## Chronic lymphocytic leukemia and prolymphocytic leukemia. Two coins or two sides of the same coin?

Chronic lymphocytic leukemia (CLL) has a distinct morphology and immunophenotype. Despite lacking specific genetic abnormalities, the diagnosis is straightforward even in cases with atypical morphology. B-cell prolymphocytic leukemia (B-PLL) is a rare lymphoid neoplasm first described in 1974 as an aggressive variant of CLL. It differs from CLL in morphology and immunophenotype and it is recognized as a distinct entity in the World Health Organisation classification. Like CLL, B-PLL does not have recurrent genetic alterations although *MYC* abnormalities, *TP53* mutations and complex karyotypes are frequent.[1–3] The diagnosis of B-PLL relies on cytology, the immunophenotype and on investigations that rule out other splenomegalic B-cell neoplasms, particularly, mantle cell lymphoma (MCL). A debated issue is whether B-PLL represents the evolution of a CLL, particularly in cases with increased prolymphocytes (CLL/PL), or if it is a different entity. The differences in cytology, the immunophenotype and gene expression profile between CLL and B-PLL would argue against them being the same entity in two phases of disease evolution.[4]

We describe a patient that manifested with a "*bona fide*" B-PLL and who was successfully treated with immunochemotherapy. Upon "relapse" there was evidence of a different B-cell clone with a morphology and immunophenotype consistent with CLL. This case reinforces the notion of the existence of B-PLL as an entity distinct from CLL.

A 57-year-old male presented with a 6-week history of weight loss and fatigue. Examination revealed splenomegaly and no lymphadenopathy. The white blood cell count (WBC) was $89.61 \times 10^9$/L with 91% lymphocytes, hemoglobin 118 g/L and a normal platelet count. The biochemistry was normal except for lactate dehydrogenase that was raised to 894 UI/L (250-450 UI/L). No monoclonal band was detected by immunofixation. Peripheral blood (PB) showed medium to large sized lymphoid cells with a round prominent single nucleolus, and moderately basophilic cytoplasm. The morphology was consistent with the cell being a prolymphocyte (Figure 1A). Flow cytometry showed that 93% of cells were clonal B-lymphocytes $\lambda^+$, $CD5^+$, $FMC7^{++}$. CD10, CD23, CD200 and CD43 were negative. The anti-

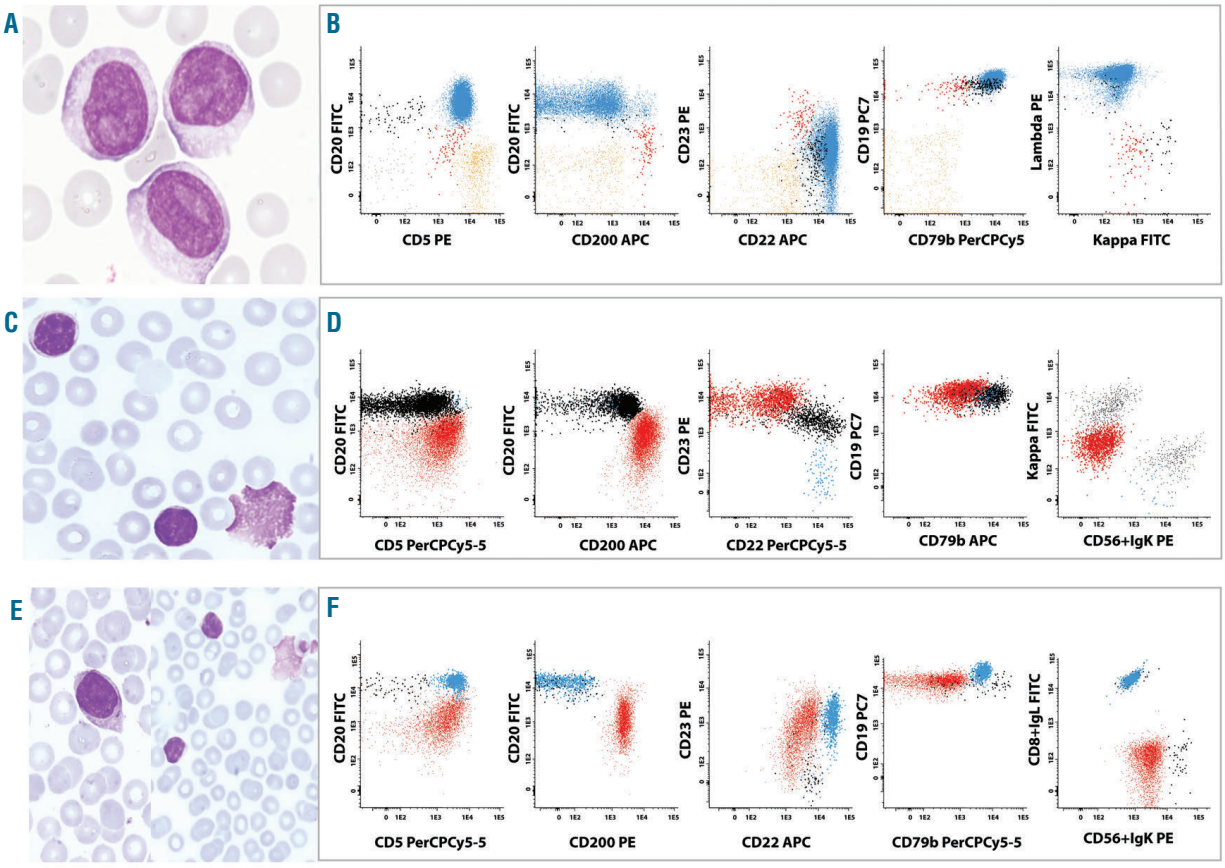


**Figure 1. Morphology and phenotype at diagnosis and through the follow up of the patient.** In flow cytometry images, B-cell prolymphocytic leukemia (B-PLL) is shown in light blue, chronic lymphocytic leukaemia (CLL) in red and polytypic B lymphocytes in black. At diagnosis, the morphology of lymphoid cells was consistent with prolymphocytes (A) and flow cytometry identified a predominant population of $CD5^+$ B-cells with λ chain restriction not typical of CLL corresponding to B-PLL, but also a small percent of residual polytypic B-cells (0.6% of B-cells) and CLL-like $\kappa^+$ cells (0.7% of the B-cells) (B). At "relapse", the peripheral blood morphology was typical of CLL (clumped chromatin and smudge cells) (C), and by immunophenotype the most prominent B-cell populations corresponded to CLL $\kappa^+$ cells (68%) and polytypic B-cells. A small amount of $\lambda^+$ $CD5^+$ B-PLL (3.6% of B-cells) was also seen (D). After ibrutinib, morphology showed a mixture of prolymphocytes (E, left) and CLL cells (E, right). These two populations (75% of CLL and 23% of B-PLL), along with polytypic B-cells, were demonstrated by immunophenotype (F).

gen intensity of CD20, CD22 and CD79b was normal (Figure 1B). A positron emission tomography scan confirmed the splenomegaly and showed infracentimetric widespread lymphadenopathy without F-18 fluorodeoxyglucose uptake.

A bone marrow aspirate (BMA) demonstrated infiltration by 43% prolymphocytes. The karyotype was normal. Fluorescence *in situ* hybridization (FISH) revealed a *MYC* (8q24) rearrangement with the λ light chain in 92% of nuclei (Figure 2A). *BCL6, BCL2, CCND1* and *CCND2* were not amplified or rearranged and *TP53* was not deleted. The t(11;14)(q13;q32) was absent. Single nucleotide polymorphism (SNP) array 6.0 of tumor DNA showed a subclonal trisomy of chromosome 4 and alterations in 3q consistent with chromothripsis (Figure 2B). A gene expression quantification study was performed by real time quantitative-PCR using three gene sets frequently overexpressed in conventional MCL (*CCND1, SOX11, HDGFRP3* and *DBN1*),[5] cyclin D1-negative MCL (CCND2 and CCND3),[6] or in other B-lymphoproliferative disorders (*FMOD, KSR2, MYOF, MME, CXCR4*, and *CAMSAP2*).[7] Overexpression in these genes was not found. No mutations were detected in the *TP53* coding region (exons 2-11) or in hotspot regions of *BRAF, MYD88, NOTCH2, NOTCH1*, and *MAP2K1*. Analysis of the immunoglobulin heavy chain (*IGHV*) gene rearrangement at the FR1 region showed a single monoclonal peak (Figure 3A). Cerebrospinal fluid was not infiltrated. On the basis of all these investigations, the patient was diagnosed with B-PLL.

The patient received one cycle of Hyper-CVAD (cyclophosphamide, vincristine, adriamycin and dexamethasone) followed by five cycles of R-CHOP (rituximab, cyclophosphamide, doxorubicin, vincristine and prednisone) achieving a complete response (CR). Four months after documenting CR, B-lymphocytes comprised 3% of PB lymphocytes ($4.31x10^9$/L) but 40% of them showed a different morphology and immunophenotype from that at diagnosis and consistent with CLL: $CD5^+$, $CD23^+$, $CD43^+$, $CD200^+$, $CD10^-$, CD20 and CD22 weakly positive and weak κ light chain restriction (Figures 1C-D). Analysis of the *IGH* gene (FR1 region) demonstrated a monoclonal pattern with a different peak than the one at diagnosis of B-PLL (Figures 3A-B). Sequencing of these products demonstrated that at diagnosis the clonal peak belonged to the *IGHV5* family (*IGHV5-51*01F/D3-22*01F/J6*02F*) with 96.88% identity to the germ line while at relapse the family was *IGHV1-69*13/D5-12*01F/J6*02F* with a 100% of identity.

A watch and wait policy was adopted. The CLL lym-

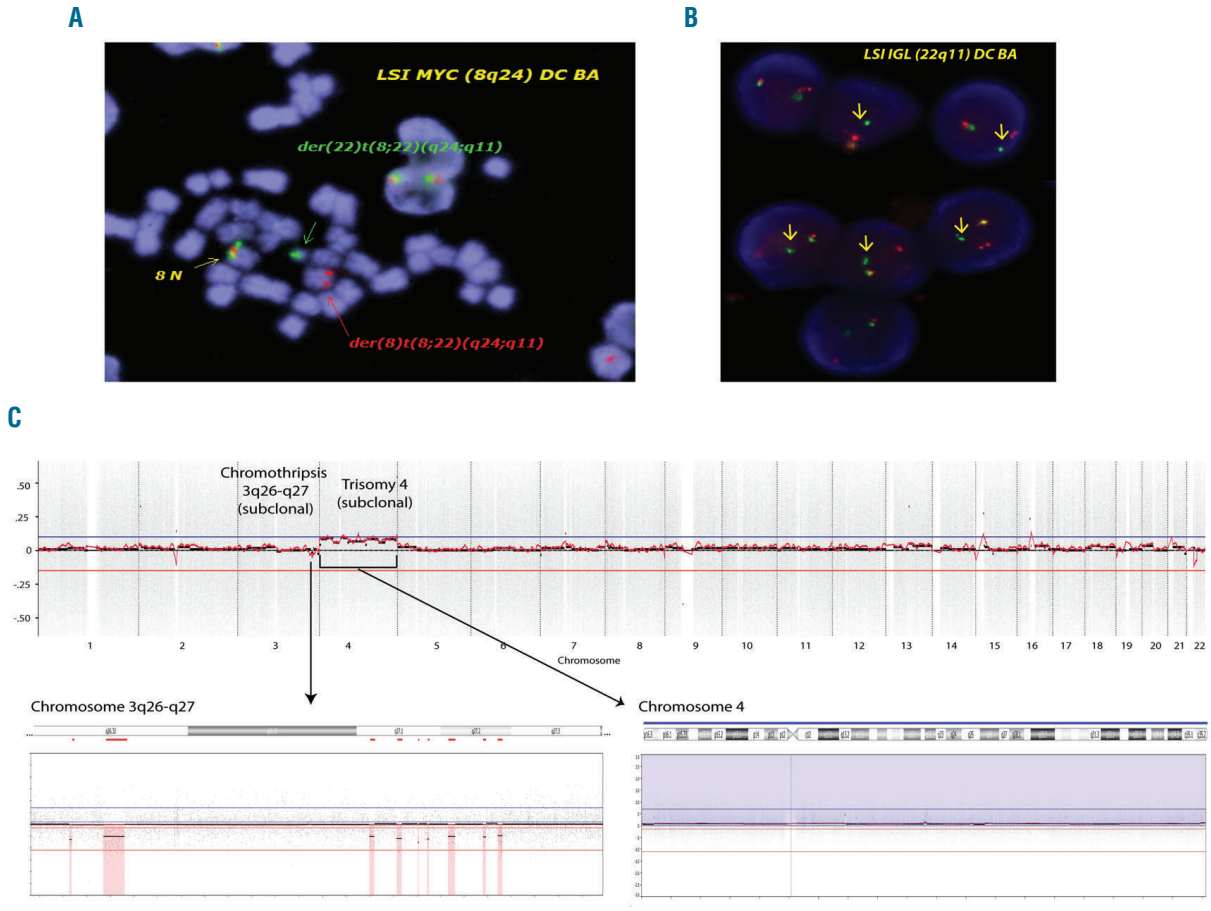


**Figure 2. Fluorescence *in situ* hybridization and single nucleotide polymorphism array 6.0 studies.** (A) Fluorescence *in situ* hybridization (FISH) on a metaphase using a *MYC* dual color break apart probe. Split signals green and red confirms the presence of *MYC* rearrangement. (B) FISH on nuclei using *IGL* dual color break apart probe, the split signal green and red confirms the *IGL* rearrangement. (C) The Single nucleotide polymorphism (SNP) array 6.0 shows the presence of chromothripsis pattern in chromosome 3 with nine losses (red) in the q-arm (zoom of 3q26-q27) and a subclonal trisomy 4 (zoom of whole chromosome 4).

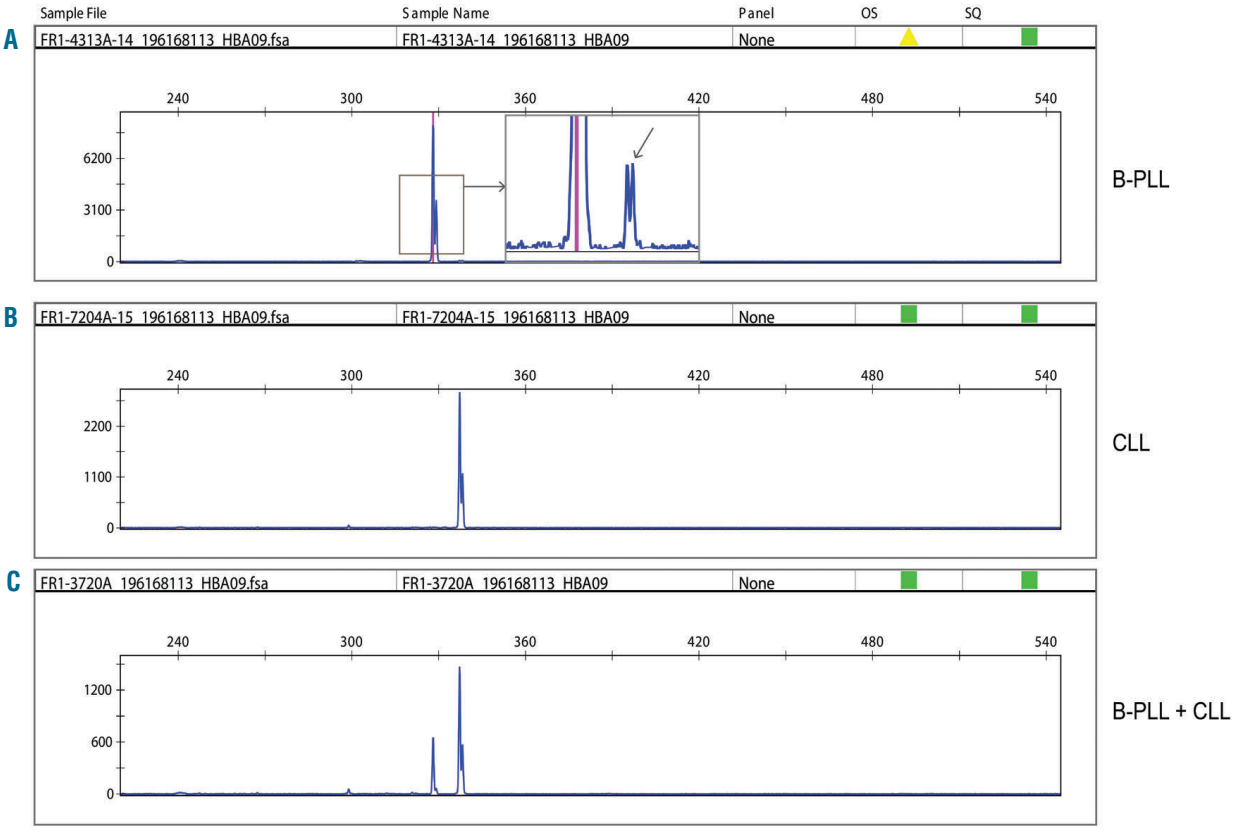


**Figure 3. B-cell clonality assessment.** Comparison of B-cell clonality at different time points of the disease evolution; at diagnosis (A), 4 months after complete response (B) and 2 years after the diagnosis (C). The clonality study at diagnosis were retrospectively reviewed, detecting a second peak corresponding to the chronic lymphocytic leukemia (CLL) population. B-PLL: B-cell prolymphocytic leukemia.

phocytes progressively increased to 215.4x10$^9$/L within 2 years; there was a progressive thrombocytopenia and hemoglobin was normal. The BMA showed infiltration by 78% lymphocytes with a CLL phenotype. Cytogenetics showed a normal karyotype and FISH for *ATM, D12Z3, DLEU, LAMP1* and *TP53* was normal. *MYC* (8q24) rearrangement was not detected. A computerized tomography scan showed supra and infradiaphragmatic lymphadenopathy, up to 16 mm in diameter and 20 cm splenomegaly. Due to CLL progression the patient started on ibrutinib 420 mg/day and achieved a partial response with normalization of the lymphocyte count and improvement in the platelet count. At the last control, 2 years after starting ibrutinib, the patient is asymptomatic, the spleen is not palpable and the lymphocyte count is 1.3x10$^9$/L, hemoglobin 150 g/L and the platelet count 91x10$^9$/L. Flow cytometry shows 18% B lymphocytes with three populations: a population with a CLL phenotype and κ light chain restriction, a second with a phenotype of the B-PLL cells (CD5$^+$, FMC7$^{++}$, negative for CD10, CD23, CD200 and CD43 and λ light chain restriction) and a third with a polytypic B-cell phenotype (Figures 1E-F). Molecular *IGHV* analysis (FR1 region) confirms the presence of two clones with identical peaks to those detected at diagnosis of B-PLL and CLL phases (Figure 3C). At that time, flow cytometry data was retrospectively reviewed, detecting a small population with CLL phenotype already present at onset, consistent with low count monoclonal B-cell lymphocytosis (Figures 1B and Figure 3A) and a small amount of B-PLL present at CLL diagnosis (Figure 1D). All laboratory investigations are summarized in the *Online Supplementary Table S1*.

The co-existence or sequential occurrence of two unrelated B-lymphoid neoplasms is well recognised.[8] In this setting, CLL has been described together with various B-cell diseases.[9] Only one case with concurrent CLL and B-PLL with two separate B-cell clones has so far been described.[10] However, this is the first extensively investigated patient who manifested as B-PLL that "relapsed" as CLL shown to be clonally unrelated. The differences in cytology and immunophenotype of the neoplastic cells in both phases of the evolution and the demonstration of two B-cell clones with different *IGHV* rearrangements confirmed the different nature of the two diseases. This association is of interest considering the controversial relationship between CLL and B-PLL and the debate if B-PLL is a subgroup of CLL.

Diagnosis of B-PLL based on clinical, morphological and immunophenotype data can be difficult because B-PLL shares some features with other splenomegalic B-lymphoproliferative disorders. In the presence of a CD5+ B-cell disorder it is important to consider the diagnosis of MCL. MCL was ruled out in our case on the basis of the absence of the translocation t(11;14) and the overexpression of *SOX11, CCND1, CCND2, CCDN3, HDGFRP3, DBN1*.[5,6] Van der Velden *et al.* suggested that B-PLL could be a specific subgroup of t(11;14)-negative MCL, based

on similarities in the immunophenotype and gene expression profile (GEP).[11] However, we did not find any expression of genes related with MCL in our patient who had instead *MYC* rearrangement, a frequent feature in B-PLL and rare in MCL.[12]

In agreement with the previously described data documenting that 50-80% of B-PLL have mutated *IGHV*,[3,13] our case showed a mutated *IGHV* pattern in the B-PLL clone. These previous studies also described that the *IGHV* rearrangements were predominantly involving *IGHV3* or *IGHV4* in most cases (89-100%).[3,13] However, the neoplastic B-PLL cells in our case were *IGHV5*, until now not described in B-PLL. In CLL, the mutational status of the *IGHV* genes allow the stratification of patients into two prognostic groups and the cases with unmutated *IGHV* genes having a worse prognosis. An exception is the CLL subset #2, defined by the expression of stereotyped *IGHV3-21/IGLV3-21 BcR IG* that has a poor outcome regardless the mutational status.[14,15] Overrepresentation of certain *IGHV* genes such as *IGHV1-69, IGHV3-07, IGHV3-21* and *IGHV4-34* has been documented in CLL. Biased usage of the *IGHV1-69* gene has been confirmed by several groups and associated with an unmutated profile, as in our case.[15] Unlike CLL, the *IGHV* mutational status has no prognostic impact in B-PLL.[13]

Genomic differences between B-PLL and CLL were also demonstrated by GEP. A study identified 46 genes which most efficiently differentiated B-PLL from CLL-CLL/PL. One of the genes over-expressed in B-PLL was *MYC* whilst the most characteristic transcripts of CLL were the costimulatory molecule CTLA4/CD152 and the transcription factor LEF1.[4]

Recently, a prognostic model for B-PLL has been proposed considering *MYC* and 17p status. This model identifies three risk groups: low risk (no *MYC* aberration), intermediate risk (*MYC* aberration but no del17p), and high risk (*MYC* aberration and del17p). The median overall survival (OS) is 125.7 months for the intermediate risk *versus* a median OS of 11.1 months for the high risk group.

In conclusion, there are solid clinical and genetic data that support that B-PLL is a biologically distinct disease from CLL and is considered as such in the World Health Organisation classification. In this context, the case described here reinforces this notion.

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