

# Chronic Lymphocytic Leukemia

## *DETECTION OF PROGNOSTICALLY SIGNIFICANT GENOMIC ABERRATIONS IN CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) BY FLUORESCENCE IN SITU HYBRIDIZATION (FISH)*

### Test Highlights

- FISH is more sensitive than conventional cytogenetics in detecting genomic aberrations.
- This FISH panel detects prognostically important genomic abnormalities in CLL and is suitable for widespread use.

### Clinical Background

- Chronic lymphocytic leukemia (CLL) is the most common form of adult leukemia in the Western world. The disease is characterized by the accumulation of mature-appearing lymphocytes in the blood, bone marrow, lymph nodes, and spleen. CLL has a highly variable clinical course, and some patients die from the disease within a few months from diagnosis, whereas others live for 20 years or more.
- The commonly used clinical staging systems developed by Rai, et al and Binet, et al have been effective in classifying patients into broad prognostic groups that appear to correlate with the gross tumor burden and its effect on the function of the bone marrow.<sup>1,2</sup> However, these staging systems do not accurately predict the clinical course of the disease in individual patients, especially those patients who have a low tumor burden at the time of diagnosis. Therefore, there is considerable interest in characterizing molecular markers that could identify patients with more rapidly progressive forms of the leukemia for whom the “watch and wait” approach may not be appropriate.
- Molecular markers with prognostic value in CLL include somatic hypermutation of the immunoglobulin heavy-chain variable-region (IgVH) gene in the leukemic cells, surface CD38 expression, and chromosomal abnormalities identified by cytogenetic analysis. Patients with unmutated IgVH genes have much more aggressive disease, with a predicted median survival of 95 months, whereas those with mutated IgVH genes have a relatively benign course, with a predicted median survival of 293 months.<sup>3</sup> However, establishing the mutation status is technically challenging, time consuming, and not readily available to most clinicians. High CD38 expression, originally described as a surrogate for unmutated IgVH genes, correlates with aggressive disease, but expression levels can change over time during the course of the disease, a fact that affects the predictive value of this marker.
- Conventional cytogenetic analysis yields up to a 40–50 percent abnormality rate, and the success rate has been hampered by the lack of growth of CLL cells in cell cultures and chromosomal abnormalities that are often missed by this technique. However, the standard fluorescent in situ hybridization (FISH) panel has dramatically improved the detection rate of clonal abnormalities in CLL to more than 80 percent. Some of these abnormalities are significant predictors of disease progression and patient survival. FISH allows detection of chromosomal aberrations in both actively dividing cells and interphase nuclei. Recently, this technique has been used to detect the most common genomic abnormalities in CLL, including

trisomy 12, rearrangements involving 14q32, and deletions of 13q14, 6q21, 17p, and 11q22-23. Five major prognostic groups currently exist, including those with a median survival time of 32 months (the p53 deletion), 79 months (the ATM deletion), 111 months (normal FISH), 114 months (trisomy 12), and 133 months (the 13q14 monoallelic deletions).<sup>4</sup>

- Unfortunately, the FISH analysis reported by Dohner, et al used 13 probes and is too cumbersome for routine clinical use in most laboratories. Our group has therefore compiled a simplified CLL FISH panel designed to detect the genomic aberrations with proven clinical significance.<sup>5</sup> This panel utilizes only four commercially available probes and reliably detects the prognostically important genomic abnormalities, thus allowing clinicians to assess the biological risk of disease progression in patients with CLL.

### Indications for Ordering

FISH testing for CLL is indicated in individuals who have been diagnosed with CLL by clinical criteria based on a lymphocytosis of greater than  $5 \times 10^9$  cells/l with greater than 50 percent mature-appearing lymphocytes, as well as the characteristic immunophenotype of CD5, CD19, CD20, and CD23 expression, monoclonal kappa or lambda expression, and dim surface immunoglobulin expression. FISH testing serves as a screen to prognostically stratify the risk of CLL patients.

### Methodology

- Peripheral blood or bone marrow specimens are analyzed by FISH using a set of commercially available FISH probes specific for ATM (11q22.3), chromosome 12 centromere, D13S319 (13q14.3), and p53 (17p13.1) loci (Vysis, Downers Grove, IL). These four probes constitute the CLL FISH panel.
- All four probes are set up separately for each patient.
- Hybridization and detection of hybridization signals are performed according to the manufacturer’s protocols.
- 200 nuclei are evaluated for each probe.
- Peripheral blood samples from 20 individuals without hematologic diseases and with normal karyotypes are used as controls. Means and standard deviations (SD) of the percentages of nuclei with one, two, and three hybridization signals are calculated. Results are considered abnormal if the percent of nuclei with the abnormal hybridization signal is greater than three SD from the mean.

### Additional Ordering Notes

A sodium heparin (green top) tube of peripheral blood (minimum 5 mL of whole blood) or 2–3 mL of bone marrow is required. Samples should be stored and transported at room temperature to the laboratory within two days of draw.

### Limitations

- This probe panel only detects prognostically important imbalances (gain or loss of DNA) in the chromosomes of interest.
- Chromosome alterations outside the regions complementary to these FISH probes will not be detected.

### References

1. Rai KR, et al. Clinical staging of chronic lymphocytic leukemia. *Blood* 1975;46:219–34.
2. Binet JL, et al. A new prognostic classification of chronic lymphocytic leukemia derived from a multivariate survival analysis. *Cancer* 1981;48:198–206.
3. Hamblin TJ, et al. Unmutated IgVH genes are associated with a more aggressive form of chronic lymphocytic leukemia. *Blood* 1999;94:1848–54.
4. Dohner H, et al. Genomic aberrations and survival in chronic lymphocytic leukemia. *N Engl J Med* 2000;343:1910–16.
5. Goorha S, et al. A set of commercially available fluorescence in situ hybridization probes efficiently detects cytogenetics abnormalities in patients with chronic lymphocytic leukemia. *Genet Med* 2004;6:48–53.

## Test Information

**2002295**

**Chromosome FISH, CLL Panel**

For specific collection, transport, and testing information, refer to the ARUP Web site at [www.aruplab.com](http://www.aruplab.com).

For information on test selection, ordering, and interpretation, refer to ARUP Consult® at [www.arupconsult.com](http://www.arupconsult.com).