Pre-therapeutic assessment of chronic lymphocytic leukaemia: what are the genetic tests and why?

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Bilan préthérapeutique de la leucémie lymphoïde chronique : quel bilan génétique et pourquoi ?

CLL, predictive mutationnal status, TP53, IGHV

Abstract

In the era of targeted therapies, the management of chronic lymphocytic leukaemia (CLL) has evolved. A pre-therapeutic genetic assessment is now indispensable to guide the choice of therapeutic strategy adapted to each patient. On the one hand, CLL is clinically heterogeneous, with one third of patients never being treated. On the other hand, relapses are frequent, and patients often receive several lines of therapy. There are two types of genetic markers, those that are predictive of the response to treatment and guide the therapy and those that are prognostic and help to stratify and improve patient follow-up. The decision to initiate treatment remains based on the clinical stage. Genetic analysis, based on the mutational status of *TP53* and *IGHV,* can help to choose between immunochemotherapy and targeted therapy.

Résumé

A l'ère des traitements ciblés, la prise en charge de la leucémie lymphoïde chronique (LLC) a évolué. Le bilan génétique préthérapeutique est indispensable aujourd'hui pour le choix de la stratégie thérapeutique adaptée à chaque patient. La LLC est une hémopathie dont l'évolution clinique est hétérogène, un tiers des patients n'étant jamais traité. D'autre part, les rechutes sont fréquentes et les patients reçoivent souvent plusieurs lignes de traitement. Les marqueurs génétiques sont de deux types: (1) prédictifs de la réponse au traitement, qui orientent le choix thérapeutique et/ou (2) pronostiques, qui aident è stratifier les patients pour un meilleur suivi. La décision d'entreprendre un traitement reste basée sur le stade clinique. Le bilan génétique va permettre d'orienter le traitement vers la chimiothérapie ou les traitements ciblés. En effet, il est basé sur le statut mutationnel de *TP53* et des *IGHV* (gène de la partie variable des chaînes lourdes des immunoglobulines). Le statut muté de *TP53* contre-indique la chimiothérapie basée sur les analogues de purines comme la fludarabine. Le statut *IGHV* muté est prédictif d'une très bonne réponse à cette dernière. D'autres marqueurs sont pronostiques et vont aider à stratifier les patients en fonction du risque d'évolution. Les recommandations actuelles, en dehors des essais cliniques, sont d'évaluer le statut mutationnel de *TP53*–déletion 17p (del[17p]) et mutations–avant toute ligne de traitement et le statut mutationnel des *IGHV* ainsi que la présence de la délétion 11q, 13q, la trisomie 12 par hybridation *in situ* en fluorescence (FISH), un caryotype conventionnel étant recommandé.

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Chronic lymphocytic leukaemia (CLL) is a haemopathy with a heterogeneous clinical course. Some patients will remain stable for many years and will not need to be treated, others will progress with the appearance of cytopenias or adenopathies and will need to be treated, and still others will present with aggressive disease from the start. In addition, relapses are frequent, and patients often receive several lines of treatment. This clinical heterogeneity reflects the genetic heterogeneity between patients as well as within the clonal population. The development of targeted therapies–involing kinase inhibitors (Bruton's tyrosine kinase [BTK], phosphoinositide 3-kinase [PI3K]), the B antigen receptor (BCR) pathway and BCL2–has revolutionised the therapeutic management of CLL. Pre-therapeutic genetic testing is now essential for guiding the choice of therapeutic strategy adapted to each patient.

There are two types of genetic markers: (1) those that are predictive of treatment response (theranostics), which guide the choice of treatment and (2) those that are prognostic, which help stratify patients for better follow-up. CLL is not defined by a single genetic abnormality; some variants recur affecting more than 5% of patients, and a large number of genes are mutated at low frequencies with no known biological or clinical significance.

Somatic mutations and therapeutic choice

Anomalies in the *TP53* gene lead to resistance to purine analogues and therefore contraindicate fludarabine-cyclophosphamide-rituximab (FCR) chemotherapy. The mutational status of *TP53* is the most important predictive marker of the response to chemotherapy which, in the era of targeted therapies, guides the choice of therapy towards BTK, PI3K or BCL2 inhibitors. The acquisition of *TP53* abnormalities in CLL cells increases as the disease progresses: they are present in 10% of cases before treatment, in 30%–40% at relapse and in 50%–60% of cases at the time of Richter transformation. Anomalies in *TP53* are associated with a poor prognosis, with a median survival of three to five years. These patients progress more rapidly, are resistant to chemotherapy and have a greater risk of Richter transformation [1]. In the majority of cases, a 17p deletion (del[17p]) is associated with a mutation in the other allele, but in 30% of cases a single mutation is present. In rare cases (<10%) only one del(17p) is identified. These results are based on Sanger sequencing studies, which have a sensitivity of about 15%. The use of sensitive sequencing techniques that can detect genome variants with a sensitivity close to 1% will allow more mutated patients to be detected. Indeed, mutations in *TP53* are often present in only a portion of CLL cells and may be subclonal (present in less than 10% of cells) [2]. However, several studies have shown that the presence of a subclone leads to resistance to FCR-type chemotherapy, with the expansion of the resistant clone [3]. Current recommendations are, therefore, prior to any line of treatment, to test for del(17p) by fluorescence *in situ* hybridisation (FISH) and *TP53* mutations by NGS sequencing [4]. Sequencing analysis of exons 2 to 11 is recommended, although the majority of mutations are found between exons 4 and 9, corresponding to the DNA binding domain of the protein. The p53 protein (known as the "guardian of the genome") is a transcription factor that acts as a tetramer. Frame-shift mutations (small deletions or insertions), stop mutations or splice-site mutations are mutations that result in the loss of p53 expression, and their combination with a del(17p) on the other allele results in the loss of total p53 expression in the cell and thus loss of function as a transcription factor. Heterozygous missense mutations result in the expression of a mutated protein, and the tetramer formed by mutated and non-mutated p53 subunits can no longer bind to DNA (dominant-negative effect) and the mutation therefore results in a loss of function of the transcription factor. Furthermore, mutated forms of p53 have a different conformation and can interact with other transcription factors such as

early growth response 1 (EGR1) or nuclear factor-kB (NF-kB) and lead to the acquisition of new functions that promote tumour proliferation [5, 6]. Anomalies in *TP53* allow the cell to survive despite the acquisition of DNA anomalies. This generates genetic instability with the accumulation of subclonal abnormalities and the appearance of a complex karyotype [3]. Clinically-speaking, do the various mutations in *TP53* have the same impact? Are the variants of *TP53* all pathogenic? The European ERIC group has published methodological recommendations for analysing and interpreting *TP53* variants [4].

In patients who have no *TP53* abnormalities, the presence of somatic mutations in the immunoglobulin heavy chain variant (*IGHV*) gene has been shown to be a highly predictive marker of a very good response to FCR therapy in patients with no contraindications to intensive therapy [7, 8]. Several studies on patients with mutated *IGHV* treated with FCR, with very long periods of remission–beyond six years–associated, in 50% to 60% of patients, with negative minimal residual disease, have shown plateaued progression-free survival, an absence of relapse and an overall survival close to that of the healthy population. Conversely, patients treated with the BTK inhibitor ibrutinib or the P13K inhibitor idelalisib, whether or not they have mutated *IGHV*, achieve the same progression-free survival rate [9].

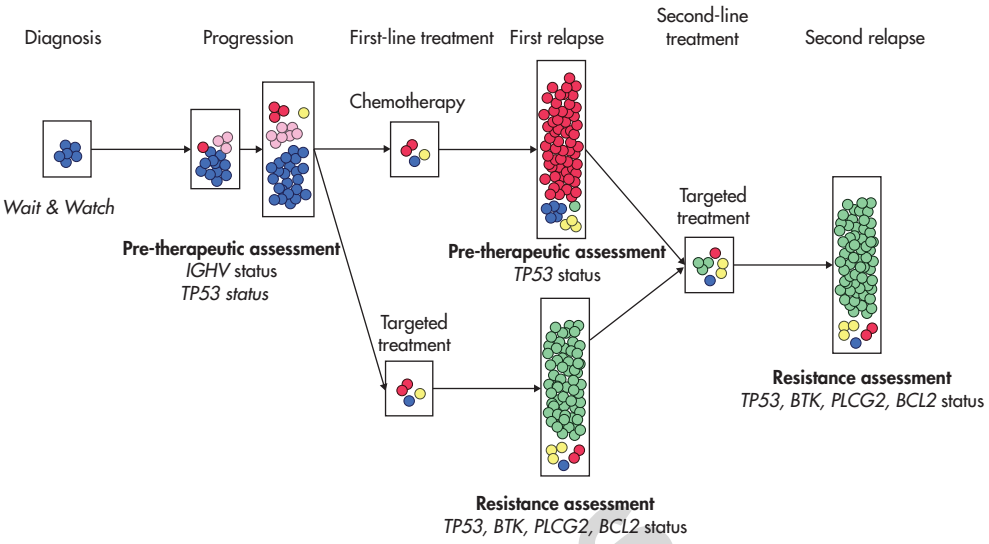
The European ERIC group has published methodological recommendations for analysing the mutational status of *IGHV* [10]. The rearrangement of *IGHV* in B lymphocytes, from which the CLL-defining clone is derived, is analysed by sequencing. The sequence of the PCR VDJ product is compared with *IGHV* sequences in germline conformation in the international immunogenetics information system (IMGT) database (http://www.imgt.org/). Thus, the unmutated haracter is defined by a sequence of *IGHV* genes in the leukaemia cells with 98% or more identity to the reference sequence. Conversely, an identity below 98% defines mutational status. Mutated *IGHV* status remains the same throughout the disease, and is determined only once. The prevalence of mutated *IGHV* is 60% in both diagnosed and asymptomatic patients and the prevalence of unmutated *IGHV* non-mutation increases in patients who progress (60%) and relapse (70%–80%). Patients with mutated *IGHV* have a favourable outcome with longer treatment-free periods, better progression-free survival after chemotherapy, lower risk of transformation and overall better survival. *IGHV* mutational status is the most robust prognostic marker in CLL [11]. In contrast, the B repertoire and the use of the different *IGHV* genes in clonal rearrangement are biased with the preferential use of certain genes. Some *VH* genes are preferentially used in unmutated *IGHV* patients (IGHV1-69 and IGVH4-39 in particular), while others are more frequently observed in mutated *IGHV* patients (IGHV4-34, IGHV3-7 and IGHV3-23).

However, analyses of hypervariable CDR3 heavy and light chain sequences, the main determinant for the antigen, have identified almost identical amino acid sequences in groups of patients, defining stereotyped CDR3 subsets [12].

The identification of stereotyped BCRs in CLL patients suggests a key role for the antigen in leukaemogenesis. To date, there are about 100 CDR3 subsets and about 33% of patients have a stereotyped BCR and share 19 subsets. Within certain subsets, patients have common clinical features. Subset 1 includes patients who all carry unmutated *IGHV* genes with a particularly poor prognosis, whereas Subset 4 includes patients with mutated IGHV genes, who are young at diagnosis and rarely need treatment. Subset 2, associated with the IGHV3-21 rearrangement, has a poor prognosis regardless of *IGHV* mutational status [13].

Identification of *IGHV* mutational status in combination with the search for abnormalities in *TP53* prior to treatment is now essential in the therapeutic process. Indeed, the role of immunochemotherapy has decreased significantly since the advent of targeted therapies and, while it remains an option for *IGHV*-mutated patients without *TP53* abnormalities, especially in patients who are

Figure 1



Predictive genetic testing to assist in treatment choice. Example of clonal evolution of CLL, with the acquisition of genetic abnormalities at different stages of the disease. Clonal heterogeneity is represented by different coloured circles. Treatments can reduce or eliminate certain clones or, conversely, favour the emergence of other clones. For example, the red clones may correspond to *TP53*-mutated clones. The choice of treatment will depend on the results of the pre-therapeutic assessment.

eligible for FCR, targeted therapies are preferred for patients with unmutated *IGHV* (*figure 1*) [14].

Mutations leading to resistance to targeted treatment and therapeutic choice in relapsing patients

After three years of treatment with ibrutinib, approximately 10% of patients progress and 10% have a Richter transformation. In patients who progress, a *BTK* mutation and/or a *PLCG2* mutation is found in more than 80% of cases [15]. In patients still on treatment who have not yet progressed, more than 50% have a detectable *BTK* mutation and/or *PLCG2* mutation [16]. These mutations are detectable nine months before progression. To date, there are no recommendations concerning the biological and therapeutic management of these patients. Should patients with persistent lymphocytosis be tested for mutations? Should targeted therapy be changed when resistance mutations are detected? Early change in treatment should be evaluated in clinical trials.

Similarly, *BCL2* resistance mutations have been detected in more than 50% of patients who progressed after more than two and a half years of treatment [17]. New clinical trials combining inhibitors with limited treatment periods should help avoid the selection of resistant mutated clones.

Genetic prognostic markers in chronic lymphocytic leukaemia

Since the 1980s, the first genetic studies focusing on copy number abnormalities using conventional karyotyping, FISH and, later, SNP array techniques have demonstrated the presence of recurrent cytogenetic abnormalities in CLL in more than 80% of patients at diagnosis, revealing a first level of genetic heterogeneity between patients. All studies since then have confirmed that the most common

chromosomal abnormalities are partial chromosome deletions, such as del(13q) (50%–60% of cases), del(17p) (5%–10%) and del(11q) (6%–20% of cases), or chromosomal gains such as trisomy 12 (tri[12], 10%–16% of cases). Balanced translocations are also described in rarer cases. These studies have also shown that the presence of these cytogenetic abnormalities allows patients to be classified into prognostic groups [18]. Patients with del(17p) have the worst prognosis, followed by patients with del(11q) and those with tri(12). Patients with del(13q) as the only abnormality have the longest overall survival, which is greater than that of patients for whom no such abnormality was found. Furthermore, it has been shown that as the number of genomic abnormalities increases, the disease appears to have a worse prognosis [19].

Del(13q14) is the most common cytogenetic abnormality, found in over 50% of patients. Its presence, when isolated, is associated with a good prognosis. The minimal deleted region includes the microRNAs miR-15a/16-1, encoded in an intron of the DLEU2 (deleted in lymphocytic leukaemia) gene. These microRNAs play a role in the negative regulation of the expression of the anti-apoptotic protein BCL2 by inducing the degradation of its transcript. The loss of DLEU2 and miR-15a/16-1 would therefore play a role in B clonal expansion by causing a defect in the regulation of apoptosis [20, 21].

Although the minimal deleted region always includes *DLEU2* and miR-15a/16-1, the size of the del(13q) is variable. Larger deletions can induce loss of the *RB1* tumour suppressor gene. This gene plays a role in cell cycle control and its loss is associated with a worse prognosis than the isolated deletion of miR-15a/16-1. However, the prognostic impact of these biallelic deletions is not well known [22].

Tri(12) is considered to be an intermediate biological risk marker but its functional impact on disease development has not been elucidated. It is strongly associated with the presence of a *NOTCH1* mutation and unmutated *IGHV* status [23]. Pathogenically, it is difficult to clearly establish a set of candidate genes affected by trisomy 12 since, unlike deletions which target a critical region, it is the whole chromosome that is duplicated. However, studies have correlated tri(12) with the over-expression of genes carried by the chromosome such as *P27, CDK4, HIP1R, MYF6* and *MDM2* but the pathophysiological link is not always clear. Among these genes, *MDM2* is involved in p53 degradation and therefore its over-expression could affect the cell cycle [24].

Monoallelic deletion of the long arm of chromosome 11 is found in 6%–20% of cases. The minimal deletion region is located at 11q22.3-q23.1 which contains the ataxia telangiectasia mutated tumour suppressor gene (*ATM*). The remaining copy of *ATM* is usually targeted by mutations, hence the association of del(11q) and *ATM* mutation frequently found in patients. Del(11q) may also include the loss of *BIRC3* at position 11q22, a negative regulator of the NF-kB pathway also affected by inactivating mutations. Clinically, del(11q) is associated with a more node-based disease with a large, progressive tumour mass and is a poor prognostic factor [25].

The incidence of complex karyotypes (CK) with more than three abnormalities is approximately 15% at diagnosis and increases with clinical course, the presence of *TP53* anomalies, unmutated *IGHV*, and the presence of del(11q) or tri(12). Some studies have shown that the presence of CK is associated with a poor prognosis with a poor response to conventional chemotherapies and to new targeted therapies [15, 26]. A recent study showed that CK is a predictive marker of poor response to ibrutinib or venetoclax, independently of *TP53* anomalies [27].

The study of a European cohort of 5,476 CLL patients further defined the prognostic impact according to the number of CK abnormalities. CKs with more than five abnormalities are associated with a poor prognosis regardless of clinical stage, *IGHV* mutational status and *TP53* abnormalities, whereas CKs with three to four abnormalities have a prognostic impact related to *TP53* abnormalities. Interestingly, tri(12)- and tri(19)-containing CKs represent a very good prognostic

group. The combination of CK markers, *IGHV,* and *TP53* status allows the identification of different prognostic groups, ranging from excellent prognosis for CK +12, +19, mutated *IGHV* status, with no *TP53* abnormalities and no CK abnormalities, to very poor prognosis for CK with more than five abnormalities. This stratification needs to be validated in clinical trials for routine use [28].

Over the past decade, the development of high-throughput genome-wide sequencing approaches has transformed our understanding of the genetics of CLL and has allowed us to characterise the mutational landscape of the disease. High-throughput exome or whole-genome sequencing studies of patients have identified a high number of recurrent genetic abnormalities, which has provided new insights into the mechanisms underlying disease development, progression and resistance to treatment [29].

Two recent studies have collectively reported the results of genome and tumour exome sequencing of approximately 1,000 CLL patients and have identified a total of 75 recurrent somatic mutations [30, 31]. Combining the results of these two studies, *SF3B1, ATM, NOTCH1* and *TP53* were identified as the most frequently mutated genes. The high number of cases has allowed us to achieve sufficient statistical power to identify mutations with a frequency of less than 1% and to discover new mutated genes such as *RPS15, IKZF3* and *PTPN11.* In the vast majority of cases, the set of somatic mutations identified affect exons, but in the series by Puente *et al.* [31], extensive genome sequencing revealed recurrent mutations in non-coding regions, including mutations in the 3'UTR region of NOTCH1 or in an enhancer of the PAX5 gene. Mutations in NOTCH1, SF3B1, BIRC3, FPXW7, POT1, RPS15, and XP01 were reported as pathogens with an unfavourable prognostic value [32, 33]. Additional data from clinical trials is necessary to validate this and there is currently no indication to systematically search for them.

Several prognostic markers have been described and prognostic scores have been proposed to identify high-risk patients and those with a very good prognosis. The international prognostic index (CLL-IPI) integrates the clinical stage, age, IGHV status, and ß2-microglobulin and TP53 anomalies. The indication for treatment is currently based on clinical criteria, but prognostic markers could help identify patients who should be treated and those who risk remaining resistant to treatment.

Table 1

List of mandatory and recommended tests by iWCLL and FILO at the time of treatment.

	Mandatory	Recommended	Not recommended
Karyotype		X	
4-probe FISH			
- Del(11q)	X		
- Del(13q)	X		
- Tri(12)	X		
- Del(17p)	X		
IGHV mutational status	X		
TP53 mutation (NGS > Sanger)	X		
Targeted NGS (NOTCH, SF3B1, BIRC3, ATM...)			Clinical trials

Conclusion

The pre-therapeutic clinical assessment integrates prognostic and predictive markers, some of which are currently being evaluated in clinical trials.
Mandatory examinations in routine practice, outside clinical trials, and those recommended by the International Workshop on Chronic Lymphocytic Leukemia (iWCLL) [34] and the cooperating group the French Innovative Leukemia Organization (FILO) when treatment begins are summarised in *table 1*.
The evolution of treatments in parallel with technologies now allows us to propose predictive markers in oncohaematology, which will allow the best therapeutic choice for the patient. In addition, extensive sequencing methods have revealed the extreme genetic complexity of CLL and its heterogeneity between patients regarding cell biology. The accumulation of anomalies at different stages of the disease will require analysis of relapse and transformation to be adapted in order to ensure optimal treatment.

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