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Cytogenetics in the management of chronic lymphocytic leukemia: Guidelines from the Groupe Francophone de Cytogénétique Hématologique (GFCH)

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ABSTRACT

Chromosomal abnormalities are frequent in chronic lymphocytic leukemia (CLL), and most have prognostic value. In addition to the four well-known abnormalities (13q, 11q and 17p deletions, and trisomy 12), other recurrent aberrations have been linked to the disease outcome and/or drug resistance. Moreover, the complex karyotype has recently emerged as a prognostic marker for patients undergoing immunochemotherapy or targeted therapies. Here, we describe the main chromosomal abnormalities identified in CLL and related disorders (small lymphocytic lymphoma and monoclonal B-cell lymphocytosis) by reviewing the most recent literature and discussing their detection and clinical impact. Lastly, we provide technical guidelines and a strategy for the cytogenetic assessment of CLL.

Introduction

Chronic lymphocytic leukemia (CLL) is the most common form of chronic leukemia in Western countries. It mainly occurs in older adults and is characterized by the clonal proliferation and accumulation of mature CD5+CD23+ B lymphocytes in the bone marrow, peripheral blood, lymph nodes, and spleen. The immunophenotypic Matutes score can be used to diagnose CLL [1]. CLL is distinguishable from its predominantly nodal variant (small lymphocytic lymphoma, SLL) by its leukemic appearance. CLL is a very heterogeneous disease biologically and clinically: some patients never require therapy, whereas others experience an aggressive clinical course, a poor response to therapy, and death within months of diagnosis. Unmutated immunoglobulin heavy chain variable region genes (IGHV) status is associated with a poor prognosis, as is IGHV3-21 gene usage (in stereotype subset 2) independently of the IGHV status. In about 5 % of cases, CLL evolves into a lymphoma known as Richter transformation (RT). Furthermore, CLL can be preceded by an asymptomatic precursor state known as monoclonal B cell lymphocytosis (MBL); the MBL-to-CLL conversion rate appears to be 1 %–2 % a year [2].

Since the 1970s, many chromosomal abnormalities (CAs) have been identified through the use of a variety of techniques: chromosomal banding analysis (CBA), fluorescent *in situ* hybridization (FISH), array-based comparative genomic hybridization (aCGH), and array-based single nucleotide polymorphism (aSNP). CAs are present in more than 80 % of patients with CLL. Some of the recurrent CAs have clear pathogenic importance because they influence the course of the disease and are used in routine clinical practice to identify patients at high risk of disease progression and poor survival [2]. Döhner et al.'s hierarchical prognostic model (published in 2000) is based on FISH detection of the four most recurrent CAs: 13q14 deletion [del(13q)], 17p13 deletion [del (17p)], 11q22 deletion [del(11q)], and trisomy 12 (+12) [3]. The del (13q) (when isolated) is associated with the best prognosis, while del (17p) is predictive of a short median survival time and a poor response to chemotherapy. The karyotype is now being considered increasingly in the workup for CLL. Over the last 10 years, a growing number of studies have highlighted the negative prognostic value of a complex karyotype (CK) in CLL ([4], for a review see Ref. [5]).

The treatment of CLL/SLL has evolved markedly in recent years, thanks to our better understanding of the disease's biology and the development of targeted therapies. Bruton tyrosine kinase inhibitors (BTKi) and the anti-apoptotic BCL-2 protein inhibitor (BCL2i) venetoclax are the most widely used targeted drugs and are gradually replacing immunochemotherapies (ICTs) [1,6]. New (non-covalent) BTKis and BCL2is are now emerging, and potentially curative combinations of targeted agents are being tested in clinical trials in CLL. Deletion 17p and a highly CK (HCK) are strong factors for a poor prognosis in the setting of ICT. There is clear evidence to show that these markers also have prognostic value when targeted therapies are used [7–11]. Moreover, some CAs (such as 2p gain or 8p deletion) are reportedly associated with resistance to targeted therapies [12,13]. Cytogenetic assessment is therefore still essential in the management of CLL, in order to stratify the patients and personalize the treatment strategies.

Chromosomal abnormalities (Table 1)

Common chromosomal abnormalities

Approximately 80 % of all patients with CLL carry at least one of the four common CAs: del(13q), +12, del(11q), and del(17p) [3,14].

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Deletion 13q

Frequency. The most frequent CA is del(13q). This specifically involves band 13q14 and occurs in approximately 55 % of all cases of CLL.

Cytogenetic description. This deletion is often cryptic and not visible by CBA. Reciprocal translocations involving 13q14 [t(13q)] and various chromosomes have been described. The absence of recurrent translocation partners suggests that these genomic rearrangements result in the loss of the 13q14 band [15]. According to FISH, the del(13q) is most frequently monoallelic but may be biallelic or occur as a mixture of mono- and bi-allelic cell populations. Del(13q) seems to be an early event [16].

Genes involved. A minimal deleted region of 29 kb has been identified: it encompasses the non-coding RNA deleted in the leukemia (*DLEU*) 2 gene and the microRNA *(MIR)-15A/16-1* cluster located within the intronic region of *DLEU2* [17]. The cluster induces apoptosis by targeting and downregulating BCL2 on the posttranscriptional level [18].

Prognosis. CLL with an isolated del(13q) (36%) detected by the four classical FISH probes (for del(13q) (the DLEU/D13S319 probe), del (11q) (the ATM probe), del(17p) (the TP53 probe) and +12 (the D12Z1 probe)) is characterized by an indolent course [3]. This good prognosis can be modulated by a biallelic del(13q), a high number of cells (>65%) with del(13q) [19], and a large deletion including the tumor suppressor retinoblastoma 1 gene [20] which could result from clonal evolution and may represent a more aggressive disease. The presence of additional CAs and/or a CK detected by CBA can also worsen the prognosis [14].

Associated gene mutations. In untreated CLL patients, isolated del(13q) is associated with *MYD88* mutation and mutated IGHV status [21].

Detection. As del(13q) is often cryptic, FISH is the most sensitive detection technique.

Trisomy 12

Frequency. Trisomy 12 is observed in 10 % to 25 % [3] of patients with CLL.

Cytogenetic description. In more than 50% of cases, +12 is associated with one or several CAs, such as +18 or +19, del(14q), or translocations (t) t(14/v;18), t(14/v;19), t(8;14/v) [22–27]. Trisomy 12 might be an early clonal abnormality [16].

Genes involved. It is not known which genes are involved in the pathogenesis of CLL with +12.

Prognosis. Trisomy 12 is associated with an intermediate outcome, according to Döhner et al.'s prognostic model [3]. CLL with +12 often has atypical morphological and immunophenotypic features, high proliferation rates, unmutated IGHV genes, and higher incidences of thrombocytopenia, RT and other secondary cancers [28–30]. Additional CAs might modulate this prognosis, with notably a good prognosis when +12, +19 and/or +18 are combined (see below) [4,11,31].

Associated gene mutations. Trisomy 12 is associated with *NOTCH1* and *BIRC3* mutations [29].

Detection. Trisomy 12 is easily detected by CBA using the recommended culture (72 h with CpG-oligonucleotides and interleukin-2 (IL2)).

Deletion 11q

Frequency. Deletion 11q is found in 5–10 % of patients with early-stage CLL and approximately 25 % of patients with advanced CLL [3,32].

Cytogenetic description. The deletion covers a large region (greater than 20 Mb and involving the loss of over a hundred genes) in most patients. However, the size of the deletion varies. Importantly, a small del(11q) can be overlooked - particularly when it is subclonal. The del(11q) is most frequently subclonal [16].

Genes involved. The minimal deleted region includes *ATM* (11q22.3), which encodes the proximal DNA damage response kinase ATM [33]. In 80 % of del(11q) patients, the *BIRC3* gene at 11q22.2 (about 6Mb upstream of *ATM*) is also impacted, leading to its monoallelic deletion [34,35]

Prognosis. Patients carrying a del(11q) clone typically present with bulky lymphadenopathy and rapid progression and show poor overall survival (OS) and progression-free survival (PFS) when receiving chemotherapy (CT) or ICT, respectively. Del(11q) has no adverse impact on the response to targeted therapies [1,6].

Associated gene mutations. The del(11q) is associated with mutations in the remaining *ATM* allele in more than one-third of patients [33]. Furthermore, in 10 % of patients with a *BIRC3* deletion, truncating mutations in the remaining *BIRC3* allele may lead to biallelic inactivation; this should be a marker of poor survival in CLL [34,36].

Detection. As del(11q) can be subclonal, FISH using an ATM probe is recommended (along with CBA).

Deletion 17p

Frequency. Deletion 17p is found in 5 % to 8 % of chemotherapy-naïve patients and in up to 40 % of relapsed or treatment-refractory (R/R) patients [3,37].

Cytogenetic description. Typically, 17p losses involve the entire short arm of chromosome 17 - mainly due to unbalanced translocations (in 70 % of cases) with several chromosome partners (the most frequent being chromosome 18q, followed by 8q or 8p), rather than interstitial or terminal deletions, isochromosome i(17q), monosomy (-) 17, or a ring chromosome [38,39]. i(17q) is reportedly associated with a worse prognosis within the subgroup of patients with del(17p) CLL [38,40]. In rare cases, the del(17p) is cryptic. Deletion 17p is associated with CK [38] and is often subclonal - especially at diagnosis [16].

Genes involved. 17p deletions encompass the 17p13 band and include the prominent tumor suppressor gene *TP53* [3].

Prognosis. CLL patients with del(17p) show poor treatment-free survival, poor median OS, and a poor response to CT that cannot be overcome by the addition of anti-CD20 antibodies [2,3]. As ICT is not effective in patients with p53 pathway inactivation, targeted therapies are the standard of care in that setting [1]. Ibrutinib and venetoclax are beneficial in patients with del(17p) in both first-line and R/R settings. However, these patients have a poorer response to ibrutinib and venetoclax than those without del(17p) [9,10,41].

Associated gene mutations. The majority of patients with del(17p) have mutations in the remaining *TP53* allele (>90 %). However, both situations exist: a *TP53* mutation without del(17p) and a del(17p) without *TP53* mutation, in 30 % and 10 % of cases, respectively [42]. Overall, *TP53* disruption (including del(17p) and/or *TP53* mutations) are the

Table 1
Characteristics of recurrent chromosomal aberrations in CLL.

Recurrent CA	Frequency	Involved genes/MIR	Karyotype: main abnormalities	Main ACA	FISH probes[§]	Main associated molecular features	Prognosis	Refs.
del(13q)/t(13;v)	55 %	*MIR15A/16-1* (13q14)	-Cryptic -Deletion -Non recurrent translocations		13q14 (D13S319)	When isolated -*MYD88*mut (65 %) -M-IGHV (60 %)	Favorable when isolated	[14,17, 20,21, 91]
+12	10–25 %	Unknown (12q13-15)	-Mostly complete -Rare +12q	Isolated: 50 % ACAs: +19, +18 t(14;19)(q32; q13), t (14;18)(q32; q21) del(14q)	D12Z1	-*NOTCH1*mut (24–42 %) -*BIRC3*mut (45 %) -UM-IGHV (67 %)	Depends on the associated CAs	[3,22,29, 67,91, 92]
del(11q)	-Stage A: 5–10 % -Stages B/C: 25 %	*ATM* (11q22) *BIRC3* (11q22)	Deletion		11q22 (*ATM*)	-*ATM*mut (30 %) -*BIRC3*mut (<5 %) -UM-IGHV (73–90 %)	-Unfavorable: short PFS with CIT -Neutral with targeted therapies	[32–35, 78,91]
del(17p)	Treatment naive: 5–8 %	*TP53* (17p13)	-Unbalanced translocation, including der/dic(17;18), i(17q) -Deletion -Cryptic	CK	17p13 (*TP53*)	-*TP53*mut (>90 %) -UM-IGHV (81 %)	-Unfavorable: short PFS and OS with CIT -Remains to be evaluated with targeted therapies	[3,38,39, 93]
2p gain	-Stage A: 6 % -Stages B/C: 16 %	*MYCN* (2p24) *REL* (2p16) *XPO1* (2p15)	-Unbalanced translocation (whole 2p arm; chromosome partners: 18, 20, 22) -2p duplication	CK del(11q) del(17p)	2p24 (*MYCN*)	UM-IGHV (80 %)	Unfavorable: short OS	[12,35, 43,46]
del(8p)	5 %	*TNFRSF10* (8p21)	Unbalanced translocation (t(8;8)/i(8q); other chromosome partners: 17, 3, 13, 2)	CK del(11q) del(17p)	8p22 (*LPL*)	UM-IGHV (73 %)	Unfavorable: short OS and TTFT	[46,47, 49, 52–54]
8q24 gain	5–6 %	*MYC* (8q24)	Unbalanced translocation	CK del(11q) del(17p)	8q24 (*MYC*)	UM-IGHV (90 %)	Unfavorable: short OS and TTFT	[38, 47–49]
t(8;14)(q24; q32)/t(8;v)	<1 %	IG::*MYC*[¤] Non-IG::*MYC*[¤]	Balanced translocations	del(11q) del(17p)	14q32/ 22q11/2p12 (IGH/K/L) 8q24 (*MYC*)	not reported	Unfavorable: short OS	[25,50]
t(14;19)(q32; q13)/t(19;v)	<5 %	IG::*BCL3*[‡]	Balanced translocations	+12	14q32/ 22q11/2p12 (IGH/K/L) 19q13 (*BCL3*)	UM-IGHV (90 %); subset 8 (IGHV4-39)	Unfavorable: short TTFT	[23]
t(14;18)(q32; q21)/t(18;v)	<5 %	IG::*BCL2*[£]	Balanced translocations	+12	14q32/ 22q11/2p12 (IGH/K/L) 18q21 (*BCL2*)	M-IGHV (20 %)	Favorable?	[27,58]
del(6q)	6 %	unknown	Deletion		6q21 (*SEC63*) 6q23 (*MYB*)	UM-IGHV (93 %)	Neutral?	[3,61,62]
del(14q)	<5 %	*ZFP36L1* (14q24) *3'*IGH (14q32) *TRAF3* (14q32)	Deletion	+12	14q32 (IGH)	-*NOTCH1*mut (31 %) -UM-IGHV (77 %)	Unfavorable: short TFS	[26,64]
del(15q)	4 %	*MGA* (15q15)	Cryptic				Not reported	[45]
+19	1–2 %	unknown	Mostly complete	+12		M-IGHV (96 %)	Favorable	[31,67, 94]

(*continued on next page*)

Table 1 (*continued*)

Recurrent CA	Frequency	Involved genes/MIR	Karyotype: main abnormalities	Main ACA	FISH probes[§]	Main associated molecular features	Prognosis	Refs.
+18	1 %	unknown	Mostly complete	+12 +19		M-IGHV (78 %)	Favorable	[67]
Complex karyotype	Low/ intermediate CK: 10 % Highly CK: 4–8 %		Low CK = 3 CAs Intermediate CK = 4 CAs Highly CK ≥ 5 CAs	del(17p)		-*TP53*mut (70 %) -UM-IGHV (64–77 %)	Unfavorable: short OS	[4]
Chromothripsis	1–9 %		Chromosomes 2, 3, 6, 8, 9, 11, 13, and 17*	CK del(11q) del(17p)		-*TP53*mut (30–100 %) -UM-IGHV (74 %)	Unfavorable: short TTFT, PFS, and OS	[45,46, 63]

Note: the majority of these CAs can be subclonal.
OS: overall survival; TTFT: time to first treatment; PFS: progression-free survival; M-IGHV: mutated IGHV; UM-IGHV: unmutated IGHV; CK: complex karyotype; HCK: highly complex karyotype; CA: chromosomal abnormality; CIT: chemoimmunotherapy; ACA: additional chromosomal abnormality.
§ commercial probes. It should be noted that in-house probes for genes of interest can be built.
¤ Driver derivative chromosome (the derivative chromosome that drives the deregulation of the pathological (onco)gene): variable.
° Driver derivative chromosome: t(14;19): mainly der(14); t(19;v): not investigated.
£ Driver derivative chromosome: t(14;18): mainly der(14); t(18;v): mainly der(18).
* Chromothripsis is detected using CMA or OGM only.

most important single factors associated with a poor prognosis [2].

Detection. Use of a TP53 FISH probe to assess del(17p) is mandatory before therapy.

2p gain

Frequency

2p gain is a recurrent, frequent CA in advanced CLL (in 6 % of cases of CLL overall, 16 % of cases of advanced CLL, and 22 % of R/R CLL cases) [12,43].

Cytogenetic description

2p gain often involves the entire short arm and can be a consequence of unbalanced translocations (the most frequent partners being chromosomes 18, 20 and 22) or duplications in the short arm. 2p gain is virtually never isolated, and may be missed within a CK [35]. It is strongly associated with del(11q) [12,44]. 2p gain is a late event [12, 16].

Genes involved

At least two minimal regions of gain have been described: 2p24 and 2p15-16 [12,45,46]. The 2p24 region encompasses *MYCN*, and the 2p15-16 encompasses *XPO1* and *REL*, all of which are overexpressed in CLL with 2p gain [12,43]. The 2p15-16 region is more frequently gained than the 2p24 region, which is rarely gained alone [35].

Prognosis

2p gain is associated with factors linked to a poor prognosis (such as del(11q), del(17p), CK and unmutated IGHV genes [12,43,45]) and poor OS [46]. It has been reported that 2p gain was associated with drug resistance, including resistance to fludarabine and ibrutinib [12,35].

Associated gene mutations

Landau et al. have reported an association between 2p gain and *ATM* mutations [44]. No association has been found between 2p gain and *XPO1* mutations [12].

Detection

Since 2p gain in a CK can be overlooked by CBA, we recommend performing FISH. Using at least one probe encompassing REL or XPO1 is the ideal first choice. Using a commercially available MYCN probe initially is an easier option, which detects the majority of 2p gains.

Chromosome 8 abnormalities

8q abnormalities

The 8q abnormalities found in CLL mainly involve the 8q24 region encompassing the *MYC* gene. Two types of 8q24 aberrations can be observed: translocations [t(*MYC*)] and gains.

Frequency. t(*MYC*) are observed in fewer than 1 % of cases of CLL [25]. Gain of one or more copies of the *MYC* gene is found in 5–6 % [47,48].

Cytogenetic description and genes involved. Translocations t(*MYC)* can involve the *IGH* locus (14q32), kappa (2p12) or lambda (22q11) light chain genes, or (more frequently, in 61 % of cases) a non-immunoglobulin gene partner, which leads to the overexpression of *MYC*. The most frequent non-IG translocation partner is the long arm of chromosome 9 [25]. *MYC* gains result from unbalanced translocations rather than duplications. The 8q gain regions translocate to random chromosomes, the most recurrent being chromosome 4 [48]**.** *MYC* aberrations (translocations or gains) are associated with del(11q) and del (17p) [25,38,48]. *MYC* gain is more frequently found in a CK (85 %) [49] than t(*MYC*) is (15–60 % according to studies) [25,50]. In rare cases, a *MYC* gain can co-exist with a t(*MYC*) in separate clones [48]. *MYC* aberrations can be clonal or subclonal and can occur secondarily during the progression of CLL [25,38].

Prognosis. t(*MYC*) is associated with an elevated prolymphocyte count and an aggressive clinical course [25]. Survival is better when the t (*MYC*) is present in a non-CK, rather than a CK. *MYC* gains are significantly associated with poor OS [46,48] and a short time to first treatment (TTFT) [47]. When *MYC* gain is associated with del(17p) (in "double-hit" CLL), the prognosis is particularly poor [38]. *MYC* abnormalities contribute to the transformation into diffuse large B-cell lymphoma (DLBCL)-RT: they are detected in about 30 % of RTs and acquired at the time of the transformation in 75 % of cases [51] (see above in the section on RT).

Associated gene mutations. Not reported.

Detection. Since they can be subclonal, *MYC* aberrations can be

challenging to detect using CBA only [22]. Performing FISH with a MYC probe is useful if these aberrations are not to be missed. In cases of translocation involving the 8q24 region, FISH is required to confirm the involvement of the *MYC* locus - especially when the partner is non-IG.

Deletion 8p

Frequency. 8p loss is found in approximately 5 % of CLL cases overall [47] and up to 28 % of CLL with del(17p) [52].

Cytogenetic description. The majority of 8p losses result from an unbalanced translocation; the most recurrent partner is chromosome 8 [t(8;8) or isochromosome 8q (with subsequent 8q24 gain), followed by chromosomes 2, 3, 13, and 17. Deletion of the short arm of chromosome 8, -8 or ring of chromosome 8 alone are rarer. del(8p) is often present in a CK [49,53]. The del(8p) is a subclonal event [16].

Genes involved. A minimal deleted region of 4.2Mb has been delineated at 8p21, including the *TNFRSF10A/B* apoptotic TRAIL receptor genes [46].

Prognosis. Cases of CLL with del(8p) have a high prevalence of poor prognostic factors (del(11q), del(17p), CK), a shorter time from diagnosis to first treatment, a higher risk of RT, and poor survival [46,47, 52–54]. Burger et al. have suggested that the haploinsufficiency of *TNFRSF10A/B* resulting from 8p deletion contributes to ibrutinib resistance [13]. Jondreville et al. showed that *TNFRSF10B* loss is associated with fludarabine resistance [53]. Moreover, it has recently been reported that acquired del(8p) is involved in venetoclax resistance [55].

Associated gene mutations. None reported.

Detection. The del(8p) can be overlooked in a CK, and we recommend performing FISH in that case. Using at least one probe encompassing *TNFRSF10A/B* genes is the best first choice, and using a commercially available LPL probe is an easier initial option that detects the majority of del(8p) cases [49,53].

Chromosomal translocations

Frequency

Reciprocal translocations are recurrent events in CLL with frequencies varying among studies (ranging from 5 to 30 %) [22,56,57].

Cytogenetic description

All chromosomes can be involved, and recurring translocations (whether unbalanced or balanced) are rare. Unbalanced translocations frequently involve chromosome 13q (see the section above).

Genes involved

Balanced translocations particularly involve the immunoglobulin gene loci (IGH in 14q32, or IGK/IGL at 2p11 and 22q11, respectively) and lead to the juxtaposition of an IG enhancer and an oncogene, the expression of which is deregulated. They are reported in 5 to 7% of treatment-naïve patients with CLL [22]. The main partners are *BCL3* (19q13), *BCL2* (18q21), and *MYC* (8q24) (see the section above). t(14;22)(q34;q11)/IGH::IGL translocations have also been described. In these rare cases, the oncogenic mechanism has not been elucidated but might involve the deregulation of a nearby oncogene. In CLL, IG translocations are associated with +12 [22,58]. IG and t(13q) translocations are mainly clonal [15,24,27].

Prognosis

Chromosomal translocations as a whole were initially described as being independently associated with a shorter TTFT and worse OS [59]. However, a more recent study showed that translocations negate the improved prognosis in IGHV-mutated patients but do not have an effect on the TTFT in IGHV-unmutated patients [57]. Unbalanced translocations are associated with CK, unmutated IGHV, and *TP53* abnormalities [57]. The prognostic value of IG-translocations appears to depend on the IG partner. IG::*BCL3* cases often harbor atypical morphological and immunophenotypic features and are associated with unmutated IGHV status, frequent IGHV4-39 usage (subset #8), worse OS, and a shorter TTFT [23,24]. Conversely, IG::*BCL2* is associated with mutated IGHV status, and these patients have the same prognosis as those without IG-translocations [27,60].

Associated gene mutations

Mutations in *NOTCH1, BCL2, FBXW7, ZMYM3* and *MGA* are more frequent in CLL with IG translocations. It is noteworthy that IG::*BCL2* cases have a lower median mutation frequency.

Detection

Translocations are easily detected by CBA. FISH can be useful for specifying recurrent loci (especially IGH, IGK, IGL, *BCL2, BCL3* and/or *MYC*) and the presence of a deletion at breakpoints (especially for translocations involving 13q14/*DLEU*, 11q22.3/*ATM*, and 17p13.1/*TP53*).

Other chromosomal abnormalities

Deletion 6q

Frequency. A del(6q) has been described in approximately 6% of cases [3,61,62].

Cytogenetic description. del(6q) is rarely found as an isolated CA and is often part of a CK [61]. At least two minimal deleted regions have been described: 6q21-23 (encompassing the *MYB* gene) and 6q25-q27 [61–63]. del(6q) is mainly subclonal [44].

Genes involved. The genes have not yet been identified, although one study suggested that *FOXO3* is involved [61].

Prognosis. del(6q) is associated with unmutated IGHV genes [46,61]. The prognostic significance is still subject to debate [61]. It is noteworthy that the del(6q) was not included in Dohner et al.'s hierarchical model [3].

Detection. del(6q) can be detected by CBA. However, additional FISH with 6q21/6q23 (SEC63/MYB) probes might be of value - especially in the context of a CK.

Deletion 14q

Frequency. Interstitial 14q deletion [del(14q)] is a recurrent but rare (<5 %) event in CLL.

Cytogenetic description. del(14q) is the sole abnormality in 25–30% of cases (62), and is associated with +12. No minimal deleted region has been described but del[14](q24.1q32.33) is the most frequent del(14q) observed; it involves *ZFP36L1* in 14q24.1 and the IGH gene at 14q32.33 [26,64].

Telomeric deletions of the 5′IGH (corresponding to the IGHV region) detected by FISH have also been described [65]. Further analysis of these cases showed that the pattern reflects the physiological loss of an IGHV portion during the VDJ rearrangement and is not an oncogenic event [65].

Genes involved. Given the frequent involvement of IGH, transcriptional

activation of a partner gene located at 14q24.1 has been suggested but not found [26,64]. Perez-Carretero et al. found an association between 3′IGH deletion and *TRAF3* mutations, resulting in biallelic inactivation of this gene located at 14q32 [66].

Clonal evolution. del(14q) is usually a clonal abnormality [26,64].

Prognosis. del(14q) is associated with unmutated IGHV status, *NOTCH1* mutations, and a short PFS [26,64,66].

Detection. del(14q) is detected by CBA. FISH using an IGH (14q32) break-apart probe can be useful for distinguishing between del(14q) and 14q32 translocations with IGH rearrangement.

Deletion 15q

Deletion of the region 15q15 (encompassing the *MGA* gene) has been detected (using aSNP) in approximately 4% of a cohort of cases of CLL [45].

Trisomy 19 and trisomy 18

Frequency. Trisomy 19 occurs in 1 to 2% of CLL cases and trisomy 18 in ~1% [31,67].

Cytogenetic description. Around 10% of cases of trisomy 12 have +19 as a secondary event. Trisomy 19 without +12 is rare. Patients with +12,+19 are typically quite young [31]. In cases with +12,+19, additional trisomies are frequent (notably +18, in 62 % of cases). Trisomy 18 is rarely found alone and is mostly associated with +19; it is considered to be a subclonal event [4].

Genes involved. There are no minimal gain regions and no putative causative genes have been identified, although an extra copy of *BCL2* on chromosome 18 or an extra copy of *BCL3* on chromosome 19 might be involved in the physiopathology of CLL.

Prognosis. Cases with +12,+19,+/−18 are associated with mutated IGHV genes and must not be considered as a CK with regard to the prognosis [5]; they have an indolent course [11,31].

Detection. Trisomies are easily detected by CBA.

The complex karyotype

Definition, frequency, and cytogenetic description

As we have suggested previously [68], one can define a low CK, an intermediate CK and highly complex karyotype (HCK) as having 3 CAs, 4 CAs, and 5 or more CAs, respectively. Furthermore, Rigolin et al. have suggested that there are two types of CK: CK1 includes balanced translocations, deletions, monosomy or trisomy, and CK2 includes unbalanced translocations, chromosome additions, insertions, duplications, and ring, dicentric and marker chromosomes [69]. This distinction has not yet been validated in the context of clinical trials or large cohorts.

Overall, a CK is present in 11% to 19% of treatment-naive CLL patients and in up to 40% of patients with R/R CLL. An HCK is observed in 4 to 8% of treatment-naive patients (for a review, see Refs. [4,5,8,68]). A CK is typically associated with del(17p) and del(11q) [22,69,70].

Associated gene mutations

A CK is associated with *TP53* mutations [4].

Prognosis

A CK is associated with factors for a poor prognosis, such as unmutated IGHV and *TP53* aberrations. Except in cases with +12,+19,+other aberrations, HCK is considered to be an independent factor for a poor prognosis (shorter PFS and OS times) in patients receiving targeted therapies or CTs/ICTs, regardless of IGHV and *TP53* status [4,11]. Indeed, a CK counters the good prognosis associated with an isolated del (13q) (detected by FISH) or unmutated IGHV status. A CK also makes the poor prognosis associated with *TP53* aberrations even worse [38,49].

Detection

A CK is detected by CBA only. Genomic complexity (as evaluated by other techniques, such as chromosomal microarray analysis (CMA) and optical genome mapping (OGM)) has yet to be validated in clinical practice ([46] and the dedicated section below).

Chromothripsis

Frequency and cytogenetic description

CLL was the first cancer in which chromothripsis (CTH) was reported [71]. In CLL, CTH is observed with frequencies from 1 to 9% and most frequently impacts chromosomes 2, 3, 6, 8, 9, 11, 13, and 17 [45,46,63,72]. CTH is associated with high-risk genomic aberrations like del(11q), del(17p) and CK [45,46,63].

Associated gene mutations

CTH is associated with *TP53* and *SETD2* mutations [46,72]. A *TP53* disruption is found in approximately 70–80% of cases with CTH [45,46,63,73].

Prognosis

CTH is associated with unmutated IGHV genes [45]. It has been reported that CLL patients with CTH have shorter TTFTs [63,73], PFS times [45], and OS times [45,46].

Detection

CTH is detected using CMA. A recent study showed that OGM is also a valuable cytogenomic tool for detecting CTH [73]. The resolution of CBA is too low to reveal CTH. However, most of the chromothriptic chromosomes are altered in the karyotype and show monosomy, deletion, or involvement in unbalanced rearrangements [73].

Gene mutations

Only a few gene mutations occur in more than 5% of patients with CLL. The most frequently mutated genes in newly diagnosed CLL are NOTCH1 (10~15%), SF3B1 (10~15%), *TP53* (4~8%), *XPO1* (~5%), FBXW7 (2~6%), MYD88 (2~5%) and BIRC3 (2~5%) [74]. Actual incidence may vary depending on the technique used and the limit of detection. The prevalence of mutations increases over the course of the disease. In particular, the frequency of *TP53* mutations rises to 10% at the time of the first-line treatment, 30% to 40% at relapse, and up to 60% in RT because *TP53* abnormalities can be acquired or selected during the progression.

Many of these recurrently mutated genes (such as *NOTCH1, SF3B1, TP53, BIRC3, EGR2, POT1 or XPO1*) have a unfavorable prognostic impact [74]. The risk of RT is specifically affected by the presence of *NOTCH1* mutations at CLL diagnosis [75]. Moreover, the concomitant presence of several mutations [76], and mutational complexity more broadly [77] might help to identify CLL patients with the worse prognosis.

Some mutated genes are predictive biomarkers with consequences for the choice of treatment. A *TP53*-inactivating mutation reportedly lowers the benefits of CT/ICT [78]. *NOTCH1* mutations appears to be associated with refractoriness to anti-CD20 agents [79].

More recently, treatment-emergent mutations leading to drug resistance were discovered in the majority of patients treated with BTKi and BCL2i. This finding might be of interest when deciding on a new line of treatment and seeking to overcome resistance [80,81].

Primary and secondary CAs and clonal evolution

Clonal evolution is a basis for progression and relapse [16]. Among the driver events, clonal aberrations (e.g. +12, del(13q), *NOTCH1* or *MYD88* mutations) arise early in the course of the disease, and subclonal aberrations (e.g. *SF3B1, TP53* or *ATM* mutations, and homozygous del (13q) or 2p gain) expand over time and represent later events in the course [44]. Clonal evolution at progression can be predictive of subsequent survival [8]. It is therefore of great importance to count all the CAs according to Jondreville et al.'s [5] guidelines and not only those present in the most complex clone – in contrast to what is stated in the ISCN 2020 (for a review, see Ref. [68]).

CAs and Richter transformation

RT corresponds to the progression of CLL or SLL to DLBCL (in 90–95% of cases) or Hodgkin lymphoma (in 5–10%; for a review, see Condoluci and Rossi [82]). Although the DLBCL-RT clone generally arises from the CLL clone, 10%−20% of cases of RT are clonally unrelated to CLL. RT and CLL have common cytogenomic abnormalities, whereas RT only shares a few characteristics with *de novo* DLBCL - even in clonally unrelated cases of RT [83]. Approximately 5–10% of CLL patients develop RT during long-term follow-up but the mechanisms driving this evolution are not well understood.

At the time of CLL diagnosis, a CK (and especially a CK2 or an HCK) is the strongest predictor of RT, followed by del(11q), del(17p), unmutated IGHV, and Binet stage B or C [84]. Moulin et al. have reported that most mutations in *TP53, NOTCH1, SF3B1, EGR*2 and *XPO1* observed at the time of RT-time are inherited from the CLL stage [85]. Moreover, Nadeu et al. demonstrated that minor subclones harboring genomic characteristics of RT cells were already present at CLL diagnosis, up to 19 years before transformation [86]. Taken as a whole, these data emphasize the importance of karyotyping and mutational analyses at the time of CLL diagnosis and the need for close monitoring of patients with an RT-associated chromosomal or mutational profile.

A whole-genome sequencing study of longitudinal samples revealed a concordant increase in complexity from CLL diagnosis to relapse and RT [86].

At time of DLBCL-RT, four highly prevalent genomic abnormalities have been reported: *TP53* aberrations, *MYC* deregulations, *NOTCH1* mutations, and *CDKN2A/B* deletions. Conversely, the mutations or CAs frequently observed in *de novo* DLBCL without prior CLL are usually rare in RT [83]. Two groups of RT patients have been described: the first (50% of cases) exhibits *TP53* alterations and (in some cases) *CDKN2A* (9p21) deletion, and the second (30 % of cases) is characterized by the association of +12 and *NOTCH1* mutations. When the *TP53* and *NOTCH1* variants are not inherited from CLL, they are frequently acquired at RT and so may well be important in the transformation [85]. *MYC* alterations (translocations or gains) are reported in 30% of RTs [87]. Overall, the RT genome is characterized by a compendium of driver alterations that frequently arise as single, catastrophic events like kaetegis and chromothripsis [86]. Furthermore, whole-genome doubling occurs in 15% of cases [88].

Unlike *de novo* DLBCL (with mostly mutated IGHV status), RT harbors unmutated IGHV (in 80% of cases) and has a high prevalence of stereotypic B-cell receptors [85]. Stereotypic subset #8 has been particularly linked to an increased risk of RT [89].

Small lymphocytic lymphoma

Lymph node infiltration by small lymphocytes with a CLL phenotype and in the absence of hyperlymphocytosis $>5 \times 10^9$/L should prompt a diagnosis of SLL. In fact, SLL and CLL are considered to be same entity in the WHO classification and are studied together in virtually all published genome analyses. The very few studies to have compared the cytogenetic profiles of SLL and CLL concluded that there were fewer FISH-detectable genomic aberrations in SLL [90]. One study showed that +12 was more frequent in SLL than in CLL (36% vs 13%, respectively) and that del(13q) was less frequent in SLL than in CLL (9% vs 44%, respectively) [90].

Monoclonal B-cell lymphocytosis

MBL is defined by a monoclonal B-cell population with a count below 5×10^9/L in the peripheral blood, and the absence of palpable lymphadenopathy or other clinical features characteristic of a lymphoproliferative disorder (anemia, thrombocytopenia, constitutional symptoms, and organomegaly). MBL is further categorized into three subtypes: low-count MBL (a monoclonal CLL/SLL phenotype B-cell count below 0.5×10^9/L), which rarely progresses to CLL; CLL/SLL-type MBL (a monoclonal CLL/SLL-phenotype B-cell count $\geq 0.5 \times 10^9$/L), which progresses to CLL at a rate of 1%–2% per year; and non-CLL/SLL-type MBL (WHO-HAEM5 WHO's classification; https://tumourclassification.iarc.who.int/chapters/63). Only a few studies have evaluated the cytogenetics of MBL patients, and most of these used interphase FISH to test for the four "CLL-associated" CAs. del(13q) and +12 are the most frequent CAs in CLL/SLL-type MBL, with frequencies similar to those seen in early stage CLL, while the prevalence of del(11q) and del(17p) is low [95,96]. The karyotype is more likely to be normal in MBL than in CLL, and CKs are significantly less frequent in MBL [96]. The risk of progression from CLL/SLL-type MBL to CLL does not appear to be influenced by the type of CA. However, the acquisition of CAs may be a strong determinant of disease progression [97].

Techniques

Chromosome banding analysis

Techniques

CBA in CLL has long been hampered by the low *in vitro* proliferative activity of malignant cells. The use of a 72 h culture with immunostimulatory CpG-oligonucleotides and IL2 has renewed interest in karyotyping in this disease. This B-cell mitogen combination enhances the proliferation of CLL malignant cells, improves metaphase generation and thus allows the detection of CAs in up to 80–90% of cases, with a median of one CA per treatment-naïve patient [22]. CBA is usually performed on a peripheral blood sample but a bone marrow sample can also be used. Lymph nodes can be analyzed in SLL. However, tumor cells are often circulating in peripheral blood (albeit at a low level) and so karyotyping could be attempted with this sample.

The GFCH recommends harvesting 15 ml of peripheral blood (the amount should be adapted to lymphocytosis) in a heparinized tube (preferably with heparinate lithium). After a WBC count, $1–2 \times 10^6$/ml cells are cultured with medium, CpG-oligonucleotides and IL2 for 72 h, in two flasks. A 48 h culture may be possible. Likewise, a 96 h culture with adjunction of CpG-oligonucleotides and IL2 after 24 h could be attempted. Particular attention should be given to high WBC counts, which are prone to culture failure because of a relative excess of cells vs. the volume of medium. If the lymphocyte count is low (e.g. $<20\times10^9$/L), Ficoll-Paque density gradient centrifugation or a buffy-coat step could be applied before culture to increase the proportion of mononuclear cells. The use of 12-O-tetradecanoylphorbol-13-acetate (TPA) as a mitogen is no longer recommended.

Value in CLL

CBA can reveal a bundle of aberrations undetected by targeted FISH with the four classical CLL probes. Balanced events (like translocation, inversion or insertion) and unbalanced aberrations (particularly 2p gain, del(8p) or CK/HCK) may add value to the FISH results and the prognostic assessment. Sequential karyotyping over time can also usefully demonstrate clonal evolution.

Indications (Table 2)

If typical CLL with no need for treatment is diagnosed, karyotyping is optional. However, if the diagnosis is uncertain, CBA is mandatory - especially for distinguishing between atypical CLL and other lymphoproliferative disorders, such as mantle cell lymphoma with a t(11;14)(q13;q32) translocation [1].

According to the guidelines issued by the international workshop on CLL (iwCLL), CBA is not mandatory for CLL management at diagnosis or before first-line treatment - with the exception of clinical trial assessments [2]. The European Research Initiative on CLL (ERIC) experts also recommended systematic CBA in prospective clinical trials but did not mention use in "real-life" situations [98].

The GFCH recommends CBA before treatment initiation in patients with disease progression (as defined by the iwCLL criteria) and who are not participating in clinical trials and at each subsequent symptomatic relapse; this helps the physician to evaluate karyotype complexity, other cytogenetic abnormalities, and the prognosis. In clinical trials, CBA should be mandatory.

FISH

Four-set CLL probes

FISH with the four classical probes (DLEU/D13S319, ATM, TP53, D12Z1) can be performed on peripheral blood lymphocytes; it identifies targeted cytogenetic lesions in >80 % of all cases of CLL.

Indications (Table 2)

According to the iwCLL, a prognostic FISH assessment with the four classical probes is mandatory [2] at baseline. In patients with disease progression or relapse, testing for del(17p) is mandatory before the initiation of each new line of treatment [1,2,6,99]. As mentioned above, many other aberrations (such as 8q24 gain or translocation (*MYC*), 2p gain (*MYCN*), 6q deletion (6q21/6q23 probe), del(8p) (*LPL*) or IG translocation (IGH, IGK, and IGL)) might have prognostic value and can be easily explored with commercial probes. However, their assessment at diagnosis or before treatment remains optional in routine clinical practice [25,27,35,38,43].

The GFCH recommends the mandatory evaluation of del(17p) (and *TP53* mutation status) in patients with disease progression and before each new line of treatment. It is noteworthy that FISH is a sensitive method (sensitivity threshold: 1 to 5%, depending on the abnormality detected) and is the most appropriate method for the detection of subclones. Along with CBA, the use of FISH to evaluate the sometimes cryptic del(13q) and del(11q) might be of great interest. In patients with a CK, 2p gain, del(8p) and 8q gain can be difficult to detect, and so FISH can be helpful.

In clinical trials, the application of an exhaustive panel of probes is highly recommended.

Table 2
Strategy for cytogenetic testing in CLL.

	Mandatory	Highly recommended	Optional
Initial staging at diagnosis			
CBA			x
FISH 17p13 (*TP53*)			x[a]
FISH 13q14 (*DLEU/D13S319*), cen12[b], 11q22 (*ATM*)			x
Prior to treatment			
CBA		x	
FISH 17p13 (*TP53*)	x		
FISH 13q14 (*DLEU/D13S319*), cen12[b], 11q22 (*ATM*)		x	
FISH 2p, 8p, 8q[c]		x[d]	
Clinical trial (depending on the design)			
CBA/enlarged FISH panel		x	

[a] If the CLL international prognostic index score is requested.
[b] Trisomy 12 is always detected by karyotyping. This probe could be optional if CBA is performed.
[c] See Table 1.
[d] According to CBA
CBA: chromosomal banding analysis; cen: centromeric probe.

Other techniques used routinely

Many CMA studies have enabled characterization of the size and the minimal deleted region of known CLL abnormalities and the discovery of new recurrent CAs with clinical signification, such as 2p gain, 8p losses, 8q gains, and chromothripsis. The CMA technique is also a powerful approach for assessing genomic complexity, which is linked to a poor prognosis [46]. However, given the technique's low sensitivity for detecting known markers of a poor prognosis and the absence of validation in prospective studies, CMA has not been widely implemented in routine diagnostic practice. FISH remains the gold standard for the detection of del(17p). A very recent study showed that OGM is a valuable tool in CLL: it detects most of the abnormalities defined by combinations of standard methods in a single test and provides a more comprehensive analysis of the genome [100]. However, the sensitivity threshold of about 15 % means that some high-risk CAs can be overlooked, together with small, abnormal subclones that have to be counted when defining genome complexity. These issues must be considered if OGM is to be included in cytogenomic assessments of CLL in the future.

Scores and classifications that include cytogenetic variables

Döhner's hierarchical prognostic model [3] was based on the use of five risk categories and four FISH probes to detect 17p, 11q and 13q deletions and +12 in patients receiving CT del(17p) and del(11q) were associated with a poor prognosis, del(13q) was associated with the best prognosis when isolated, and +12 was associated with an intermediate prognosis.

Rossi et al. [34] suggested an integrated mutational-cytogenetic model, based on *TP53, BIRC3, NOTCH1* and *SF3B1* abnormalities, del(11q), +12 and del(13q), with four CLL subgroups: (i) high-risk, harboring *TP53* and/or *BIRC3* abnormalities (10-year survival rate: 29 %); (ii) intermediate-risk, harboring *NOTCH1* and/or *SF3B1* mutations and/or del(11q)22-q23 (10-year survival rate: 37 %); (iii) low-risk, harboring +12 or a normal genetic profile (10-year survival rate: 57 %); and (iv) very low-risk, harboring del(13q)14 only, whose 10-year survival rate (69.3 %) did not significantly differ from a matched set of people from general population.

More recently, Baliakas et al. [4] included the CK and +12,+19, *TP53* and IGHV status in their prognostic scoring system. They identified five groups, ranked from the shortest OS to the longest: (i) HCK; (ii) low and intermediate CK with *TP53* abnormalities (deletion and/or mutation); (iii) non-CK/*TP53* abnormalities; (iv) non-CK/non-*TP53* abnormalities/unmutated IGHV; and (v) non-CK/non-*TP53* abnormalities/mutated IGHV and CK+12+19.

The CLL international prognostic index (CLL-IPI) uses a weighted grading of five independent prognostic factors: *TP53* disruption (deletion and/or mutation), IGHV mutational status, serum β2-microglobulin, clinical stage, and age [101]. The CLL-IPI score was recently used to predict the TTFT and the OS time in patients with MBL. Patients with MBL and a high-risk CLL-IPI have a 6-fold greater risk of needing treatment in the next 12 months than those with a low-risk CLL-IPI [102].

Conclusion

At present, only testing for del(17p) (and *TP53* mutations) is mandatory before treatment. We believe that both FISH and CBA are essential in the prognostic assessment of patients with CLL, and we highly recommend performing these tests before treatment. In clinical

trials and those in patients on targeted therapies in particular, CBA and FISH are important to precisely evaluate the signification of a CK and specific CAs that are not targeted by the standard FISH panel (such as 2p gain, del(8p), and *MYC* aberrations).

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