Genomic Features: Impact on Pathogenesis and Treatment of Chronic Lymphocytic Leukemia

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\section*{Keywords}
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\section*{Summary}
Genomic markers are among the strongest prognostic factors in chronic lymphocytic leukemia (CLL). Chromosomal aberrations, \textit{IGHV} and \textit{TP53} mutation status are well-established and essential to discriminate between a more indolent course of disease and a high-risk CLL, which requires an alternative treatment regimen. In addition, a variety of gene mutations with unclear prognostic value have been identified: \textit{SF3B1}, \textit{ATM}, and \textit{BIRC3} may describe CLL with adverse outcome, whereas \textit{NOTCH1} is predictive for resistance against CD20 antibodies. Integration of novel drivers into a small set of key pathways forms the basis for future pathogenetic and therapeutic implications.

\section*{Introduction}
Several prognostic factors have been established in chronic lymphocytic leukemia (CLL) within the last years, but only few have found their way into daily clinical practice. Among those, genomic markers play an important role, particularly chromosomal aberrations, \textit{IGHV} hypermutation, and mutation status for \textit{TP53}. These were found to affect clinical outcome in a variety of analyses including different CLL trials. They correlate with short time to first treatment, worse response to therapy, and shorter progression-free survival (PFS) and overall survival (OS) \cite{1–4}. Determination of \textit{TP53} deletion and mutation status is highly recommended for every CLL patient at baseline and in advance of every new line of therapy to better predict the course of disease and avoid early relapses. This becomes particularly important as novel compounds rather than chemotherapeutics are being recommended for patient with mutated \textit{TP53} or del17p CLL to avoid the accumulation of therapy-induced mutations in patients with impaired DNA repair mechanisms \cite{5}. However, especially in patients with Binet A and a watch-and-wait regimen, biomarkers are both a blessing and a curse: They can on one hand assist patient guidance but also enhance psychological stress and lead to premature therapy initiation.

Besides these well-established markers, other genomic defects were identified as drivers in CLL, but their clinical significance is less clear. Recently, 2 fundamental comprehensive whole exome and whole genome sequencing studies covering together more than 900 patients with CLL and monoclonal B-cell lymphocytosis (MBL) deciphered the genomic landscape of the disease and sorted drivers into early and late events in tumorigenesis \cite{6, 7}. Thus, the genetic profile may not only be useful to predict tumor growth and aggressiveness but also play a role in the stepwise transformation of naive or mature B cells into MBL and finally CLL.

\section*{IGHV Mutation Status}
The recombination of variable (V), diversity (D), and joining (J) immunoglobulin gene segments as well as somatic hypermutations are important steps in physiological B cell differentiation \cite{8, 9}. Although MBL and CLL cells were considered to derive from naïve, pre-germinal center lymphocytes without prior antigen contact, a significant number of somatic mutations are found in the heavy chain genes in every second CLL patient pointing to a maturation in germinal centers. A high number of acquired mutations within the heavy chain correlates with low homology to the closest \textit{IGHV} germline gene. A cutoff of 98% homology is chosen widely to sepa-
rate cases with mutated IGHV (<98%) from those with unmutated IGHV status (>98%), and this cutoff is based on the different clinical outcomes of both groups [8]. However, as some publications rely on a distinction of lower cutoffs, the transition range off 96–98% homology should be handled with care.

IGHV mutation status is a strong prognostic factor: Early-stage CLL cases with unmutated IGHV show shorter time to first treatment and decreased OS in multivariate analysis [10]. In patients treated with the current standard chemotherapy regimen FCR (fludarabine, cyclophosphamide, rituximab), unmutated IGHV was an independent prognostic factor for shorter PFS with a hazard ratio (HR) of 1.51 (p = 0.008) [2] (fig. 1). The survival advantage of an unmutated immunoglobulin on the surface of a CLL cell may arise from a more unspecified stimulation via a variety of different (auto)antigens and thus a permanent activation of B cell receptor (BCR) signaling [11]. Intriguingly, stimulation also occurs in specific IGHV subsets via recognition of epitopes on the BCR molecule itself by the heavy chain complementarity-determining region HCDR3 [12].

In line with this hypothesis, it was observed that novel compounds targeting BCR signaling via BTK or PI3K inhibition diminish this survival advantage: Early study results suggest no impact of IGHV mutation status on the efficacy of idelalisib + rituximab and ibrutinib, as well as the apoptosis-inducing BCL2-inhibitor ABT199 [13–15]. However, as the number of IGHV-mutated cases within these relapse/refractory patient studies is low, bigger study cohorts are necessary to clarify the context.

In addition to the number of mutations, the usage of a specific VH gene is also prognostic for outcome. V3–21 usage was shown to be a prognostic factor in heterogeneous cohorts outside clinical trials independently of mutation status [16].

Chromosomal Aberrations

CLL is characterized by a stable genome with a low number of mutations and aberrations in comparison to other hematologic malignancies and especially to solid tumors. Nevertheless, 4 of 5 CLL cases harbor at least 1 chromosomal aberration typically characterized by recurrent deletions or – less frequently – by chromosomal gains. In 2000, the most common aberrations were integrated into a hierarchical system that reflects the clinical impact of different aberrations even when existing in co-occurrence [1]. Thus, for daily practice, a small set of fluorescence in situ hybridization probes is sufficient to assess the presence of deletions of 13q, 11q, and 17p, and trisomy 12 and to determine disease progression and poor survival based on the Döhner classification as follows: del17p > del11q > tri12 > normal karyotype > del13q (fig. 1). Cases with more than 1 abnormality behave according to the aberration with the poorest survival, i.e. del17p dominates all other subgroups [1].

Deletions of chromosome 13q and gain of chromosome 12 affect together more than 80% of CLL cases and are mutually exclusive. Both are typically detected at clonal levels which categorizes them as early initiating events in CLL pathogenesis [6, 17]. Furthermore, the early occurrence was also confirmed by clonal-sub-
clonal pairs in a more complex lineage analysis as well as by the high prevalence of these aberrations in MBL [6, 18]. Not much is known about the environmental step originated by deletion of 13q, but the tiny deleted fragment includes the loci of mir15 and mir16. Both microRNAs can induce apoptosis by targeting BCL2 [19, 20], and thus their depletion may result in an imbalance between apoptosis and proliferation. The pathogenic mechanism of trisomy 12 for CLL evolution is still unknown.

In contrast to these early initiating events, deletion of 11q and more pronounced 17p occur at subclonal levels affecting only a subset of cells. These aberrations result in a heterozygous loss of the tumor suppressor genes TP53 and ATM, respectively, inducing an increase in the affected subclone over time and thus a more aggressive disease. As both diminish the DNA repair machinery, they are highly associated with treatment failure not only with chemotherapeutics but also novel compounds such as ibrutinib [3, 13]. With a HR of 7.49 for PFS and 9.32 for OS (both p < 0.001) within the CLL8 trial, deletion of 17p is the strongest prognostic factor, and thus it is not surprising that there are specific recommendations for patient care for this ultra-high-risk group, including the approval for treatment with novel drugs upfront. Disrupted TP53 is associated with genomic instability and complex karyotype [21]. In a recent report, the presence of complex karyotype within a 17p-deleted subgroup strongly decreased survival, drafting the provoking thesis that complex karyotype rather than co-occurrence of deleted 17p results in adverse clinical impact [22]. This data has not been confirmed by other studies so far.

Several additional genomic aberrations were found in CLL at lower incidence with enhanced techniques like comparative genomic hybridization (CGH) array or whole exome sequencing (WES). These include deletions of 6q, 8q, 10q, 14q, and 15q, and amplification of 8q and 2p containing driver genes like MGA, IRF4, XPO1. Also cases of chromothrypsis were detected in CLL in 3–5% of cases [7, 23]. However, these aberrations occur with a low incidence and lack independent prognostic value.

**Gene Mutations**

The genomic landscape of CLL was delineated fundamentally in 2015 with 2 comprehensive studies covering the whole exome or even the whole genome of more than 900 patients with CLL and MBL. In comparison to other hematologic malignancies and solid tumors, the CLL genome is characterized by a low mutation rate of 21.5 mutations per exome, which did not differ between CLL and MBL [6, 7]. In total, more than 40 recurrently mutated driver genes were identified with a high overlap between both studies. The high number of cases allowed for the first time to reach statistical power to detect CLL driver genes with an incidence as low as 1% [6] and to register novel drivers like RPS15, PTPN11, or IKZF3 previously not associated with CLL (fig. 2).

Although most mutations were found in both IGHV-mutated and -unmutated cases, there were also drivers associated with only 1 subgroup: Mutations in the BCR and Toll-like receptor (TLR)-signaling pathway were only found in IGHV-mutated patients. However, the number of mutated drivers was higher in cases with unmutated IGHV [7]. Furthermore, some of the drivers were found predominantly at clonal and others at subclonal levels while subclonality itself was an independent prognostic factor for short PFS [6].
Among all of these drivers, TP53 has a special role in CLL: It was among the first recurrently mutated genes identified in CLL, and characterizes patients with very poor PFS and OS in different clinical trials independently of other prognostic factors [3, 24]. Although about 60% of all mutated cases co-occur with deleted 17p, the prognostic impact was verified independently of chromosomal aberrations through multivariate analysis [3]. Thus, it is not surprising that chemotherapy selects for TP53-mutated subclones which explains the low incidence of about 5% in untreated but about 40% in heavily pretreated or chemotherapy-refractory patients [25]. As chemotherapy lacks efficacy in this high-risk subgroup and may induce durable DNA damage upon dysfunctional DNA damage repair, novel compounds should be chosen over fludarabine or bendamustine-based chemo(immuno)therapy regimens in all phases of the disease including first-line therapy. This has been acknowledged in international guidelines, and thus it is recommended to determine TP53 deletion and mutation status before each line of therapy [26].

SF3B1 and NOTCH1 mutations – both identified via early WES approaches in CLL – cluster in a few spots within the gene and affect a relatively high number of cases (about 10–20%) [27–30]. Due to this fact, there are currently a number of publications presenting the results of Sanger sequencing-based mutation screening for NOTCH1 and SF3B1 and their relation to clinical characteristics and outcome [31–35]. In particular 2 clinical trials serve perfectly to determine the prognostic value of both: In the UK LRF CLL4 trial 777 untreated patients were assigned to chlorambucil (CHL), fludarabine (F), or fludarabine and cyclophosphamide (FC) treatment. In CLL8 of the German CLL study group (GCLLSG), 817 patients were randomized to first-line chemotherapy with FC or chemo-immunotherapy with rituximab and FC [2, 36].

Splicing factor 3 subunit 1 (SF3B1) is part of the splicing machinery and thus an evolutionarily conserved hotspot. Mutations in SF3B1 were initially described in myelodysplastic syndrome and its precursors and are characterized by single nucleotide variants located at hotspots in exons 14–16 [37]. Although the impact of SF3B1 mutations on RNA processing has not been confirmed in a functional setting, mutated SF3B1 coincided with different alternative splicing of protein-coding and non-coding genes [38]. Furthermore, it may also diminish DNA damage repair showing transcriptional and apoptotic responses to DNA-damaging agents comparable to defective ATM and TP53 [39]. Regarding the prognostic value, SF3B1 mutated cases were associated with decreased PFS in CLL8 and decreased OS in LRF CLL4 [3, 40] (fig. 3, table 1). SF3B1 is a subclonal driver characterized as an intermediate to late event in the clonal evolution of CLL. In longitudinal analysis, different SF3B1 mutations are found at different time points within the same patient indicating convergent evolution [6]. Mutated SF3B1 associates with unmutated IGHV and deletion of 11q [3, 30, 31, 40]. Interestingly, it is mutually exclusive with mutated NOTCH1, an observation that is not explained so far.

With NOTCH1, another evolutionarily highly conserved gene is mutated in 10–15% of CLL cases. The specific mutation pattern is a 2bp deletion within the PEST domain that leads to a premature stop codon resulting in an abbreviation and constitutive activation of the intracellular domain [33, 41]. Meanwhile, a mutation in the 3’ UTR of NOTCH1 was found to induce the same effect increasing the incidence of mutated NOTCH1 by 3% [7].

Fig. 3. Prognostic impact of the driver gene mutations in TP53, NOTCH1, and SF3B1 on progression free survival (PFS) and overall survival (OS) in the GCLLSG CLL8 trial. Kaplan-Meier estimates for PFS (top) and OS (bottom) according to the status of 3 recurrent mutations in CLL. Color code and line structure characterize the treatment arms [3].
**NOTCH1** was initially identified as a prognostic factor for short time to first treatment, decreased OS, and transformation to an aggressive lymphoma [31, 33, 42, 43]. Moving from this early analysis of heterogeneous cohorts to well-characterized populations from clinical trials, the role of **NOTCH1** has been refined. Notably and in contrast to previous reports, **NOTCH1** was not an independent prognostic factor for PFS in both LRF CLL4 and CLL8. In fact, mutated **NOTCH1** was identified to be the first predictive factor in CLL in the CLL8 trial, which was only possible due to the study design: the addition of rituximab to FC in the experimental arm improved PFS and OS in comparison to the FC standard (fig. 3, table 1). The **NOTCH1**-mutated subgroup lacked the beneficial effect of rituximab on response to therapy and PFS, which was confirmed via multivariate analysis of the FCR-**NOTCH1** interaction (HR 1.65, p = 0.02 for PFS) (table 2). Acknowledging the importance of study design for the exploration of this predictive effect, **NOTCH1** was also subject of the analysis in the COMPLEMENT1 trial with untreated unfit patients randomly assigned to CHL or ofatumumab + CHL. Again, the HR of 2.01 (p < 0.01) confirmed an impact of **NOTCH1** mutations on PFS in ofatumumab + CHL but not in CHL (HR 1.14, p = 0.59) [44].

For other recurrently mutated genes, prognostic value data from clinical trials is less mature. This is due to a low incidence and an extensive effort for conventional sequencing approaches as these drivers typically lack hotspot mutations. **ATM** mutations for example do not cluster in hotspots and are dispersed over 62 exons. Furthermore, a reliable distinction of mutations and rare polymorphisms is hard due to the lack of a detailed database comparable to IARC for **TP53**. Thus, the interpretation of single nucleotide variants is error-prone without non-tumor controls, and results with regard to the prognostic impact are therefore contradictory. In LRF CLL4, mutated **ATM** decreased PFS in 11q-deleted patients, but not in cases with an intact second allele [45]. In a WES-based analysis of a subgroup of CLL8, these results were not confirmed [6]. **BIRC3**, like **ATM** located on the long arm of chromosome 11 and affected in about 80% of 11q deletions, is another driver with unclear prognostic value. Initial results brought **BIRC3** in context with aggressive CLL and predisposition for non-response to therapy [46]. In trials, the incidence was as low as 3% in untreated patients and lacks impact on survival so far [6, 47].

Meanwhile, with **NFKBIE**, **XPO1**, **RPS15**, **BRAF**, **EGR2**, and others, another set of infrequently mutated genes associated with adverse outcome emerged, also yet unconfirmed in their prognostic value [6, 7, 43, 48]. As the genomic landscape characterization of CLL expands with every new study, it is worth stepping back and looking at the bigger picture instead of details. Like in other malignancies, the number of affected drivers adds genetic instability and is thus correlated with adverse outcome [6, 7, 30]. Furthermore, in CLL, recurrent mutations are not homogeneously spread across the genes in a human genome but affect drivers that could be integrated into a small set of pathways. They include the receptor-triggered signaling through **NOTCH**, Wnt, BCR, and inflammatory receptors, MAPK-ERK and NFκb signaling, as well as intracellular core mechanisms including DNA damage and cell cycle control, chro-

### Table 1. Outcome in patient subgroups defined by mutations (columns) and treatment (rows) after FC and FCR from the GCLLSG CLL8 trial [3].

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mutation</th>
<th>TP53wt (n = 556)</th>
<th>TP53mut (n = 72)</th>
<th>p valuea</th>
<th>NOTCH1wt (n = 560)</th>
<th>NOTCH1mut (n = 62)</th>
<th>p valuea</th>
<th>SF3B1wt (n = 507)</th>
<th>SF3B1mut (n = 114)</th>
<th>p valuea</th>
</tr>
</thead>
<tbody>
<tr>
<td>PFS, months</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FC</td>
<td></td>
<td>35.9</td>
<td>12.1</td>
<td>&lt;0.001</td>
<td>32.8</td>
<td>33.9</td>
<td>0.743</td>
<td>33.9</td>
<td>28.6</td>
<td>0.008</td>
</tr>
<tr>
<td>FCR</td>
<td></td>
<td>59</td>
<td>15.4</td>
<td>&lt;0.001</td>
<td>57.3</td>
<td>34.2</td>
<td>0.013</td>
<td>59.1</td>
<td>42.9</td>
<td>0.033</td>
</tr>
<tr>
<td>p valueb</td>
<td></td>
<td>&lt;0.001</td>
<td>0.001</td>
<td></td>
<td>&lt;0.001</td>
<td>0.996</td>
<td>&lt;0.001</td>
<td>0.001</td>
<td>0.004</td>
<td></td>
</tr>
<tr>
<td>OS, months</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FC</td>
<td></td>
<td>89.6</td>
<td>30.4</td>
<td>&lt;0.001</td>
<td>83.7</td>
<td>85.9</td>
<td>0.597</td>
<td>86.4</td>
<td>75.6</td>
<td>0.172</td>
</tr>
<tr>
<td>FCR</td>
<td></td>
<td>NR</td>
<td>42.2</td>
<td>&lt;0.001</td>
<td>NR</td>
<td>79.2</td>
<td>0.112</td>
<td>NR</td>
<td>NR</td>
<td>0.301</td>
</tr>
<tr>
<td>p valueb</td>
<td></td>
<td>0.014</td>
<td>0.166</td>
<td>&lt;0.001</td>
<td>0.793</td>
<td>0.004</td>
<td>0.1</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

aVariables included in the models: FC, FCR, TP53mut, NOTCH1mut, SF3B1mut, and the interactions of each gene mutation with treatments [3].

### Table 2. Multivariable analyses of mutations, treatments, and their interactions for survival after FC (fludarabine, cyclophosphamide) and FCR (fludarabine, cyclophosphamide, rituximab) treatment [3].

<table>
<thead>
<tr>
<th>Parameter</th>
<th>HR</th>
<th>95% CI</th>
<th>p valuea</th>
</tr>
</thead>
<tbody>
<tr>
<td>PFS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FC</td>
<td>0.544</td>
<td>0.445–0.665</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>FCR</td>
<td>0.544</td>
<td>0.445–0.665</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TP53mut</td>
<td>3.607</td>
<td>2.737–4.755</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>SF3B1mut</td>
<td>1.355</td>
<td>1.070–1.717</td>
<td>0.012</td>
</tr>
<tr>
<td>NOTCH1mut</td>
<td>1.652</td>
<td>1.076–2.535</td>
<td>0.022</td>
</tr>
<tr>
<td>NOTCH1mut interaction</td>
<td>1.652</td>
<td>1.076–2.535</td>
<td>0.022</td>
</tr>
<tr>
<td>OS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FC</td>
<td>0.654</td>
<td>0.498–0.860</td>
<td>0.002</td>
</tr>
<tr>
<td>FCR</td>
<td>0.654</td>
<td>0.498–0.860</td>
<td>0.002</td>
</tr>
<tr>
<td>TP53mut</td>
<td>4.47</td>
<td>3.234–6.177</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Variables included in the models: FC, FCR, TP53mut, NOTCH1mut, SF3B1mut, and the interactions of each gene mutation with treatments [3].

HR = Hazard ratio; CI = confidence interval; PFS = Progression-free survival; OS = overall survival.
matin modification, transcription and splicing, and ribosomal processing. Notably, none of these pathways were uncharted in CLL, and novel drivers integrate perfectly in the established landscape indicating the importance of these categories for CLL development and growth [6, 7, 28]. 2 operational conclusions follow from these observations: First, we need to enhance our understanding of the role of specific pathways rather than of individual genes for tumor pathogenesis and progression of CLL. In parallel, there is an urgent need for a more complete genomic characterization in current clinical trials to identify the prognostic value of specific drivers and pathways for distinct kinds of therapy.

Conclusion

Among dozens of more or less established prognostic factors, genetic markers show high reliability and strong impact on outcome reproducible in several different clinical trials. There were attempts to integrate clinical characteristics, lab parameters, and genomic parameters including novel mutations into a combined hierarchical model [4, 49, 50]. And although some of these models are used in clinical trials, they never made their way into daily clinical practice.

IGHV mutation status, cytogenetics based on the Döhner’s hierarchical model, and TP53 mutation status remain our gold standard to predict independently the aggressiveness of the disease. Furthermore, mutated/deleted TP53 status identifies a subset of high-risk patients with a need for special care and specific therapy options without chemotherapy.

Although we can contemplate a more complete genomic landscape of CLL and a couple of novel candidate genes after recent publications in 2015, currently none of them are recommended to be assessed in a routine clinical setting. This is mostly due to our insufficient knowledge about the pathogenetic background of novel drivers and their impact on outcome. However, this sets the basis for vast numbers of future studies to clarify not only the clinical impact of these novel markers but also their biologic background.

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References


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