

CORRESPONDENCE



MicroRNA in Chronic Lymphocytic Leukemia

TO THE EDITOR: Calin et al. (Oct. 27 issue)¹ report on genomic mutations within microRNA loci in patients with chronic lymphocytic leukemia (CLL). We believe that the frequency with which such mutations can be found was not accurately estimated and that some of the cell-based data were unsound. Among 69 samples of CLL cells, in which one copy of chromosome 13q was lost, we did not identify mutations within a 581-bp segment encompassing the *miR-15a-16-1* gene. We also consider it very surprising that a single substitution outside the pri-miR-16-1 hairpin abolished the expression of both *miR-15a* and *miR-16-1* as presented in Figure 2E of the original article. Figure 2E is inconclusive and mislabeled. It appears that *miR-15a* (mislabeled as U6, which is a 100-nucleotide small nuclear RNA) is present in all samples and that *miR-16-1* is absent from even the control 293 cells. *MiR-15a* represents about 5 percent, and *miR-16-1* about 10 percent, of the total microRNA content of the 293 cells. It remains unexplained why *miR-15a* was detected in the left portion of Figure 2E but *miR-16-1* was not detected in the right portion, showing the control human embryonic kidney 293 cells.

Arndt Borkhardt, M.D.

Uta Fuchs, Ph.D.

Ludwig-Maximilians-Universität München
80337 Munich, Germany
arndt.borkhardt@med.uni-muenchen.de

Tom Tuschl, Ph.D.

Rockefeller University
New York, NY 10021

1. Calin GA, Ferracin M, Cimmino A, et al. A microRNA signature associated with prognosis and progression in chronic lymphocytic leukemia. *N Engl J Med* 2005;353:1793-801.

TO THE EDITOR: Calin et al. found a significant association between a microRNA expression signature and ZAP-70 expression, mutational status of the immunoglobulin heavy-chain variable-region (*IgV_H*) gene, and time to initial treatment in patients with CLL. They suggest that microRNA expression can be included as a prognostic marker in CLL. This proposal is premature, since the microRNA signature appears to be a surrogate for high ZAP-70 expression and unmutated *IgV_H*, which are known to be markers of a poor prognosis in patients with CLL.¹⁻³ Was a multivariate analysis done to show that microRNA is an independent prognostic factor? Furthermore, it is not clear that the list of microRNA genes that predict time to treatment completely overlaps with the microRNAs that are associated with ZAP-70 expression and *IgV_H* mutational status (only five are the same), especially since the chip used to detect microRNA can discriminate between similar isoforms of microRNAs. Finally, it is not clear whether the set of nine microRNAs that predict progression was validated in the independent set of 50 patients with CLL.

Wee J. Chng, M.D.

National University Hospital
Singapore 119074
chngwj@nuh.com.sg

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1. Rassenti LZ, Huynh L, Toy TL, et al. ZAP-70 compared with immunoglobulin heavy-chain gene mutation status as a predictor of disease progression in chronic lymphocytic leukemia. *N Engl J Med* 2004;351:893-901.
2. Crespo M, Bosch F, Villamor N, et al. ZAP-70 expression as a surrogate for immunoglobulin-variable-region mutations in chronic lymphocytic leukemia. *N Engl J Med* 2003;348:1764-75.
3. Hamblin TJ, Davis Z, Gardiner A, Oscier DG, Stevenson FK. Unmutated Ig V(H) genes are associated with a more aggressive form of chronic lymphocytic leukemia. *Blood* 1999;94:1848-54.

THE AUTHORS REPLY: In our article, we reported the sequences of 42 microRNA genes in 75 patients with CLL and found mutations in 5 of the 42 genes in 11 patients. For this reason, we are surprised that Borkhardt et al. questioned our estimates on the basis of their failure to find mutations in the *miR-15a-miR-16-1* transcript in 69 cases of CLL. That Borkhardt et al. did not find this rare germ-line mutation, which we identified in only 2 of 75 patients, is not unexpected. Moreover, the frequency of germ-line mutations may vary in different populations,¹ and one of our patients with a germ-line mutation in *miR-15a-miR-16-1* had familial CLL. The reduction in gene expression induced by this mutation was reproduced in two independent experiments and by a quantitative reverse-transcriptase-polymerase-chain-reaction assay. The mechanism responsible for this effect remains to be identified. Borkhardt

et al. are correct that Figure 2E is mislabeled. The headings were reversed; the left portion should read “*miR-16-1*,” and the right portion should be labeled “*miR-15a*.”

Contrary to the assertion by Chng, our extended signature, associated with ZAP-70 expression (presented in the Supplementary Appendix to the article) and composed of 19 microRNAs, includes all 9 microRNAs that predict time to treatment (and not 5, as stated by Chng). We did not conclude that microRNA expression should substitute for ZAP-70 expression and *IgV_H* mutation status as a prognostic marker in cases of CLL but, rather, that it is a new prognostic marker. Whether it is an independent prognostic factor has yet to be determined. Finally, since we had no survival data for the test set of 50 samples of CLL, we restricted our analyses to cases with data on ZAP-70.

George A. Calin, M.D., Ph.D.
Amelia Cimmino, M.D., Ph.D.
Carlo M. Croce, M.D.

Ohio State University
Columbus, OH 43210
carlo.croce@osumc.edu

1. Liede A, Karlan BY, Narod SA. Cancer risks for male carriers of germline mutations in BRCA1 or BRCA2: a review of the literature. *J Clin Oncol* 2004;22:735-42.

Response of Glioblastomas to EGFR Kinase Inhibitors

TO THE EDITOR: Mellinghoff et al. (Nov. 10 issue)¹ identified coexpression of epidermal growth factor receptor (EGFR) deletion mutant variant III (EGFRvIII) and the tumor-suppressor protein PTEN as a molecular signature in pretreatment glioblastoma tissue that predicts sensitivity to EGFR inhibitors. However, several tumors that were responsive to erlotinib in the validation data set exhibited PTEN loss, and one of these did not express EGFRvIII, suggesting that alternative mechanisms of action exist. Although the effects of EGFR inhibitors on EGFR activity and signaling were analyzed in vitro, ideally tissue from patients in the training and validation sets would also be analyzed for molecular effects during therapy in order to correlate clinical with molecular outcomes. We analyzed tissue resected from patients who were undergoing erlotinib or gefitinib therapy, and we did not observe marked inhibition

of EGFR phosphorylation.² However, none of our patients responded robustly to erlotinib or gefitinib. Future studies should use a two-pronged approach in order to identify both pretreatment molecular predictors of outcome and molecular effects during treatment. To expedite clinical trials, investigators might consider biopsy after several weeks of treatment with targeted therapeutics, and they might consider withdrawing therapy if pathway inhibition is not observed.³

Andrew B. Lassman, M.D.
Lauren E. Abrey, M.D.

Memorial Sloan-Kettering Cancer Center
New York, NY 10021
lassmana@mskcc.org

Mark R. Gilbert, M.D.
University of Texas M.D. Anderson Cancer Center
Houston, TX 77030