The genomic landscape of chronic lymphocytic leukemia: clinical implications

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A B S T R A C T

Chronic lymphocytic leukemia (CLL) is a molecularly heterogeneous disease as revealed by recent genomic studies. Among genetic lesions that are recurrent in CLL, few clinically validated prognostic markers, such as *TP53* mutations and 17p deletion, are available for use in clinical practice to guide treatment decisions. Recently, several novel molecular markers have been identified in CLL, including *NOTCH1*, *SF3B1*, *MYD88* and *BIRC3* mutations. Though these mutations have not yet gained the qualification of predictive factors for treatment tailoring, they have shown to be promising to refine the prognostic stratification of patients. The introduction of targeted drugs is changing the genetics of CLL, and has disclosed the acquisition of previously unexpected drug resistant mutations in signaling pathway genes. Ultra-deep next generation sequencing has allowed us to reach deep levels of resolution of the genetic portrait of CLL providing a precise definition of its subclonal genetic architecture. This approach has shown that small subclones harboring drug resistant mutations anticipate the development of a chemorefractory phenotype. Here we review the recent advances in the definition of the genomic landscape of CLL and the ongoing research to characterize the clinical implications of old and new molecular lesions in the setting of both conventional chemo-immunotherapy and targeted drugs.

Learning goals

At the conclusion of this activity, participants should:
- understand the genetics of CLL and its relevance for disease biology;
- understand the clinical implications of CLL genetics for disease prognostication, prediction of response to therapy and treatment tailoring;
- understand the current evidence-based minimal requirements for CLL genetics characterisation in the daily clinical practice.

Introduction

The chronic lymphocytic leukemia (CLL) genome carries approximately 1000 molecular lesions per tumor, including 10-20 non-synonymous mutations and approximately 5 gross structural abnormalities (Figure 1).[1-3] At variance with other indolent B-cell lymphoproliferative disorders, where one predominant gene is molecularly altered in virtually all cases, CLL has a heterogeneous genetic profile and no unifying lesions have so far been identified. Few molecular alterations recur at a frequency of over 5% in CLL, while a large number of biologically and clinically uncharacterized genes are mutated at lower frequencies.[1-3] The most recurrent molecular lesions of CLL point to the deregulation of cellular programs of clinical importance (Figure 2), namely: i) apoptosis and cell cycle; ii) cell signaling; and iii) mRNA splicing.

Apoptotic and cell cycle checkpoints

Deletion 13q14 is the most frequent genetic lesion of CLL occurring in 50%-60% of cases.[4] The minimal deleted region on 13q14 contains the *miR15A* and mi*R16A* microRNAs.[5] In normal cells, *miR15A* and *miR16A* inhibit the expression of multiple genes, including *BCL2*, the cyclins *CCND1* and *CCND3*, and cyclin-dependent kinase 6 (*CDK6*).[6,7] Deletion of *miR15A* and *miR16A* abrogates this inhibitory effect, favors the constitutive survival and cycling of tumor B cells, and causes CLL in mouse models.[6,7] In a relevant fraction (approx. 25%) of CLL patients, deletion of 13q14 occurs in the absence of any concomitant driver genetic lesion. Patients harboring solely 13q14 deletion have an excellent clinical outcome with a progression rate of less than 1% per year and an expected survival only slightly lower than that of the general population.[8] *BCL2* is one of the genes that are up-regulated in CLL as a consequence of *miR15A*/mi*R16A* deletion. Consistent with the central contribution of *BCL2* activation in the pathogenesis of CLL, selective inhibition of BCL2 through the BH3 mimetic ABT-199 results in high response rates in relapsed or refractory patients, including those harboring high-risk genetic abnormalities.[9]

TP53 codes for a central regulator of the DNA-damage-response pathway and, when functional, triggers CLL cell apoptosis in response to chemotherapy. *TP53* may be disrupted in CLL by deletions, mutations, or a combination of both. The deletion of 17p13 always contains the *TP53* locus, and is found

Figure 1. Mutated genes in chronic lymphocytic leukemia. Word clouds representing genes that are affected by mutations in CLL (COSMIC v71). The size of the font is proportional to the prevalence of gene lesions.

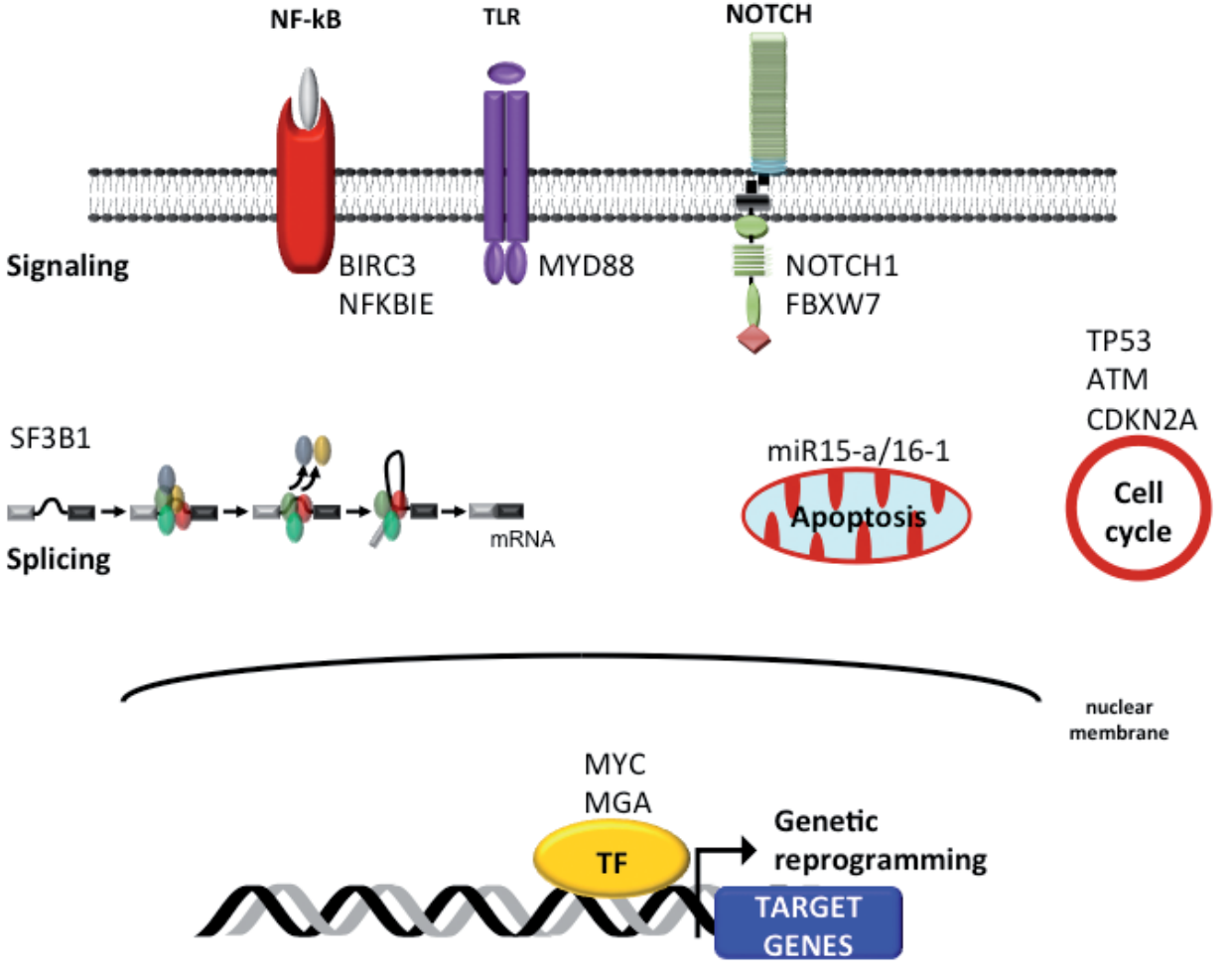

Figure 2. Pathways associated with recurrently mutated genes in chronic lymphocytic leukemia.

in 3%-8% unselected CLL at diagnosis and in 8%-12% CLL at the time of first-line treatment.[4,10,11] On the other hand, specific subgroups of patients affected by fludarabine-refractory CLL or Richter syndrome show a higher incidence of 17p13 deletion that may be detected in up to 30%-40% of cases.[12,13] Mutations represent the most frequent form of *TP53* inactivation in CLL and are frequently (80%-90% of cases) accompanied by the loss of the second allele through 17p13 deletion. At diagnosis, the incidence of *TP53* mutation has been reported to be 4%-8%.[14-16] As disease progresses, the incidence of *TP53* mutations rises to 10%-12% at the time of first-line treatment, 40% in fludarabine-refractory CLL, and 50%-60% in Richter syndrome.[13,17-20] Overall, 95% of mutations are localized within the central DNA-binding domain of TP53, impairing DNA binding and transactivation of target genes.[21]

The clinical importance of *TP53* abnormalities in CLL is tightly linked to the poor prognosis marked by this genetic lesion and its close association with chemorefractoriness, as documented by a number of observational studies and prospective trials led in both the chemotherapy and immunochemotherapy era. Among newly diagnosed CLL, patients harboring 17p13 deletion have the worst outcome, with an estimated median overall survival (OS) of 3-5 years (approx. 30% of cases are alive at ten years, accounting for an approx. 70% reduction of the expected survival compared to the general population).[4,8] This is consistent with the notion that newly diagnosed CLL with 17p13 deletion frequently harbor unmutated *IGHV* genes, present in advanced stage, or show a rapidly progressive disease that requires treatment shortly after diagnosis (median time to first treatment: 9 months). However, it is important to stress that there is a small subgroup of patients with 17p13 deletion (and mostly mutated *IGHV* genes) who may exhibit stable disease for years without indications for treatment.

The outcome of patients with 17p13 deletion who need treatment is poor and there are no definitive data on the most efficacious first-line treatment. Patients with 17p13 deletion will very rarely achieve complete response after chemo/chemoimmuno-therapy (Table 1), as demonstrated by: i) the German CLL Study Group CLL8 trial,[11] in which CLL harboring 17p13 deletion had a complete response rate of only 2% compared to 21% and 44% among CLL lacking 17p13 deletion and treated with fludarabine-cyclophosphamide (FC) or fludarabine-cyclophosphamide-rituximab (FCR), respectively; and ii) the UK Leukemia Research Foundation CLL4 trial,[10] in which the group of patients with 17p13 deletion had a very low (5%) rate of complete/nodular partial responses. Such poor response to chemo+/- immunotherapy translates into an estimated OS in the range of 2-3 years from the time of front-line treatment for 17p13 deleted patients.[10,11]

A number of retrospective studies suggest that, in addition to 17p13 deletion, also *TP53* mutations, even in the absence of 17p13 deletion, predict poor outcome in CLL. These observations have been confirmed within the frame of two large prospective studies conducted in the setting of previously untreated cases (Table 1). In the UK Leukemia Research Foundation CLL4 trial,[18] *TP53* mutated patients have a poorer response rate (27% *vs*. 83%), shorter progression-free survival (PFS) (5-year PFS: 5% *vs*. 17%) and shorter OS (5-year OS: 20% *vs*. 59%) compared to *TP53* wild-type patients. In the CLL8 trial of the German CLL study Group,[20] the prognostic impact of *TP53* mutation is observed for all efficacy end points of the trial, and is similarly evident in both treatment groups (FC and FCR), suggesting that the addition of rituximab to chemotherapy does not alleviate the negative impact of *TP53* lesions. Indeed, *TP53* mutated patients are less like-

Table 1. Clinical outcome of chronic lymphocytic leukemia patients harboring high-risk genetic lesions.[a]

	TTFT	ORR	MRD neg	PFS	OS
Watch and wait[16]					
TP53 mutation/deletion	34 mos	-	-	-	-
NOTCH1 mutation	39 mos	-	-	-	-
SF3B1 mutation	37 mos	-	-	-	-
11q deletion	32 mos	-	-	-	-
FC[11,20]					
17p deletion	-	34%	0%	0% at 3 yrs	37% at 3 yrs
TP53 mutation	-	51%	7%	12 mos	30 mos
NOTCH1 mutation	-	87%	50%	33 mos	85 mos
SF3B1 mutation	-	89%	23%	18 mos	75 mos
11q deletion	-	87%	23%	32% at 3 yrs	83% at 3 yrs
FCR[11,20]					
17p deletion	-	68%	14%	18% at 3 yrs	38% at 3 yrs
TP53 mutation	-	75%	18%	15 mos	42 mos
NOTCH1 mutation	-	90%	46%	34 mos	79 mos
SF3B1 mutation	-	96%	66%	42 mos	NR
11q deletion	-	93%	64%	64% at 3 yrs	94% at 3 yrs
CLB, F, FC[22]					
TP53 mutation/deletion	-	-	-	4 mos	26 mos
NOTCH1 mutation	-	-	-	22 mos	72 mos
SF3B1 mutation	-	-	-	27 mos	58 mos

[a]FC: fludarabine, cyclophosphamide; FCR: fludarabine, cyclophosphamide, rituximab; F: fludarabine; CLB: chlorambucil; NR: not reached; TTFT: time to first treatment; ORR: overall response rate; MRD: minimal residual disease; PFS: progression-free survival; OS: overall survival; yrs: years; mos: months.

ly to respond (FC arm: 51% *vs*. 92%; FCR arm: 98% *vs*. 75%) and reach minimal residual disease (MRD) negativity (FC arm: 7% *vs*. 37%; FCR arm: 18% *vs*. 68%) compared to *TP53* wild-type patients. Accordingly, *TP53* mutated patients show a significantly shorter PFS (FC arm: 12 months *vs*. 35 months; FCR arm: 15 months *vs*. 59 months) and OS (FC arm: 30 months *vs*. 89 months; FCR arm: median not reached *vs*. 42 months) than *TP53* wild-type cases.

Combinations of alemtuzumab with steroids are amongst the most potent therapies for patients harboring *TP53* abnormalities, yielding response rates of 88% in previously untreated cases, with 65% of cases achieving a complete response.[23] The BTK inhibitor ibrutinib as single agent or combined to rituximab induces a response rate of 68%-97% in patients with *TP53* abnormalities.[24-27] A similar proportion of responses is observed with the PI3Kδ inhibitor idelalisib combined to rituximab.[28] Although these results appear significantly better than every previous historical control in CLL with *TP53* abnormalities, alemtuzumab, ibrutinib and idelalisib *per se* do not promise long-lasting remissions. Therefore, these patients should be considered for alternative treatments within clinical trials whenever possible. Use of FCR, alemtuzumab-based regimens or new drugs may be considered as a de-bulking strategy, but allogeneic stem cell transplantation should still be offered and discussed in patients who are in adequate physical condition and who have an available donor.[29]

Given their value as biomarkers of treatment resistance, current guidelines recommend testing 17p13 deletion and *TP53* mutations in CLL patients requiring therapy.[30-33] Sanger sequencing is the currently recommended approach for *TP53* mutation analysis.[31] However, due to its limited sensitivity, conventional Sanger sequencing misclassifies as wild-type those CLL cases harboring *TP53* mutations of low clonal abundance (<10% of the alleles). Such small *TP53* mutated subclones occur in a significant fraction of CLL, have the same unfavorable prognostic impact as clonal *TP53* defects, and anticipate the development of a chemorefractory phenotype among CLL patients requiring treatment.[34] Thanks to its high sensitivity (down to 1-0.1%), deep next generation sequencing is capable of detecting these minor, but clinically relevant, *TP53* mutated subclones. Therefore, deep next generation sequencing should be considered as a useful tool for a comprehensive assessment of *TP53* disruption in CLL.

The *ATM* gene encodes a nuclear serine/threonine kinase whose activity is induced by chromosomal double-strand breaks that arise endogenously or after exposure to DNA-damaging agents, including chemotherapeutic drugs. ATM protects the integrity of the genome by regulating the cell-cycle arrest at G1/S and G2/M to prevent processing of damaged DNA, and by activating DNA-repair pathways or, alternatively, inducing apoptosis if the DNA damage cannot be repaired. As for *TP53*, the *ATM* gene is inactivated in CLL by both deletion and/or somatic mutations, which result in impaired DNA damage responses. Deletion of 11q22-23 always includes *ATM* and occurs in less than 10% newly diagnosed CLL, while its prevalence rises to approximately 20% at the time of first treatment.[4,10,11] Deletion 11q22-23 co-occurs with *ATM* mutations in 30%-40% of patients. *ATM* mutations generally consist in a mixture of missense substitutions distributed across the *ATM* coding sequence, with no clear hotspots, and have been observed in approximately 10%-15% of newly diagnosed patients and in approximately 15% of progressive CLL requiring first treatment.[35-37] By combining mutations and deletions, genetic lesions of *ATM* occur in approximately 20% of diagnostic samples of CLL and in approximately 35% cases requiring first treatment. From a clinical perspective, the presence of *ATM* deletion at the time of CLL presentation identifies a group of patients with intermediate-risk disease (approx. 40% of cases are alive at 10 years, accounting for an approx. 50% reduction in the expected survival compared to the general population).[8] Among CLL requiring treatment, the presence of 11q22-23 deletion alone or combined to *ATM* mutations associates with poor response to chemotherapy. In the UK Leukemia Research Found CLL4 trial,[37] where CLL were treated with various chemotherapy approaches not including an anti-CD20 monoclonal antibody, patients with both *ATM* mutation and 11q22-q23 deletion show a significantly reduced response rate and PFS (46% and 7 months, respectively) compared to those with wild-type *ATM* (84% and 28 months, respectively), 11q22-q23 deletion alone (72% and 17 months, respetively), or *ATM* mutation alone (87% and 30 months, respectively). Consistently, in the same trial, the OS for patients with biallelic *ATM* alterations is significantly reduced compared to those with wild-type *ATM* or *ATM* mutations alone (42 *vs*. 85 *vs*. 77 months, respectively). The addition of rituximab to chemotherapy significantly improves the outcome of CLL patients harboring *ATM* lesions. In the CLL8 trial of the German CLL study Group,[11] treatment with FCR increases both the complete response rate (51% *vs*. 15%) and PFS (64% *vs*. 32% at 3 years) in CLL patients with 11q22-23 deletion compared to FC alone. However, even among CLL treated with FCR, 11q22-23 deletion still remains an adverse factor that, similar to unmutated *IGHV* genes, identifies a group of patients with intermediate-risk disease that are projected to progress in a relatively short time.[11]

Signaling pathways

At variance with other B-cell tumors, genes encoding for components of the BCR signaling machinery are usually not targeted by somatic mutations in unselected CLL. The introduction of targeted drugs inhibiting BCR signaling is changing the genetics of the disease, and has disclosed the acquisition of previously unexpected drug resistant mutations in BCR pathway genes, including mutations affecting the *BTK* binding site of ibrutinib or gain-of-function mutations in *PLCG2*.[38,39] Ibrutinib resistant mutations of the BCR pathway are not detectable at the baseline before ibrutinib exposure, as well as in any ibrutnib naïve CLL, thus indicating that they are biologically irrelevant in the absence of selective pressures imposed by the drug.[40]

The NOTCH receptor genes encode a family of heterodimeric transmembrane proteins (NOTCH1 to NOTCH4) that function as ligand-activated transcription factors. When the NOTCH receptors interact with their ligands through the extracellular subunit, two consecutive proteolytic cleavages of the NOTCH proteins are initiated

and lead to pathway activation. Upon activation, the cleaved intracellular portion of the NOTCH receptors (ICN) translocates into the nucleus where it recruits a transcriptional complex to modify the expression of a number of target genes, including *MYC* and NF-κB signaling components. The most prominent mechanism of NOTCH signal suppression is operated through its PEST domain of the ICN, which is recognized by the FBXW7 ubiquitin protein ligase and directed towards proteasomal degradation.

In CLL, two genes of the NOTCH pathway are recurrently mutated, namely *NOTCH1* and *FBXW7*. *NOTCH1* mutations characterize approximately 10% of unselected CLL and are mainly represented by frameshift or nonsense events clustering within exon 34, including the highly recurrent c.7544_7545delCT deletion (approx. 80% of all mutations).[1,13,16,41] By taking advantage of this mutational spectrum, PCR-based strategies not requiring DNA sequencing have been designed for the rapid detection of the c.7544_7545delCT mutation for diagnostic and prognostic purposes.

NOTCH1 mutations in CLL are selected to disrupt the PEST domain of the protein, resulting in NOTCH1 impaired degradation, stabilization of the active ICN, and deregulated NOTCH signaling.[1] Consistent with this notion, a number of cellular pathways, including those controlling cell metabolism and cell cycle progression, are deregulated in CLL harboring *NOTCH1* mutations.[42,43] *NOTCH1* is preferentially targeted in specific biological groups of CLL. In fact, *NOTCH1* mutations are significantly more common in CLL with unmutated *IGHV* genes, and are enriched in CLL harboring +12.[43,44] Mutations of *FBXW7* have been detected in approximately 2% of unselected CLL, and are predicted to cause loss of function by disrupting the WD40 domain of the protein. *FBXW7* mutations are likely to functionally mimic the PEST domain mutations of *NOTCH1*, thus resulting in constitutively active NOTCH signaling. Consistently, *FBXW7* mutations in CLL distribute in a mutually exclusive manner with *NOTCH1* mutations and, similar to *NOTCH1* mutations, cluster with cases harboring unmutated *IGHV* genes and +12.[3] From a clinical perspective, the presence of *NOTCH1* mutations at the time of CLL presentation identifies a group of patients with intermediate-risk disease (approx. 40% of cases are alive at 10 years, accounting for an approx. 50% reduction of the expected survival compared to the general population) and those in whom CLL is more likely to transform into RS (cumulative incidence of transformation at 10 years of approx. 50%).[8,41,42] Among CLL requiring treatment, cases harboring *NOTCH1* mutation seem not to benefit from the addition of an anti-CD20 monoclonal antibody to chemotherapy. Indeed, among CLL harboring *NOTCH1* mutations, treatment with FCR does not result in the expected increase in MRD response (46% *vs*. 50%) nor into an improvement in PFS (median: 34 months *vs*. 33 months) or OS (median: 79 months *vs*. 85 months) compared to treatment with the sole FC (Table 1).[21] Similarly, among *NOTCH1* mutated CLL, treatment with chlorambucil plus ofatumumab does not result in an improvement in PFS (median: 17 months *vs*. 10 months) compared to treatment with the sole chlorambucil.[45] Although lower efficacy of therapeutic anti-CD20 monoclonal antibodies in *NOTCH1* mutated CLL seems to be consistent across two independent trials, the precise biological mechanisms underlying this clinical observation still have to be clarified. Elucidation of the mechanistic basis would strengthen the rationale for guiding treatment based on *NOTCH1* mutation status. Furthermore, it would be interesting to see if a similar observation can be made with other anti-CD20 monoclonal antibodies. In CLL, NF-κB signaling is generally up-regulated through specific interactions between protective micro-environmental niches and CLL cells. At least in a fraction of cases, CLL gain active NF-κB signaling by mutating NF-κB genes.

The non-canonical NF-κB pathway is engaged by CD40 and BAFF receptors. Upon receptor binding, the TRAF3/MAP3K14-TRAF2/BIRC3 negative regulatory complex of non-canonical NF-κB signaling is disrupted, allowing the cytoplasmic release and stabilization of MAP3K14, the central activating kinase of non-canonical NF-κB signaling. The stabilized MAP3K14 activates the IKKα kinase, which in turn directly phosphorylates NF-κB/p100, inducing partial proteolysis of p100 to p52 by the proteasome. The p52 protein dimerizes with RelB to translocate into the nucleus, where it regulates gene transcription.

The Baculoviral IAP repeat containing 3 (*BIRC3*) gene, which co-operates in the TRAF3/MAP3K14-TRAF2/BIRC3 negative regulatory complex of non-canonical NF-κB signaling, is mutated in approximately 2% of unselected CLL.[16,46] At the biochemical level, *BIRC3* mutations cause the truncation of the C-terminal RING domain of the BIRC3 protein, whose E3 ubiquitin ligase activity is essential for switching off MAP3K14 through proteosomal degradation, thus leading to constitutive non-canonical NF-κB activation.[46] From a clinical standpoint, *BIRC3* mutations identify a genetic subgroup of cases characterized by poor risk disease.[46] Also, *BIRC3* mutations, confer complete resistance to ibrutinib *in vitro* studies, consistent with the ability of mutant *BIRC3* to activate NF-κB signaling downstream the BCR and in a BTK independent fashion.[47]

NF-κB comprises a small family of transcription factors, including the NF-κB/Rel members RelA, RelB, c-Rel, NF-κB1, and NF-κB2. These proteins are kept inactive by cytoplasmic association with the IκB inhibitory proteins. The NF-κB inhibitor epsilon (NFKBIE) belongs to the IκB inhibitory protein family and counteracts NF-κB activation via cytoplasmic retention of the Rel proteins. The *NFKBIE* gene is affected by a recurrent 4bp deletion in approximately 5% unselected CLL.[48,49] *NFKBIE* mutation results in protein truncation, reduced inhibitory interaction with the Rel transcription factor, and enhanced NF-κB activation.[49] Though the precise clinical implication of *NFKBIE* mutations remains to be clarified, their enrichment among CLL presenting in advanced stage suggests that they might be involved in disease progression.

In B cells, Toll-like receptors (TLR) are central to the BCR-independent response to antigens by sensing a variety of pathogen-associated molecular patterns derived from bacteria, viruses, and fungi. Adaptor proteins, including the myeloid differentiation factor 88 (MYD88), are essential for initiating the TLR signaling. MYD88 has a modular structure with a death domain (DD) at the N terminus, and a Toll-IL-1 receptor (TIR) domain at the C terminus. The TIR domain of MYD88 is crucial for signal transduction since it mediates contacts with the intracellu-

lar TIR domains of the TLRs upon signaling activation. The DD domain allows oligomerization of the active MYD88 and its interaction with the respective DD of the serine-threonine kinases IRAK1-4, thus resulting in a multimeric complex. This complex propagates the signal and leads to activation of a series of cascades and transcription factors, such as NF-κB, AP-1 and STAT3. Most *MYD88* mutations in CLL are represented by the L265P missense substitution, which affects the evolutionarily conserved beta-beta loop of the TIR domain of MYD88, suggesting that it has been selected to change the structure of MYD88 to allow spontaneous homodimerization and recruitment of IRAK1 and IRAK4.[42] Consistently, in B-cell tumors, mutant *MYD88* results in uncontrolled formation of the MYD88/IRAK complex, which translates into the recruitment of TRAF6, constitutive phosphorylation of TAK1 and, ultimately, the elevation of NF-κB activity and cytokine secretion. *MYD88* gene mutations occur in approximately 3% of unselected CLL, while they are enriched in a specific clinical subgroup of patients characterized by young age at presentation, mutated *IGHV* genes and expected survival similar to that of the age- and sex-matched normal population.[50]

Splicing

Splicing of precursor messenger RNA (pre-mRNA) and formation of mature mRNA through the removal of introns in protein-encoding genes is carried out in the nucleus by the spliceosome, a complex of five small nuclear ribonucleoproteins (snRNPs). These spliceosome components are required for normal constitutive and alternative splicing. Alternative splicing can generate numerous transcript variants for each gene, thus adding to genomic complexity and potentially contributing to tumorigenesis. *SF3B1* is a core component of the U2 snRNP, that recognizes the 3' splice site at the intron-exon junctions and orchestrates the excision of introns from pre-mRNA to form mature mRNA. Structurally, the SF3B1 protein has two well-defined regions: i) the N-terminal amino acid region, that contains several protein-binding motifs and functions as a scaffold to facilitate its interaction with other splicing factors such as U2AF65 and SF3B14; ii) the C-terminal region, that contains 22 non-identical tandem repeats of the HEAT motif that meander around the SF3b complex, enclosing SF3B14.

SF3B1 mutations occur with a prevalence that ranges from 7% to 10% of unselected CLL, are enriched in cases harboring unmutated *IGHV* genes, and tend to co-occur with *ATM* deletion or mutation.[2,3,51] *SF3B1* mutations in CLL are generally represented by missense nucleotide changes that recurrently target hotspots (codons 662, 666, 700, 704, 742), with a single amino-acid substitution (K700E) accounting for approximately 50% of all *SF3B1* mutations.

The pathogenic role of *SF3B1* mutations is supported by

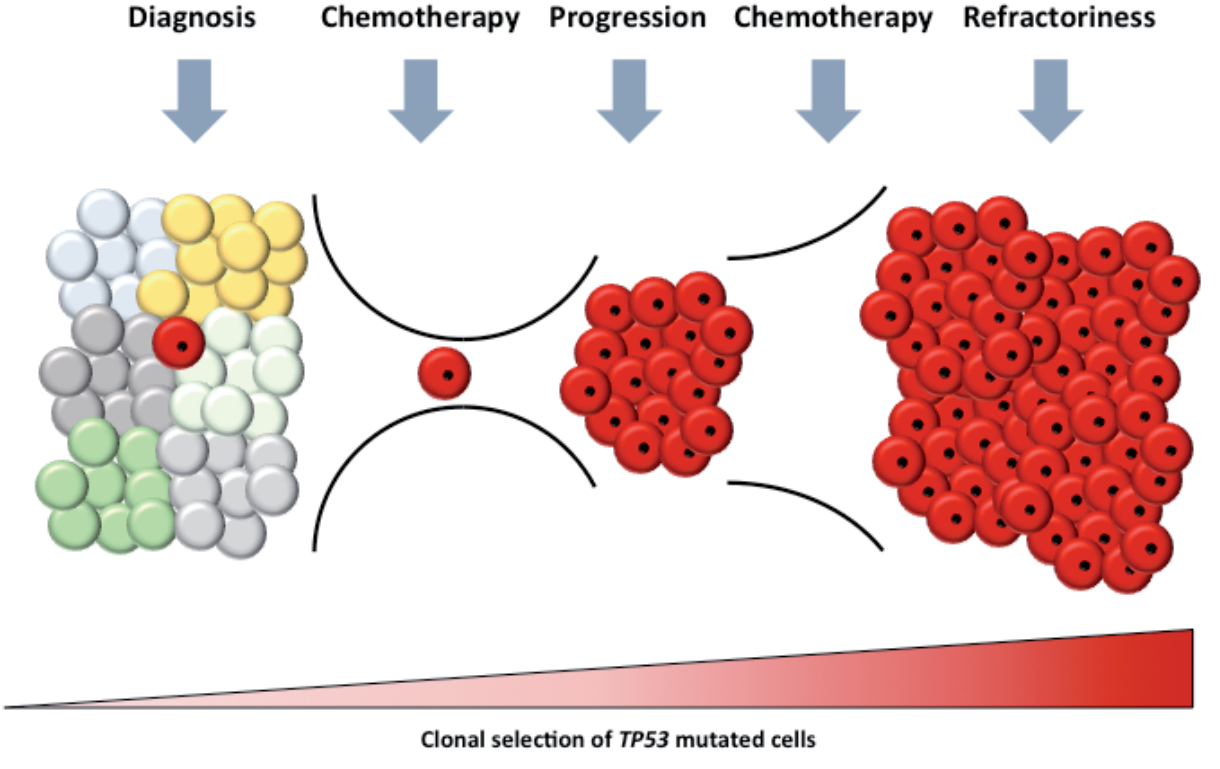

Figure 3. A model for the stepwise evolution of small *TP53* mutated clones in chronic lymphocytic leukemia.

their clustering in evolutionarily conserved hotspots within the inner surfaces of the HEAT structure at the supposed sites of interaction with RNA and co-factors.[2] This observation suggests that mutation in *SF3B1* could possibly mediate alteration in its normal function through change in the physical interactions of the SF3B1 protein with its binding partners, thus leading to a defective spliceosome complex that is incapable of performing the correct splicing steps.[3] From a clinical perspective, the presence of *SF3B1* mutations at the time of CLL presentation identifies a group of patients with intermediate-risk disease (40% of cases are alive at 10 years, accounting for an approx. 50% reduction of the expected survival compared to the general population).[8] Among CLL requiring treatment, *SF3B1* mutations can potentially help refine prognostication of treatment relapse, though they do not represent a predictive biomarker for treatment tailoring (Table 1). Indeed, in the UK Leukemia Research Found CLL4 trial,[22] the *SF3B1* status does not impact on the chance of achieving responses to chemotherapy, though patients harboring *SF3B1* mutations show a shorter PFS (29 months *vs*. 39 months) than *SF3B1* wild-type cases. Consistently, in the CLL8 trial of the German CLL Study Group,[20] the *SF3B1* status does not impact on the chance of achieving clinical or MRD responses, but strongly affects PFS, that is shorter in patients harboring *SF3B1* mutations than in wild-type cases, independent of whether they received FC (28 months vs 34 months) or FCR (42 months vs 59 months).

Conclusion and perspectives

In the era of personalized medicine, the challenges for the treatment of patients with CLL will involve correctly matching targeted therapies to the unique genetic and clonal composition of each individual tumor. Genomic studies have produced an unprecedented body of knowledge regarding the cellular programs involved in CLL pathogenesis and their implications as both prognostic/predictive factors and actionable targets. Next generation sequencing approaches have the potential of making testing of these genetic markers cheaper, faster and more widely available.

Clonal evolution studies have revealed those pathways that are altered by early genetic events in CLL and are conceivably responsible for driving the founder clone of the tumor, thus representing ideal therapeutic targets for treatments aimed at disease eradication. Technical advances, including ultra-deep next generation sequencing, have allowed an in-depth resolution of the genetic portrait of CLL, providing a precise definition of its subclonal genetic architecture.[52] This approach has shown that small subclones harboring drug resistant mutations may be admixed with a large number of wild-type cells, thus being far below the levels of detection of conventional mutation assays (Figure 3). Treatments that do not take into account such clonal diversity and target only the dominant clone leave open the possibility that one of these minor and resistant clones will then replicate and become dominant, leading to recurrence of the tumor. Thus, targeting both minor and dominant CLL clones through drug combinations incorporating novel agents could represent a pivotal strategy for the most efficient treatment of CLL.

Finally, beside the identification of actionable targets, genomic studies are also revealing new and previously unexpected mechanisms of targeted drug resistance in CLL, as exemplified by the discovery of mutation-associated ibrutinib-refractoriness.

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