To the editor:

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

We read with interest the recent paper in Blood by Sampath et al1 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 p73.2 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.3 Using HDACi, including LBH589 we confirmed the down-regulation of Itch during apoptosis. However, we also demonstrated the appearance of a 64 kDa Itch cleaved band due to the action of caspase-3, -6, and -7 on residue Asp240.3 This occurred during both HDACi-induced and spontaneous in vitro apoptosis (Figure S1A, available on the Blood website; see the Supplemental Materials link at the top of the online article) of CLL cells. However, caspase inhibitors completely abrogated Itch down-regulation after HDACi treatment (please see Figures 1A and 3B in Rossi et al3 and Figure S1B). In this model, therefore, the down-regulation of Itch is a consequence, not a cause, of apoptosis, either spontaneously occurring in CLL cells in culture or triggered by LBH589.

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. 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.3 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,4 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.1

*Pia Rivetti di Val Cervo, *Paola Tucci, Anela Majid, Anna Maria Lena, Massimiliano Agostini, Sergio Bernardini, Eleonora Candi, Gerry Cohen, Pierluigi Nicotera, Martin J. S. Dyer, and Gerry Melino

*P.R.d.V.C. and P.T. contributed equally to this study.

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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Correspondence: Prof Gerry Melino or Martin J. S. Dyer, MRC-Toxicology Unit, Hodgkin Bldg, PO Box 138, University of Leicester, Lancaster Rd, Leicester, LE1 9HN United Kingdom; e-mail: gm89@le.ac.uk or mjsd1@le.ac.uk.

References
protein (D.S., unpublished observations, June 2006). However, the
in H1299 lung cancer cells did not decrease the levels of Itch
and increase in p73 levels. In contrast, over-expression of miR-106b
repression of Itch (n
expression of miR-106b in quiescent CLL cells resulted in a
result in increases in the levels of p73 in CLL.1
We demonstrated a 1.4- to 4-fold increase in the levels of Itch
in chronic lymphocytic leukemia (CLL) cells were
incubated in presence of 10 nM LBH589 for the indicated times. Levels of actin were used as a loading control. Results from a representative experiment using CLL cells
from 1 of the 12 analyzed patients are shown. There is a decrease in the 100 kDa band but with concomitant increase in 64 kDa cleaved product. See also Rossi et al.3.

![Figure 1. Regulation of Itch in chronic lymphocytic leukemia.](image)

**A** Western blotting of expression levels of protein Itch. Primary chronic lymphocytic leukemia (CLL) cells were transfected with either pcDNA plasmids harboring the mature miR106b or a scrambled sequence, or with pre-miRs for miR106b or a scrambled sequence. Levels of actin were used as a loading control. MiR106b levels were

![Image](image)

**B** Luciferase reporter assay of miR106b on 3
UTR-Itch in Saos-2 and HEK293 cells. The results were expressed as mean ± SD from 3 independent experiments analyzed in triplicate.

![Image](image)

**C** Western blotting of endogenous levels of protein Itch in Saos-2 and HEK293 cells upon transfection. Cells were transfected either with pcDNA plasmids harboring the mature miR106b or a scrambled sequence, or with pre-miRs for miR106b or a scrambled sequence. Levels of actin were used as a loading control. Results from a representative experiment using CLL cells

![Image](image)

**D** Expression levels of TAp73, DNp73, miR34a, and miR106b in primary CLL cells exposed for the indicated times to 10 nM LBH589 as analyzed by qRT-PCR. Expression levels relative to the untreated sample at the corresponding time points are shown. Expression of TAp73 and Dn p73 was normalized to actin; expression of miR34a and miR106b was normalized to RNU6b. Statistical differences were determined by one-way analysis of variance (ANOVA) followed by Dunnett multiple comparison test, and the results were expressed as mean ± SEM from 12 independent CLL samples analyzed in triplicate.

**References**


**Response**

**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 endoge-

uous 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: miRs-17–19b targeted Bim in one lymphoma cell line but not another;2 miR-29b targeted DNMT3a/b in lung3 but not in other cell types;4 miR26a targeted EZH2 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 microRNA and target gene associations, and may not recapitulate the actions of miR-106b in primary, quiescent CLL cells. In addition, microRNAs 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 p21,7,8 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 2A1;
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