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Standardization of fluorescence in situ hybridization studies on chronic lymphocytic leukemia (CLL) blood and marrow cells by the CLL Research Consortium

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Abstract Five laboratories in the Chronic Lymphocytic Leukemia (CLL) Research Consortium (CRC) investigated standardizing and pooling of fluorescence in situ hybridization (FISH) results as a collaborative research project. This investigation used fixed bone marrow and blood cells available from previous conventional cytogenetic or FISH studies in two pilot studies, a one-day workshop, and proficiency test. Multiple FISH probe strategies were used to detect 6q-, 11q-, +12, 13q-, 17p-, and *IGH* rearrangements. Ten specimens were studied by participants who used their own probes (pilot study 1). Of 312 FISH interpretations, 224 (72%) were true-negative, 74 (24%) true-positive, 6 (2%) false-negative, and 8 (3%) false-positive. In pilot study no. 2, each participant studied two specimens using identical FISH probe sets to control for variation due to probe sets and probe strategies. Of 80 FISH interpretations, no false interpretations were identified. At a subsequent workshop, discussions produced agreement on scoring criteria. The proficiency test that followed produced no false-negative results and 4% (3/68) false-positive interpretations. Interpretation disagreements among laboratories were primarily attributable to inadequate normal cutoffs, inconsistent scoring criteria, and the use of different FISH probe strategies. Collaborative organizations that use pooled FISH results may wish to impose more conservative empiric normal cutoff values or use an equivocal range between the normal cutoff and the abnormal reference range to eliminate false-positive interpretations. False-negative results will still occur, and would be expected in low-percentage positive cases; these would likely have less clinical significance than false positive results. Individual laboratories can help by closely following rigorous quality assurance guidelines to ensure accurate and consistent FISH studies in their clinical practice and research.

1. Introduction

Studies of interphase nuclei using fluorescence in situ hybridization (FISH) are an essential part of the clinical evaluation of patients with B-cell chronic lymphocytic

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leukemia (CLL) [1–5]. FISH methods and DNA probes used to analyze cells from patients with CLL vary among cytogenetic laboratories. This is at least in part because national standards established for clinical studies generally are left to the discretion of the laboratory director — which FISH probes to use for CLL, definition of analytic details such as scoring criteria, and how to define the normal cutoff.

National guidelines to validate and use FISH assays in clinical practice have been published provided by the American College of Medical Genetics and the National Committee for Clinical Laboratory Standards [6,7]. However, not every laboratory follows these guidelines in the same way. FISH methods are accurate and reproducible when they are validated appropriately and continuous quality assurance procedures are used [8]. Multiple laboratories that work together to validate specific FISH probes can achieve excellent results following such guidelines [9–11].

The CLL Research Consortium (CRC) involves multiple institutions that work together to investigate the biology of CLL and develop treatments for CLL. The CRC FISH database currently includes results of more than 3,800 diagnostic (and many follow-up) FISH studies. Lack of FISH standardization can be problematic for cooperative groups when FISH data are pooled for clinical correlative studies. Differences among laboratories in validation procedures, FISH probes, scoring criteria, and statistical methods to define normal and abnormal results can be unintended sources of variation. This can complicate data analysis and reduce the validity of conclusions from correlative studies. To further investigate these important issues in a consortium dedicated to the study of CLL, five participating laboratories in the CRC designed and executed a joint FISH study to test for scoring variation and to identify common methods and scoring techniques that would ultimately generate more concordant FISH results.

2. Materials and methods

The selection of specimens, slide preparations, and data coding in this study were accomplished with approval of the Mayo Clinic Institutional Review Board, and informed consent was obtained in accordance with the Declaration of Helsinki. The FISH processing and analysis of coded slide preparations by each participant were performed with approval of the Institutional Review Board at each participating site.

Initially, a detailed survey questionnaire was sent to each laboratory to assess equipment, methods, and experience with FISH for CLL. Participants identified as A, B, C, D, and E listed features of their fluorescence microscopes, including filters, wattages, manufacturers, models, lenses, and digital capture systems. Each laboratory reported their clinical experience scoring FISH for CLL, including number of samples per year, types of samples (blood or bone marrow), FISH probes used, and time points of patient samples (diagnostic or follow-up). Slide preparation, pretreatment, washing techniques, and scoring practices were also compared among the participating sites.

FISH strategies used by participants in this investigation included enumeration, ND-FISH, and D-FISH (numeric and deletion FISH and double-fusion FISH, respectively) [12]. The enumeration probe strategy uses one probe per chromosome and is generally used to establish the number of chromosomes present in the interphase nucleus. The ND-FISH strategy to detect aneuploidy and chromosome deletions uses a probe of one color for a control site and a probe of another color for an interstitial target site on the same chromosome. The D-FISH strategy is used to detect a reciprocal translocation or inversion, using probes of different colors at the expected rearrangement breakpoints to produce two fused signals in the event of a rearrangement.

The FISH probes used by all five laboratories (Table 1) were designed by commercial or local institution laboratories to detect 11q, 13q, and 17p deletions, as well as trisomy

Table 1
FISH probes initially used by CRC participants in this investigation[a]

	Participant	
FISH anomaly	A, B, C, E[b]	D[c]
6q-	Not done	CEP6 (chromosome 6 centromere) *cMYB* (6q23)
11q-	*ATM* (11q22)	CEP11 (chromosome 11 centromere) *ATM* (11q22)
+12	CEP12 (chromosome 12 centromere)	CEP12 (chromosome 12 centromere) *MDM2* (12q15)
13q-	D13S319 (13q14.3) LSI13q34 (13q34)	D13S319 (13q14.3) LSI13q34 (13q34)
17p-	*P53* (17p13)	CEP17 (chromosome 17 centromere) *P53* (17p13)
t(14;?) or t(11;14)[d]	*IGH-CCND1* (14q32 and 11q13)	*IGH-CCND1* (14q32 and 11q13)

[a] All probes were made by Abbott Molecular (Des Plaines, IL) except for *cMYB* and *MDM2,* which were homebrew probes made at Mayo Clinic.
[b] These probes are used in sets to simultaneously detect 11q- and 17p-; +12 and 13q-.
[c] These probe sets were used in an ND-FISH strategy to detect 6q-, 11q-, +12, 13q- and 17p-.
[d] A D-FISH method with *CCND1* and *IGH* were used to detect translocations involving the *IGH* locus on chromosome 14.