and future requirements for therapy. The accurate classification of CLL and MBL is essential for the prospective testing of biologic markers that may, in the future, separate MBL into patients who have little likelihood of progression and those who are very likely to progress. Without the reclassification of early-stage CLL and MBL there will be continued confusion over this entity.

We strongly disagree with the final statement made by Hanson et al. At present there is confusion over the definitions of MBL and CLL, which will eventually hamper efforts to identify biologic variables that predict outcome. Inappropriately labeling individual patients with a diagnosis of “leukemia” can create major problems both psychologically and potentially financially regarding insurance coverage. In addition, clarifying the diagnosis on a population basis will allow the definition of predictors of progression and will therefore benefit patients in the future. It is true that these guidelines will “create more questions,” and that is appropriate and stimulating to further research. But at least these questions will be based on a timely consensus using the best available techniques rather than criteria established more than a decade ago when the biology of CLL was less well understood. Although any cutoff, using either lymphocyte or B-cell count, to define CLL and MBL will be somewhat arbitrary, it is important to have widely accepted definitions that are straightforward and do not overlap to bring clarity and consistency to our future studies of the disease.

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References

To the editor:

p73, miR106b, miR34a, and Itch in chronic lymphocytic leukemia

We read with interest the recent paper in Blood by Sampath et al proposing a key role for p73 in the induction of apoptosis induced by histone deacetylase inhibitors (HDACi) in chronic lymphocytic leukemia (CLL). The authors proposed that the HDAC-dependent induction of miR106b leads to translational inhibition of the Itch E3 ubiquitin ligase, and this in turn results in the up-regulation of the Itch target p73. The authors showed an inverse relationship between miR106b and Itch in CLL treated with the HDAC inhibitor, LBH589, and suggested a novel, p53-independent mechanism of CLL apoptosis, which would have important therapeutic implications.

We have previously shown that Itch is cleaved by caspases in CLL during apoptosis induced by various stimuli. Using HDACi, including LBH589 we confirmed the down-regulation of Itch in CLL expressing the mature miR106b, or with pre-miR106b (Figure 1C). In both cases we failed to detect any inhibition of Itch by miR106b. Moreover, we were not able to identify any significant increase in miR106b RNA levels in lymphocytes from 12 CLL patients treated in vitro with LBH589 (Figure 1D). Similarly, Sampath et al reported up-regulation of miR106b in only 16 patients of 47 studied. Figure 1D also shows that p73 was regulated at transcriptional level by LBH589; and not at the degradative level.

We also assessed the possibility that Itch could be a target of miR34a, expressed in CLL, as a high homology between the Itch 3’UTR and miR34a emerged from in silico analysis (Figure S1C) and miR34a was more consistently up-regulated by HDACi, even thought not reaching a strict statistical significance (Figure 1D). We did not observe any direct interaction between miR34a and Itch (Figure S1D,E).

Together, these data indicate that neither miR106b nor miR34a are involved in Itch down-regulation after exposure to HDACi, despite evidence of involvement of p73 in CLL, as suggested by Sampath et al.

To investigate further the possible interaction of miR106b and Itch, we performed luciferase activity assay on different human cell lines with 3’UTR-Itch in presence of miR106b or a scrambled sequence (Figure 1B) and we assayed the endogenous levels of Itch in different human cell lines upon transfection with a plasmid expressing the mature miR106b, or with pre-miR106b (Figure 1C). In both cases we failed to detect any inhibition of Itch by miR106b.

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Blood samples were obtained from CLL patients during routine diagnosis at the Leicester Royal Infirmary with patient consent in accordance with the Declaration of Helsinki and local ethical committee approval from the University of Leicester.

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Context-dependent actions of miR-106b in CLL

We appreciate di Val Cervo et al’s comments on our article about the role of miR-106b in repressing the ubiquitin ligase, Itch, to result in increases in the levels of p73 in CLL.1

The ability of microRNA to interact with and suppress endogenous targets likely depends on the cellular context in which it is expressed. Associations of microRNAs and their targets determined in one cell type may not predict their association in another cell type. This concept is illustrated by several examples: miR-17–19b targeted Bim in one lymphoma cell line but not another; miR-29b targeted DNMT3a/b in lung; but not in other cell types; miR26a targeted EZH2 efficiently in leukemia but minimally in embryonic kidney cells; miR29a targeted ITCH efficiently in leukemia but minimally in embryonic kidney cells.5 Our work demonstrated that ectopic expression of miR-106b in quiescent CLL cells resulted in a repression of Itch (n = 4) that was mechanistically associated with an increase in p73 levels. In contrast, over-expression of miR-106b in H1299 lung cancer cells did not decrease the levels of Itch protein (D.S., unpublished observations, June 2006). However, the levels of Itch protein were readily reduced in response to LBH589 suggesting that mechanisms unrelated to mir-106b were likely to regulate Itch in this cell line. Similarly, the findings presented in the letter show that miR-106 did not repress Itch in Saos2 osteosarcoma or HEK293T embryonic kidney cells. This result likely reflects the role of cellular context in determining miRNA and target gene associations, and may not recapitulate the actions of miR-106b in primary, quiescent CLL cells. In addition, miRNAs are likely to share a reciprocal relationship with their targets in individual cell types.6 miR-106b is over-expressed in several solid tumors and cell lines, shares a reciprocal relation with p21 and represses p21 in such cells.8 However, in CLL, miR-106b and its host gene Mcm7 were epigenetically silenced, shared a reciprocal relation with Itch, not p21, and targeted Itch, not p21, in this disease.1

We demonstrated a 1.4- to 4-fold increase in the levels of mir-106b in 12 of 19 CLL samples exposed to LBH589 (Figure 2A;
p73, miR106b, miR34a, and Itch in chronic lymphocytic leukemia

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