Several prognostic factors are used in chronic lymphocytic leukemia (CLL) to predict disease progression at diagnosis and to help guide therapeutic choices. Among these factors, the detection of deletions of the short arm of chromosome 17, where the tumor suppressor gene TP53 is located at the 17p13 locus, predicts resistance to standard treatments and poor prognosis. Deletions of 17p13 are observed in 5–7% of CLL patients at diagnosis and in 25–40% of cases with advanced refractory disease. Other markers of poor prognosis have been described, such as unmutated IGHV gene status, high levels of thymidine kinase and soluble CD23, CD38 and ZAP-70 expression, as well as other chromosomal aberrations, such as 11q23 deletion. However, defects in the TP53 pathway consistently appear as the most significant adverse prognostic factor in CLL.

About 80% of patients with 17p13 deletion display a mutation in the remaining TP53 allele, resulting in loss of function of the p53 protein, but mutations without a deletion are observed in 4–5% of cases. Mutations of TP53 have been identified that are consequently associated with an unfavorable outcome, but all mutations do not predict similar consequences on the p53 pathway. In addition, a minority of patients with 17p13 deletion have an indolent clinical course, suggesting that p53 function is preserved. Finally, it has appeared that p53 dysfunction is the result of several intricate factors that have not been clearly defined, and several questions still remain to be addressed regarding the biological consequences of TP53 deletions and the various types of mutations.

Consequently, it has become evident that new techniques to determine p53 function are required to identify patients with TP53 abnormalities (deletion and/or mutation), despite normal function, and patients who have TP53 neither mutated nor deleted, but which is dysfunctional for other reasons, including

Figure 1  Cytometric functional test for p53/p21 proteins in cells from patients with chronic lymphocytic leukemia (CLL). Profiles of response after 24-h in vitro culture of CLL cells in the presence of etoposide and nutlin-3a. (a) Type 1 profile: normal p53 function with undetectable p53 protein on day 0, p53 and p21 increased by day 1. (b) B cells from a representative type 1 profile were untreated (red) or treated with etoposide and nutlin-3a for 6 h (blue), and p53 Serine 15 phosphorylation was assessed by flow cytometry. (c) Type 2 abnormal profile, with a high baseline level of p53 protein and no increase in p53/p21 by day 1. (d) Type 3 abnormal profile, with undetectable baseline p53 protein and no increase in p53/p21 by day 1.
epigenetic gene silencing or post-translational modification. These considerations prompted us to determine in a series of 37 CLL patients the functional status of p53 by using a cytometric method that was adapted from the methodology previously described by Best et al.4 We then compared results with the cytogenetic data and mutational status of TP53 of this population of patients.

Determination of the functional status of p53 in CLL cells by flow cytometry was based on induction of p53 and p21 protein expression using etoposide and nutlin-3a. Three types of response were observed. The type 1 profile corresponded to normal function (Figure 1a): as expected the baseline expression of p53 on day 0 was undetectable (threshold: mean fluorescence intensity \( <100 \)), but by day 1, a clear-cut increase in the percentage of p53 expression was observed (increased from 100 to 600%). In parallel, a moderate increase of p21 expression was also observed on day 1 (\( +10\% \)). To confirm the functionality of p53 protein, we tested its phosphorylation at Serine 15 in response to etoposide and nutlin-3a treatment (Figure 1b). This phosphorylation, which results in p53 stabilization and accumulation in the nucleus, is a typical hallmark showing p53 activation.5 The type 2 profile (Figure 1c) was defined by a high level of p53 protein on day 0 without any (or with a slight) increase in p53 or p21 by day 1 in the presence of etoposide and nutlin-3a. The type 3 profile (Figure 1d) was characterized by undetectable p53 protein on day 0, and no change in p53 and p21 expression after in vitro culture in the presence of etoposide and nutlin-3a.

Cytogenetic data, mutational status and results of the functional tests are listed in Table 1. We observed that a normal p53/p21 expression profile (type 1) corresponded to patients without any TP53 abnormalities (20 patients, no. 1–20) or with a monoallelic TP53 abnormality (3 patients, no. 21–23), even though an ATM deletion may be present (cases no. 15–21, 23). In these patients with a normal response, the only case displaying a TP53 mutation had a monoallelic mutation on exon 6 (no. 23). It is important to note that eight patients with a normal p53/p21 response, who had been tested for p53 phosphorylation, displayed normal results in all cases: this allowed us to confirm the functionality of the protein.

Nine out of 10 patients with a cytometric type 2 expression profile had biallelic abnormalities: 8 patients (no. 24–31) had

### Table 1  Cytogenetics, mutational status and functional cytometric profiles of TP53 in patients with chronic lymphocytic leukemia

| Patient no. | Functional category (1,2,3) | FISH\(^a\) | TP53 del\(^b\) | ATM del\(^b\) | Exon/intron |
|-------------|-----------------------------|----------|----------------|----------------|-------------|
| 1           | 1                           | –        | –              | –              | UM          |
| 2           | 1                           | –        | –              | –              | UM          |
| 3           | 1                           | –        | –              | –              | UM          |
| 4           | 1                           | –        | –              | –              | UM          |
| 5           | 1                           | –        | –              | –              | UM          |
| 6           | 1                           | –        | –              | –              | UM          |
| 7           | 1                           | –        | –              | –              | UM          |
| 8           | 1                           | –        | –              | –              | UM          |
| 9           | 1                           | –        | –              | –              | UM          |
| 10          | 1                           | –        | –              | –              | UM          |
| 11          | 1                           | –        | –              | –              | UM          |
| 12          | 1                           | –        | –              | –              | UM          |
| 13          | 1                           | –        | –              | –              | UM          |
| 14          | 1                           | –        | –              | –              | UM          |
| 15          | 1                           | –        | + (72%)        | –              | UM          |
| 16          | 1                           | –        | + (65%)        | –              | UM          |
| 17          | 1                           | –        | + (65%)        | –              | UM          |
| 18          | 1                           | –        | + (92%)        | –              | UM          |
| 19          | 1                           | –        | + (95%)        | –              | UM          |
| 20          | 1                           | –        | + (91%)        | –              | UM          |
| 21          | 1                           | + (21%)  | + (97%)        | –              | UM          |
| 22          | 1                           | + (17%)  | + (76%)        | –              | UM          |
| 23          | 1                           | –        | –              | + (76%)        | Exon 6/p.R196P |
| 24          | 2                           | + (86%)  | –              | Exon 8/p.R273H |
| 25          | 2                           | + (80%)  | –              | Exon 5/p.H178D |
| 26          | 2                           | + (70%)  | –              | Exon 5/p.R175H |
| 27          | 2                           | + (94%)  | –              | Exon 8/p.R273H |
| 28          | 2                           | + (79%)  | –              | Exon 7/p.R248E |
| 29          | 2                           | + (6%)   | –              | Exon 7/p.R249S |
| 29 bis      | 1                           | –        | –              | –              | UM          |
| 30          | 2                           | + (53%)  | –              | Exon 5/p.P142R and exon 6/m.P222 L |
| 31          | 2                           | + (91%)  | –              | Exon 8/p.V272M |
| 32          | 2                           | –        | –              | Intron 9/IVS9+2A>G |
| 33          | 2                           | –        | + (84%)        | –              | UM          |
| 34          | 3                           | + (87%)  | –              | Intron 4/IVS4+1G>A |
| 35          | 3                           | + (88%)  | –              | Intron 5/IVS5+1G>T |
| 36          | 3                           | + (52%)  | –              | Intron 6/IVS7–2A>A |
| 37          | 3                           | + (17%)  | –              | Exon 8/p.Del264L |

Abbreviations: FISH, fluorescence in situ hybridization; UM, unmutated. 
\(^a\)200 Interphasic nuclei were counted. 
\(^b\)Monoallelic deletion.
and mutational data. In addition, this cytometric functional test contributes to unraveling the complexity of the p53 pathway.

Conflict of interest

The authors declare no conflict of interest.

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