications for treatment, response assessment, and supportive management of CLL. *Blood*, **131,** 2745–2760.

International Standing Committee on Human Cytogenetic Nomenclature. (2020) ISCN: An International System for Human Cytogenomic Nomenclature. Basel, NY. Karger.

Jain, N., Thompson, P., Burger, J., Ferrajoli, A., Takahashi, K., Estrov, Z., Borthakur, G., Bose, P., Kadia, T., Pemmaraju, N., Sasaki, K., Konopleva, M., Jabbour, E., Garg, N., Wang, X., Kanagal-Shamanna, R., Patel, K., Wang, W., Jorgensen, J., Wang, S., et al (2021) Ibrutinib, fludarabine, cyclophosphamide, and obinutuzumab (iFCG) regimen for chronic lymphocytic leukemia (CLL) with mutated IGHV and without TP53 aberrations. *Leukemia*, **35,** 3421–3429.

Jaramillo, S., Agathangelidis, A., Schneider, C., Bahlo, J., Robrecht, S., Tausch, E., Bloehdorn, J., Hoechstetter, M., Fischer, K., Eichhorst, B., Goede, V., Hallek, M., Döhner, H., Rosenquist, R., Ghia, P., Stamatopoulos, K. & Stilgenbauer, S. (2020) Prognostic impact of prevalent chronic lymphocytic leukemia stereotyped subsets: analysis within prospective clinical trials of the German CLL Study Group (GCLLSG). *Haematologica*, **105,** 2598–2607.

Jeromin, S., Weissmann, S., Haferlach, C., Dicker, F., Bayer, K., Grossmann, V., Alpermann, T., Roller, A., Kohlmann, A., Haferlach, T., Kern, W. & Schnittger, S. (2014) SF3B1 mutations correlated to cytogenetics and mutations in NOTCH1, FBXW7, MYD88, XPO1 and TP53 in 1160 untreated CLL patients. *Leukemia*, **28,** 108–117.

Knisbacher, B.A., Lin, Z., Hahn, C.K., Nadeu, F., Duran-Ferrer, M., Stevenson, K.E., Tausch, E., Delgado, J., Barbera-Mourelle, A., Taylor-Weiner, A., Bousquets-Muñoz, P., Diaz-Navarro, A., Dunford, A., Anand, S., Kretzmer, H., Gutierrez-Abril, J., López-Tamargo, S., Fernandes, S.M., Sun, C., Sivina, M., et al (2022) Molecular map of chronic lymphocytic leukemia and its impact on outcome. *Nature Genetics*, **54,** 1664–1674.

Lazarian, G., Guièze, R. & Wu, C.J. (2017) Clinical Implications of Novel Genomic Discoveries in Chronic Lymphocytic Leukemia. *Journal of Clinical Oncology: Official Journal of the American Society of Clinical Oncology*, **35,** 984–993.

Lepretre, S., Aurran, T., Mahé, B., Cazin, B., Tournilhac, O., Maisonneuve, H., Casasnovas, O., Delmer, A., Leblond, V., Royer, B., Corront, B., Chevret, S., Delépine, R., Vaudaux, S., Van Den Neste, E., Béné, M.C., Letestu, R., Cymbalista, F. & Feugier, P. (2012) Excess mortality after treatment with fludarabine and cyclophosphamide in combination with alemtuzumab in previously untreated patients with chronic lymphocytic leukemia in a randomized phase 3 trial. *Blood*, **119,** 5104–5110.

Malcikova, J., Smardova, J., Rocnova, L., Tichy, B., Kuglik, P., Vranova, V., Cejkova, S., Svitakova, M., Skuhrova Francova, H., Brychtova, Y., Doubek, M., Brejcha, M., Klabusay, M., Mayer, J., Pospisilova, S. & Trbusek, M. (2009) Monoallelic and biallelic inactivation of TP53 gene in chronic lymphocytic leukemia: selection, impact on survival, and response to DNA damage. *Blood*, **114,** 5307–5314.

Mansouri, L., Thorvaldsdottir, B., Sutton, L.-A., Karakatsoulis, G., Meggendorfer, M., Parker, H., Nadeu, F., Brieghel, C., Laidou, S., Moia, R., Rossi, D., Catherwood, M., Kotaskova, J., Delgado, J., Rodríguez-Vicente, A.E., Benito, R., Rigolin, G.M., Bonfiglio, S., Scarfo, L., Mattsson, M., et al (2023) Different prognostic impact of recurrent gene mutations in chronic lymphocytic leukemia depending on IGHV gene somatic hypermutation status: a study by ERIC in HARMONY. *Leukemia*, **37,** 339–347.

Michallet, A.-S., Letestu, R., Le Garff-Tavernier, M., Campos, L., Ticchioni, M., Dilhuydy, M.-S., Morisset, S., Rouille, V., Mahé, B., Laribi, K., Villemagne, B., Ferrant, E., Tournilhac, O., Delmer, A., Molina, L., Leblond, V., Tomowiak, C., de Guibert, S., Orsini-Piocelle, F., Banos, A., et al (2023) A fixed-duration immunochemotherapy approach in CLL: 5.5-year results from the phase 2 ICLL-07 FILO trial. *Blood Advances*, **7,** 3936–3945.

Nadeu, F., Delgado, J., Royo, C., Baumann, T., Stankovic, T., Pinyol, M., Jares, P., Navarro, A., Martín-García, D., Beà, S., Salaverria, I., Oldreive, C., Aymerich, M., Suárez-Cisneros, H., Rozman, M., Villamor, N., Colomer, D., López-Guillermo, A., González, M., Alcoceba, M., et al (2016) Clinical impact of clonal and subclonal TP53, SF3B1, BIRC3, NOTCH1, and ATM mutations in chronic lymphocytic leukemia. *Blood*, **127,** 2122–2130.

Puente, X.S., Beà, S., Valdés-Mas, R., Villamor, N., Gutiérrez-Abril, J., Martín-Subero, J.I., Munar, M., Rubio-Pérez, C., Jares, P., Aymerich, M., Baumann, T., Beekman, R., Belver, L., Carrio, A., Castellano, G., Clot, G., Colado, E., Colomer, D., Costa, D.,

Delgado, J., et al (2015) Non-coding recurrent mutations in chronic lymphocytic leukaemia. *Nature*, **526,** 519–524.

Rossi, D., Bruscaggin, A., Spina, V., Rasi, S., Khiabanian, H., Messina, M., Fangazio, M., Vaisitti, T., Monti, S., Chiaretti, S., Guarini, A., Del Giudice, I., Cerri, M., Cresta, S., Deambrogi, C., Gargiulo, E., Gattei, V., Forconi, F., Bertoni, F., Deaglio, S., et al (2011) Mutations of the SF3B1 splicing factor in chronic lymphocytic leukemia: association with progression and fludarabine-refractoriness. *Blood*, **118,** 6904–6908.

Rossi, D., Rasi, S., Spina, V., Bruscaggin, A., Monti, S., Ciardullo, C., Deambrogi, C., Khiabanian, H., Serra, R., Bertoni, F., Forconi, F., Laurenti, L., Marasca, R., Dal-Bo, M., Rossi, F.M., Bulian, P., Nomdedeu, J., Del Poeta, G., Gattei, V., Pasqualucci, L., et al (2013) Integrated mutational and cytogenetic analysis identifies new prognostic subgroups in chronic lymphocytic leukemia. *Blood*, **121,** 1403–1412.

Rossi, D., Rossi D, Khiabanian H, Spina V, Ciardullo C, Bruscaggin A, Famà R, Rasi S, Monti S, Deambrogi C, De Paoli L, Wang J, Gattei V, Guarini A, Foà R, Rabadan R, Gaidano G., et al, (2014) Clinical impact of small TP53 mutated subclones in chronic lymphocytic leukemia. *Blood*, **123,** 2139–2147.

Shadman, M. (2023) Diagnosis and Treatment of Chronic Lymphocytic Leukemia: A Review. *JAMA*, **329,** 918–932.

Spunarova, M., Tom, N., Pavlova, S., Mraz, M., Brychtova, Y., Doubek, M., Panovska, A., Skuhrova Francova, H., Brzobohata, A., Pospisilova, S., Mayer, J. & Trbusek, M. (2019) Impact of gene mutations and chromosomal aberrations on progression-free survival in chronic lymphocytic leukemia patients treated with front-line chemoimmunotherapy: Clinical practice experience. *Leukemia Research*, **81,** 75–81.

Tausch, E., Beck, P., Schlenk, R.F., Jebaraj, B.J., Dolnik, A., Yosifov, D.Y., Hillmen, P., Offner, F., Janssens, A., Babu, G.K., Grosicki, S., Mayer, J., Panagiotidis, P., McKeown, A., Gupta, I.V., Skorupa, A., Pallaud, C., Bullinger, L., Mertens, D., Döhner, H., et al (2020a) Prognostic and predictive role of gene mutations in chronic lymphocytic leukemia: results from the pivotal phase III study COMPLEMENT1. *Haematologica*, **105,** 2440–2447.

Tausch, E., Schneider, C., Robrecht, S., Zhang, C., Dolnik, A., Bloehdorn, J., Bahlo, J., Al-Sawaf, O., Ritgen, M., Fink, A.-M., Eichhorst, B., Kreuzer, K.-A., Tandon, M., Humphrey, K., Jiang, Y., Schary, W., Bullinger, L., Mertens, D., Lurà, M.P., Kneba, M., et al (2020b) Prognostic and predictive impact of genetic markers in patients with CLL treated with obinutuzumab and venetoclax. *Blood*, **135,** 2402–2412.

Figures legends

Figure 1. Panorama of mutational and cytogenetic abnormalities in a prospective cohort of 536 CLL patients receiving first-line chemo(immuno)therapy regimens. **(A)** Recurrent gene mutations, non-coding regions variants and cytogenetic abnormalities according to IGHV status (mutated [M-IGHV], blue; unmutated [UM-IGHV], white). Each column represents a patient sample and each row a mutated gene (italic) or cytogenetic abnormality. Colored box: presence; white box: absence; light grey box: not available. **(B)** Co-occurrence and mutual exclusion of selected mutations and cytogenetic abnormalities. **(C)** Percentages of recurrent gene mutations and cytogenetic abnormalities according to IGHV status (mutated [M-IGHV], blue bar; unmutated [UM-IGHV], white bar). CK, complex karyotype; enh, enhancer. *, P≤ 0.05; **, P≤ 0.01; ***, P≤ 0.001.

Figure 2. Somatic alterations and prognosis according to IGHV status.

(A-I). Kaplan-Meier curves for progression-free survival (PFS) according to the presence of recurrent mutations and cytogenetic abnormalities in the IGHV mutated (M-IGHV) subgroup. PFS according to the presence (red) or absence (blue) of the following recurrent molecular/cytogenetic abnormalities: CK **(A)**, del(11q) **(B)**, del(13q) **(C)**, *SF3B1* mutations **(D)**, *NOTCH1* mutations **(E)**, *SAMHD1* mutations **(F)**, *TP53* mutations **(G)**, *ATM* mutations **(H)** and DNA damage pathway **(I)**. Dashed lines represent 95% confidence interval. P values are indicated at the left bottom of each survival plot. CK, complex karyotype; enh, enhancer.

(J). Somatic alterations associated with progression-free survival (PFS, left panel) **and overall survival** (OS, right panel) **in M-IGHV** (left boxes) **and UM-IGHV** (right boxes) **in univariate analysis.** Heatmap denotes hazard ratios (HRs) and stars *P* values as indicated in the right part of the figure.