

Ilka Warshawsky

Department of Clinical Pathology, Cleveland Clinic Foundation,
Cleveland, OH

Frank Mularo

Department of Clinical Pathology, Cleveland Clinic Foundation,
Cleveland, OH

Carol Hren

Department of Clinical Pathology, Cleveland Clinic Foundation,
Cleveland, OH

Maureen Jakubowski

Department of Clinical Pathology, Cleveland Clinic Foundation,
Cleveland, OH

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Correspondence: Ilka Warshawsky, Cleveland Clinic Foundation, Dept of Clinical Pathology – L30, 9500 Euclid Ave, Cleveland, OH 44195; e-mail: warshai@ccf.org.

References

- Tefferi A, Thiele J, Orazi A, et al. Proposals and rationale for revision of the World Health Organization diagnostic criteria for polycythemia vera, essential thrombocythemia, and primary myelofibrosis: recommendations from an ad hoc international expert panel. *Blood*. 2007;110(4):1092-1097.
- IPSOGEN licenses JAK2 mutation test to improve the diagnosis of blood-based disorders. March 30, 2006; <http://www.ipsogen.com/corporate/cancer-profiler/ipsogen-news/ipsogen-news/browse/4/article/140/ipsogen-licenses-jak2-mutation-test-to-improve-the-diagnosis-of-blood-based-disorders/>. Accessed November 20, 2009.
- Murugesan G, Aboudola S, Szpurka H, et al. Identification of the JAK2 V617F mutation in chronic myeloproliferative disorders using FRET probes and melting curve analysis. *Am J Clin Pathol*. 2006;125(4):625-633.
- Xie X, Kottke-Marchant K, Lichtin A, et al. Identification of two novel mutations besides JAK2 V617F in a patient with polycythemia vera [abstract]. *J Mol Diagn*. 2007;9:H37.
- Grünebach F, Bross-Bach U, Kanz L, Brossart P. Detection of a new JAK2 D620E mutation in addition to V617F in a patient with polycythemia vera. *Leukemia*. 2006;20(12):2210-2211.
- Zhang SJ, Li JY, Li WD, et al. The investigation of JAK2 mutation in Chinese myeloproliferative diseases-identification of a novel C616Y point mutation in a PV patient. *Int J Lab Hematol*. 2007;29(1):71-72.
- Yoo JH, Park TS, Maeng HY, et al. JAK2 V617F/C618R mutation in a patient with polycythemia vera: a case study and review of the literature. *Cancer Genet Cytogenet*. 2009;189(1):43-47.
- Ma W, Kantarjian H, Zhang X, et al. Mutation profile of JAK2 transcripts in patients with chronic myeloproliferative neoplasias. *J Mol Diagn*. 2009;11(1):49-53.
- American Civil Liberties Union. ACLU Challenges Patents On Breast Cancer Genes: BRCA. February 17, 2010; http://www.aclu.org/free-speech_womens-rights/aclu-challenges-patents-breast-cancer-genes. Accessed March 16, 2010.
- Walsh T, Casadei S, Hale Coats K, et al. Spectrum of mutations in BRCA1, BRCA2, CHEK2, and TP53 in families at high risk of breast cancer. *JAMA*. 2006;295(12):1379-1388.

To the editor:

The role of CBF β in AML1-ETO's activity

Identification of interacting proteins essential for oncogenic functions of leukemia-associated transcription factors is important for understanding the underlying transformation mechanisms and designing effective cancer therapeutics.¹ We have recently found that homo-oligomerization property, but not its interaction with core-binding factor beta subunit (CBF β), is critical for AML1-ETO-mediated transformation of primary hematopoietic cells.² Strikingly, the conclusion on CBF β requirement contradicts another study by Roudaia et al, which reported an essential function of CBF β interaction for AML1-ETO activity based on an AML1-ETO double-point mutant (Y113A/T161A).³

One possible explanation for these discrepancies is the use of different point mutants in these studies.⁴ To this end, we have generated an identical AML1-ETO Y113A/T161A mutation used in the study of Roudaia et al and compared it with our M106V point mutant in the transformation assay. In contrast to cells transduced with empty vector or the AML1-ETO DNA binding mutant that rapidly lost their proliferative capacity, cells transduced with the CBF β defective mutants including Y113A/T161A could still form significant numbers of third and subsequent rounds of colonies in the serial replating assay (Figure 1A). Despite reduced number (an average of 12 different experiments), the resultant colonies exhibited very similar morphology and immunophenotypes as wild-type AML1-ETO transformed cells (Figure 1B-C and supplemental Figure 1, available on the *Blood* website; see the Supplemental Materials link at the top of the online article). This is in stark contrast to the results by Roudaia et al, where Y113A/T161A mutant when

transduced into 5'FU-treated bone marrow cells failed to give third-round colonies. It is possible that 5'FU treatment may have depleted certain AML1-ETO target cells that are responsible for the observed phenotype in the assay using positively selected c-kit cells.⁵ We also note that the transformation data in Roudaia and colleagues' study had not been normalized with the number of plated cells; that is, colony number in the second and third platings were derived from 10 times more cells (10^4) compared with the first plating (10^3).³ If presented as normalized data, the results would look quite significantly different, and the difference between the wild-type and Y113A/T161A mutant would be more modest.

In addition, one must be cautious when interpreting mutagenesis data, as it is almost impossible to engineer absolutely specific mutations that will affect only a single property of the mutated protein. Thus it is critical to have an alternative approach targeting CBF β expression without altering the structure of AML1-ETO. Consistent with our point mutant data, we further demonstrated that 2 independent shRNAs that effectively knocked down more than 95% CBF β expression at protein level in primary hematopoietic cells did not compromise AML1-ETO-mediated transformation.² Together, these results indicate that a significant reduction of CBF β activity has only modest effect on AML1-ETO-mediated transformation. However, a decisive experiment to determine whether there is an absolute CBF β dependence is to assess the behavior of AML1-ETO in a complete absence of CBF β using genetic approaches such as conditional knockout mice that will be instrumental to this issue.

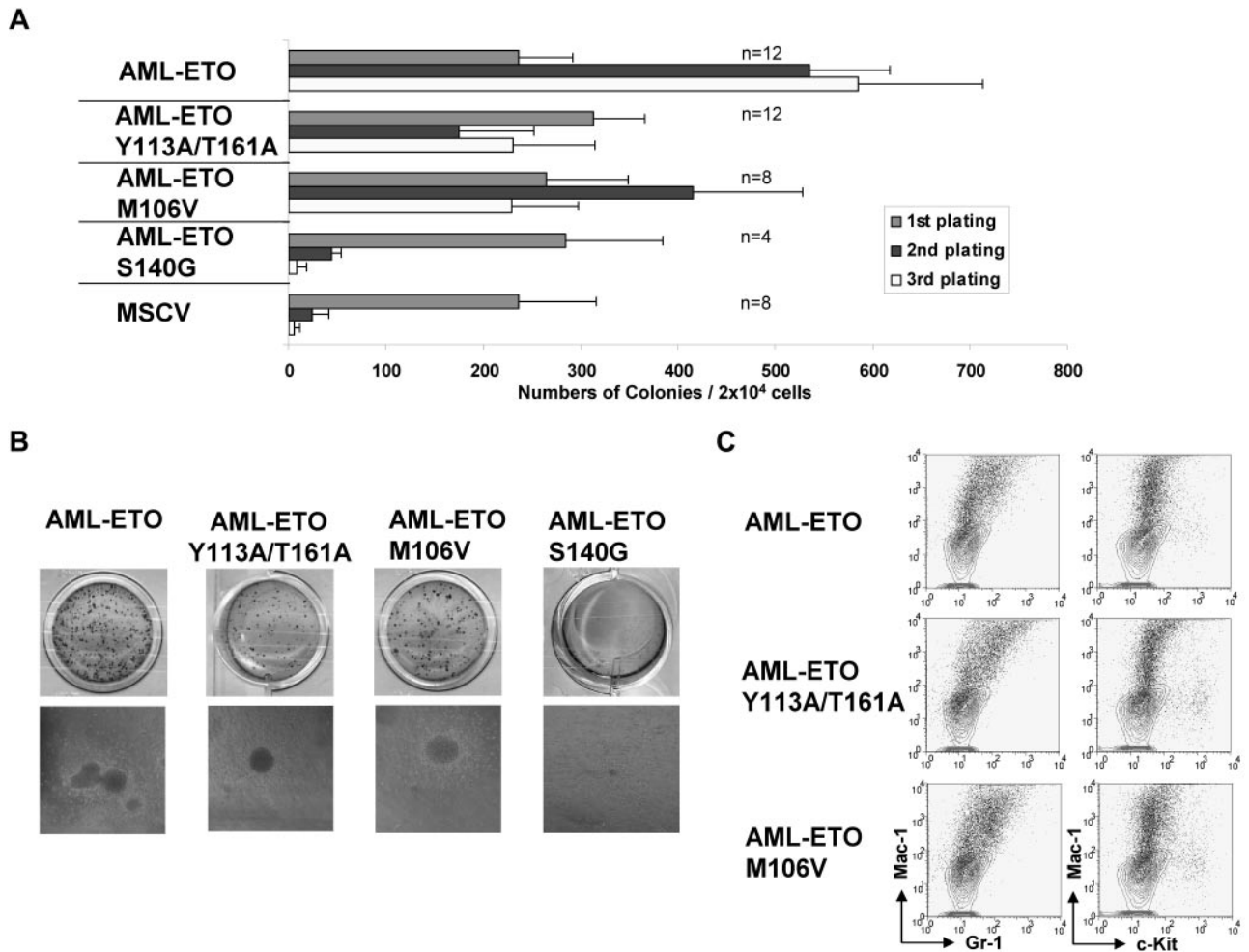


Figure 1. Analysis of transformation ability of various AML1-ETO point mutants in murine primary hematopoietic cells. (A) AML1-ETO point mutants used in the retroviral transduction/transformation assay are indicated on the left. The bar chart (right) represents the numbers of colonies after each plating in methylcellulose. Error bars show standard deviations of indicated numbers (n) of independent experiments. (B) Typical third-round colony morphology of primary transduced bone marrow cells transduced with indicated constructs. The top panel shows colonies stained with INT and the bottom panel shows unstained colonies ($\times 10$ magnification). (C) Phenotypic analysis of cells transformed by indicated constructs. Dot plots represent stainings obtained with antibodies specific for the indicated surface markers. Contour plots show unstained controls.

Colin Kwok
 Hemato-Oncology Section, The Institute of Cancer Research, Sutton; and
 Leukaemia and Stem Cell Biology Group, Department of Haematological Medicine,
 King's College London, London, United Kingdom

Bernd B. Zeisig
 Hemato-Oncology Section, The Institute of Cancer Research, Sutton; and
 Leukaemia and Stem Cell Biology Group, Department of Haematological Medicine,
 King's College London, London, United Kingdom

Shuo Dong
 Department of Medicine, Baylor College of Medicine,
 Houston, TX

Chi Wai Eric So
 Hemato-Oncology Section, The Institute of Cancer Research, Sutton; and
 Leukaemia and Stem Cell Biology Group, Department of Haematological Medicine,
 King's College London, London, United Kingdom

C.K. and B.B.Z. contributed equally to this article.

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Correspondence: Prof Eric So, King's College London, Rayne Institute, 123 Coldharbour La, Denmark Hill, London SE5 9NU, United Kingdom; e-mail: eric.so@kcl.ac.uk.

References

- So CW. An overview: from discovery of candidate mutations to disease modeling and transformation mechanisms of acute leukemia. *Methods Mol Biol.* 2009;538:1-5.
- Kwok C, Zeisig BB, Qiu J, Dong S, So CW. Transforming activity of AML1-ETO is independent of CBFbeta and ETO interaction but requires formation of homo-oligomeric complexes. *Proc Natl Acad Sci U S A.* 2009;106(8):2853-2858.
- Roudaia L, Cheney MD, Manuylova E, et al. CBFbeta is critical for AML1-ETO and TEL-AML1 activity. *Blood.* 2009;113(13):3070-3079.
- Park S, Speck NA, Bushweller JH. The role of CBFbeta in AML1-ETO's activity. *Blood.* 2009;114(13):2849-2850.
- Zeisig BB, So CW. Retroviral/lentiviral transduction and transformation assay. *Methods Mol Biol.* 2009;538:207-229.