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Karyotype Evolution on Fluorescent In Situ Hybridization Analysis Is Associated With Short Survival in Patients With Chronic Lymphocytic Leukemia and Is Related to CD49d Expression

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TO THE EDITOR: In our 2006 report in the *Journal of Clinical Oncology*,[1] we described the frequency of clonal evolution on fluorescent in situ hybridization (FISH) analysis in a prospective, longitudinal cohort study of 159 patients with previously untreated chronic lymphocytic leukemia (CLL). Clonal evolution on FISH analysis was observed in approximately 25% of patients after follow-up at 5+ years and frequently involved acquisition of specific cytogenetic defects associated with unfavorable outcome when present at baseline.[2] Given that the date of repeat FISH analysis was also the date of last follow-up at the time of our initial report, we were unable to analyze the relationship between clonal evolution and patient survival.

We continue to observe these patients and are now able to report on this critical clinical question. After a median follow-up of 2.1 years (range, 0 to 7 years) from the date of second FISH (performed minimum of 5 years after baseline FISH), median survival among patients with clonal evolution (n = 17; seven deaths) was 2.3 years compared with not yet reached for the 46 patients (seven deaths) without clonal evolution (Fig 1A; log-rank *P* value = .01). Among the 17 patients with clonal evolution, the type of cytogenetic abnormality acquired strongly related to its clinical implications (Fig 1B), where the median survival from date of second FISH among patients who acquired a del(17p13.1) or del(11q22.3) (n = 5; four deaths) was 1.3 years compared with not yet reached for those who acquired other defects such as del(13q14) (n = 12; three deaths; log-rank *P* value = .003).

Given the association between clonal evolution and survival and the fact that certain cytogenetic abnormalities such as del(17p13.1) are associated with poor response to purine nucleoside analog–based treatments,[3] it would be helpful to identify patients at increased risk of future karyotype evolution at a time when treatment may be more effective. In our original report, the only prognostic factor that predicted for subsequent karyotype evolution was ZAP-70 status, where 42% of ZAP-70–positive patients experienced clonal evolution compared with 10% of ZAP-70–negative patients (*P* = .008).

Since the time of that report, we and others have identified surface expression of CD49d (alpha 4 integrin) as an independent prognostic parameter in patients with CLL[4,5] Based on its powerful relationship to survival, we have now analyzed the relationship between CD49d assessed at or near study entry and frequency of future clonal evolution 5+ years later. Using

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the previously published 45% threshold to classify CD49d expression,[4] 12 of 55 patients with low CD49d expression experienced clonal evolution compared with five of eight patients with high CD49d expression (22% *v* 63%; *P* = .028). Similar results were also observed when the 30% threshold[4,5] was used to classify CD49d expression (20% [10 of 50] *v* 54% [seven of 13]; *P* = .031).

Use of CD49d as a prognostic marker may have several advantages over some other currently available prognostic markers because it is a surface protein that is easy to analyze and quantify by flow cytometry. Nonetheless, a variety of technical issues regarding stability and reproducibility arose shortly after the prognostic utility of some other flow-based prognostic assays (eg, CD38 and ZAP-70) were first reported. In this respect we have found CD49d expression to be reproducible on fresh versus frozen samples (n = 14) and stable over time (n = 32) in the overwhelming majority of patients with CLL (Fig 2A and B). We have also found similar results when leukemic cell expression of CD49d is assessed using a two-color (CD19, CD49d) or three-color (CD19, CD5, CD49d) flow cytometry evaluation strategy (Fig 2C).

We conclude that clonal evolution relates to survival in patients with CLL where the type of cytogenetic abnormality acquired influences its clinical implications. Our analysis of prognostic parameters indicates that ZAP-70 and CD49d status at baseline can identify patients at increased risk for both shorter survival and future clonal evolution, and could be used to identify high-risk patients for future trials of early intervention. Given that acquisition of new cytogenetic abnormalities is relatively common and may influence response to therapy, we advocate repeat FISH analysis at the time of progression (ie, before therapy selection) for patients who initially had FISH for prognostic purposes obtained at diagnosis.

Acknowledgments

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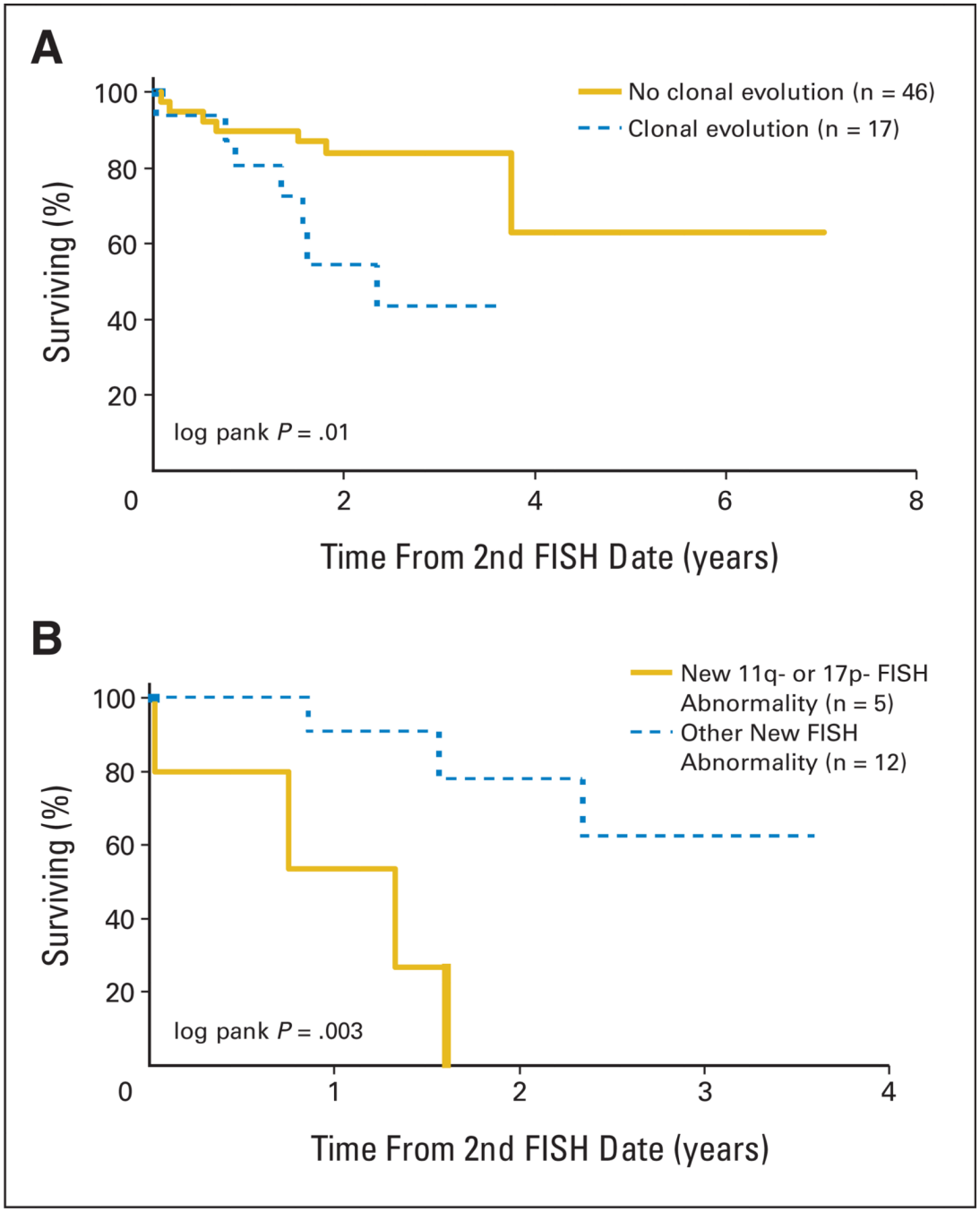

Fig 1.

Karyotype evolution and survival. (A) Overall survival from date of repeat fluorescent in situ hybridization (FISH) analysis based on whether or not patients had clonal evolution (n = 63). (B) Overall survival from date of clonal evolution based on the type of cytogenetic defect acquired (n = 17).

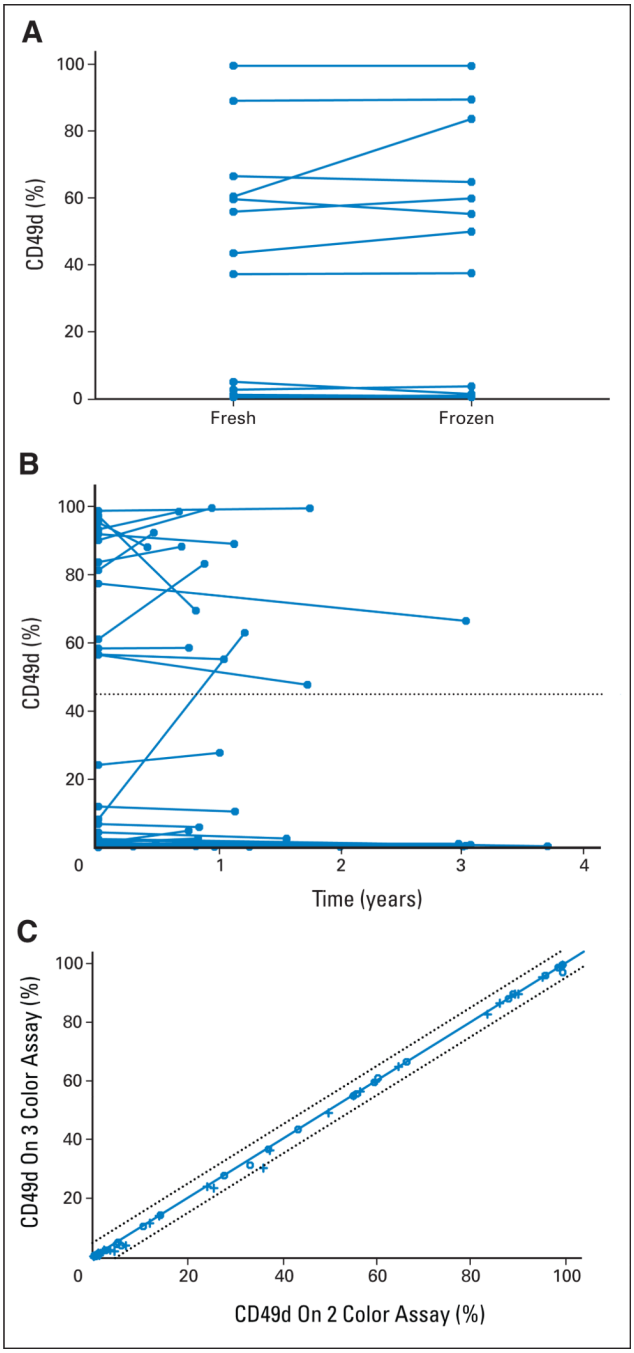

Fig 2.

Stability and reproducibility of CD49d evaluated by flow cytometry. (A) Comparison of CD49d expression on chronic lymphocytic leukemia peripheral blood samples tested as either fresh samples or on thawing after storage at −80°C (n = 14). The intraclass correlation coefficient between the two assays was 0.983 (95% CI, 0.948 to 0.995). (B) Expression of CD49d over time on sequential samples from patients (n = 32) who had samples at multiple time points over a 3-month to 4-year interval. The intraclass correlation coefficient between the two assays was 0.952 (95% CI, 0.908 to 0.976). Dotted horizontal line represents the 45% threshold used to separate patients expressing and not expressing CD49d. One (3%) of 32 patients had a change in CD49d classification over time. (C) Comparison of a two-color

(CD19, CD49d) verses three-color (CD19, CD5, CD49d) flow cytometry strategy for evaluating CD49d. (○) Fresh specimens (n = 35); (+) frozen specimens (n = 34). The solid line represents the $x = y$ line (ie, for values on this line, two-color and three-color results are the same). Dotted lines indicate a 5% difference between x and y values. The two assays yielded nearly identical results in all cases, with an intraclass correlation coefficient of 0.999 (95% CI, 0.999 to 1.000) for comparisons on both fresh and frozen samples.