A. Cuneo
F. Cavazzini
M. Ciccone
M. Dabusti
F. Cibien
G. Daghia
O. Sofritti
G.M. Viglione
G.M. Rigolin

Section of Hematology, Department of Biomedical Sciences and Advanced Therapies, University of Ferrara Ferrara, Italy

Molecular cytogenetic lesions in chronic lymphocytic leukemia

A B S T R A C T

In the 90's, only approximately 50% of chronic lymphocytic leukemia (CLL) could be shown to carry a chromosome defect, a figure reflecting inadequate cell division. The introduction of FISH allowed for the detection of chromosome aberrations in 80% of the cases and every patient could be included in a specific group according to a hierarchical cytogenetic classification as follows: 17p- > 11q- > +12 > 13q- > normal.[1] In most studies, approximately 40% of CLLs were shown to carry isolated 13q-, 10-15% of the patients carried +12 or 11q-, 2-5% 17p- or 6q- or 14q32 translocations. The variable incidence of specific lesions in different phases of the disease reflects their correlation with biologic and clinical features (Table 1). Recently, the introduction of effective mitogenic stimulation by oligonucleotides and interleukin-2 (IL-2)[2] showed that approximately 30% of CLL without chromosome defects by interphase FISH carried a chromosome lesion by CBA in regions not covered by the FISH panel of probes. Complex karyotypes could be documented in a substantial fraction of cases in association with unfavorable prognostic factors and inferior clinical outcome.[2]

13q-

13q- occurring as the sole aberration has an important prognostic value, identifying a subset of CLL patients with good prognosis.[1] This deletion occurs in the hemizygous state in approximately 75-80% of the cases and in the homozygous state in the remaining 20-25%.[3] Patients with homozygous deletion of chromosome 13q14 normally show higher lymphocyte growth kinetics than patients with hemizygous deletions.[4] Calin and colleagues identified a minimal deleted region of 29 Kb on 13q14, between the exon 2 and 5 of the *LEU2* gene containing two micro RNA genes, miR15A and miR16-1,[5] the expression of which is significantly deregulated in a fraction of CLLs. The deletion of these micro-RNA genes was recently confirmed by other investigators using a high resolution CGH array.[6] The application of high resolution techniques in the study of CLL patients revealed multiple, discrete genomic alterations in 13q region including other genes such as *RB* and *NUDT15*,[7] the role of which in the transformation process ha s not been defined yet.

+12

Trisomy of chromosome 12 is the most frequent gain of chromosomal material in CLL[8] being detected in approximately 15% of cases by FISH and by CBA. Some CLL

Table 1. Clinicobiologic significance of recurrent chromosome defects in chronic lymphocytic leukemia.

Abnormality	*Involved gene*	*Cytomorphology*	*Immunophenotype/ Ig gene status*	*Clinical and biological features*
17p-	p53	CLL/PL	CD38+++/- ZAP-70+++/- Unmutated Ig+++/-	Very poor prognosis (median survival <5 years) Resistant to purine analogues Responsive to anti CD52
11q-	ATM	Typical CLL	CD38+++/- ZAP-70++/-- Unmutated Ig+++/-	Poor prognosis (median survival 5-10 years) Massive adenopathies. Responsive to antiCD20
+12	12q13-15	Typical CLL	CD38++/-- ZAP-70+++/- Unmutated Ig ++/--	Intermediate prognosis (median survival 10-15 years)
6q-	?	Atypical CLL	CD38+++/- ZAP70++/-- Unmutated Ig ++/--	Short treatment free interval Intermediate prognosis (median survival 10-15 years)
14q32	IgH + various partners	Typical CLL	CD38++/-- ZAP70++/-- Unmutated Ig++/--	Intermediate prognosis
13q-	13q14	Typical CLL	CD38- ZAP-70+/--- Unmutated Ig +/---	Good prognosis if present as isolated change (median survival > 15 years)
Translocations	Unknown	NA	CD38+++/- unmutated Ig+++/-	Poor prognosis

+++/-: 60-80% positive; ++/-- 30-59% positive; +/--- < 30% positive; NA: not applicable.

cases carrying a partial trisomy of chromosome 12 were reported.[9] The duplicated segment extended between chromosomal bands 12q13 and 12q21.2, suggesting that this region may harbor genes important in the pathogenesis of CLL. The *MDM2* gene, mapping at 12q14.3-q15 may be upregulated in CLL bearing trisomy 12.[10] *MDM2* is a gene whose product acts as a major regulator of the tumor suppressor gene p53[11] and its overexpression may correspond to a functional deletion of the *TP53* product.

11q-

The commonly deleted segment includes the ataxia teleangiectasia mutated (*ATM*) gene which is involved in the signal transduction pathway activated in response to DNA breaks.[12] The remaining *ATM* allele is mutated in up to 36% of CLLs with 11q- and those patients with homozygous *ATM* defects may show a more aggressive disease than patients with 11q- only.[13] Patients with 11q- showed extensive adenopathy and short treatment free interval and survival as compared with other CLLs in a pivotal single centre study.[14] 11q- represented an independent adverse prognostic factor predicting for a shorter progression free survival (PFS) in several clinical trials. Patients with 11q- treated upfront with FC plus rituximab (FCR) showed a 100% overall response rate (CR rate 88%) with 77% relapse free survival at 3 years.[15]

Likewise, a good overall response rate (87% CR + Partial Remission) was described using the monoclonal antibody anti CD52 alemtuzumab upfront.[16]

17p-

The 17p- anomaly is frequently associated with additional aberrations and a complex

karyotype by CBA.[2] Over 70% of CLLs with del 17p13 may carry mutations in the remaining *TP53* allele.[17] *TP53* gene mutations may occur in patients with inferior prognosis in the absence of 17p deletion.[17] 17p-/*TP53* mutation confer genetic instability, this gene being involved in the maintenance of effective DNA-damage checkpoints, in cooperation with the DNA double-strand break sensitive *ATM* gene.[18] The clinical outcome in 17p- is severe.[1,19] Only 2 out of 8 patients (25%) with 17p- achieved CR using the FCR regimen upfront, as compared with a 72% CR rate in the remaining patients.[20] Alemtuzumab as single agent could induce responses in 7 out of 11 (64%) patients treated upfront.[16] Flavopriridol was able to induce a partial response in 7/18 relapsed/refractory patients in a recent stud.[21] Non myeloablative allogeneic transplantation may induce prolonged disease free survival in this subset of CLL.[22]

6q-

The 6q- chromosome may be frequently associated with other chromosome changes. A subset of patients, however, may carry 6q- as single or early chromosome abnormality.[23] The incidence of 6q- was found to be higher in patients requiring treatment or in relapsed/refractory patients than in patients with indolent disease. Patients with 6q- show distinct hematological features, consisting of high WBC count at presentation, atypical morphology, $CD38^+$, unmutated *IGHV* gene in 60% of the cases, shorter treatment free interval and survival as compared with CLL with favorable cytogenetic aberrations (i.e. 13q-, normal).

14q32 translocations

Recurrent partner chromosomes include 18q21/*BCL2* and 19q13/*BCL3*; other partners occasionally identified were 2p12/BCL11A; 2p13; 4p16; 4q31; 5q31; 6p21/CCND3; 7q21/CDK6, 8q11; 9q34; 17p11. The classical t(11;14)(q13;q32), indistinguishable from the translocation associated with mantle cell lymphoma was documented in CLL by several groups: these cases are likely to represent an atypical form of CLL sharing some features with leukemic mantle cell lymphoma.[24] The clinical outcome of CLL with 14q32 translocations may be worse as compared with CLL with favorable karyotype.[25,26]

Translocations (balanced and unbalanced)

Following the introduction of effective stimulation methods, a previously undescribed 34% incidence of balanced or unbalanced chromosome translocations was documented in CLL.[27] Some recurrent breakpoints were observed at 1p32, 1q21, 2p11, 6p11, 13q14, 14q32 and 18q21 and chromosome translocations were frequently found in the context of complex karyotype. A recurrent translocation t(1;6)(p35.3;p25.2) was documented in 8 patients in association with other chromosome lesions (i.e. 11q-, 17p-) and with unmutated *IGHV* and unfavorable outcome.[28]

Chromosome translocations represented the strongest predictor of an inferior clinical outcome in a study.[27]

Novel aberrations

Up to 21% of CLL investigated by automated array-based genomic profiling (matrix CGH) could be shown to harbor genomic imbalances in regions not covered by conventional FISH probe-set,[29] including, in 15% of the cases, recurrent submicroscopic deletions of chromosome 22q11.[30] Trisomy of a small

segment at 2p24 with upregulation of the *MYCN* gene mapping at this region was documented.[31] Novel regions of deletion were documented in > 10% of the patients at 9q, 10q, 15q, 22q.[32] Imbalances reflecting gain of chromosome material and/or amplification occurring in >10% of the cases involved 1p, 2q, 4q, 6q, 8q, 10p, 11p, 16q, 17q, 18q, 19q, 21q, 22q. Four distinct regions on chromosome 12 showed the highest rate of allelic imbalance and, interestingly, a gene (*CLLU1*) upregulated in CLL with aggressive clinical course was identified at 12q22.[33,34] High density single nucleotide polymorphism (SNP) arrays were employed to study LOH and allelic loss without loss of gene dosage, also referred to as uniparental disomy (UPD).[15] In 4 out of 70 cases with unmutated *IGHV* a 3,5 Mb gain at 2p16 including *BCL11A* and *REL* was detected. In 3/11 patients with homozygous 13q14 deletion, UPD of a large segment on 13q, distal to the 13q14 deleted region was identified.

Using a highly sensitive technique,[35] the presence of additional allelic losses or gains in the $CD38^+$ lymphocytes *vs.* $CD38^-$ lymphocytes was observed in 3 cases, suggesting that genetically unstable subclones spend most of the time in the activated $CD38^+$ status.

Conclusion

Molecular cytogenetic analysis in CLL revealed a number of lesions having important clinicobiologic implications. A fraction of CLL may acquire clonal chromosome changes during the natural history of the disease.[36,37] Indeed, clonal evolution was observed in 10-20% of the patients who developed del 17p13, del 6q21, del 11q23, trisomy 8q24 at follow up studies. Importantly, the late appearance of 11q- in CLL was associated with disease evolution.[38] Thus, a modern diagnostic workup in CLL should include cytogenetic and molecular cytogenetic investigations, which should be performed before first line treatment and at relapse for the selection of risk-adapted treatment.

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