

Washington University School of Medicine

Digital Commons@Becker

---

Open Access Publications

---

2005

## Dominant negative effects of the AML1/ETO fusion oncoprotein

Timothy S. Fenske

*Washington University School of Medicine in St. Louis*

Gina Pengue

*Washington University School of Medicine in St. Louis*

Timothy A. Graubert

*Washington University School of Medicine in St. Louis*

Follow this and additional works at: [https://digitalcommons.wustl.edu/open\\_access\\_pubs](https://digitalcommons.wustl.edu/open_access_pubs)

Please let us know how this document benefits you.

---

### Recommended Citation

Fenske, Timothy S.; Pengue, Gina; and Graubert, Timothy A., "Dominant negative effects of the AML1/ETO fusion oncoprotein." *Cell Cycle*. 4, 1. 33-36. (2005).

[https://digitalcommons.wustl.edu/open\\_access\\_pubs/3036](https://digitalcommons.wustl.edu/open_access_pubs/3036)

This Open Access Publication is brought to you for free and open access by Digital Commons@Becker. It has been accepted for inclusion in Open Access Publications by an authorized administrator of Digital Commons@Becker. For more information, please contact [vanam@wustl.edu](mailto:vanam@wustl.edu).

## Extra Views

# Dominant Negative Effects of the AML1/ETO Fusion Oncoprotein

Timothy S. Fenske

Gina Pengue

Timothy A. Graubert\*

Department of Internal Medicine; Division of Oncology; Stem Cell Biology Section; Washington University; St. Louis, Missouri USA

\*Correspondence to: Timothy A. Graubert, M.D.; Washington University School of Medicine; Division of Oncology, Stem Cell Biology Section; Campus Box 8007; 660 South Euclid Avenue; St. Louis, Missouri 63110 USA; Tel.: 314/747-4437; Fax: 314/362-9333; Email: graubert@medicine.wustl.edu

Received 11/18/04; Accepted 11/19/04

Previously published online as a *Cell Cycle* E-publication:  
<http://www.landesbioscience.com/journals/cc/abstract.php?id=1399>

## KEY WORDS

AML1/ETO, Runx, Sca1, hematopoietic stem cell

## ABBREVIATIONS

AE	AML1/ETO
AML	acute myeloid leukemia
CBF	core binding factor
GFP	green fluorescent protein
HSC	hematopoietic stem cell

## ACKNOWLEDGEMENTS

This work was supported by a grant from the G&P Foundation (T.A.G.).

Cell sorting was performed by the NCI-funded Siteman Cancer Center High Speed Cell Sorting core facility.

## ABSTRACT

The t(8;21)(q22;q22) translocation, present in 10–15% of acute myeloid leukemia (AML) cases results in the production of the AML1/ETO fusion protein. Expression of AML1/ETO in patients or mouse models is not sufficient to induce AML. Despite convincing evidence that AML1/ETO is directly involved in the pathogenesis of AML, the underlying mechanism is not well understood. Genetic and biochemical experiments suggest that AML1/ETO is a dominant inhibitor of the core binding factor (CBF) transcription complex that includes AML1 (RUNX1), the N-terminal fusion partner in the t(8;21). We generated and recently characterized a novel strain of transgenic mice in which the AML1/ETO cDNA was inserted into the *Ly-6A* gene that encodes Sca1, a well-characterized marker of murine hematopoietic stem cells. Unexpectedly, transgene expression assessed by flow cytometry was significantly lower than predicted in lymphocytes from these mice. We have confirmed this finding at the mRNA level and suggest that this phenotype is a consequence of dominant inhibition of transgene expression by AML1/ETO. The dominant negative characteristics of AML1/ETO may be important for AML pathogenesis and may provide a molecular target for therapeutic intervention.

## TARGETING THE SCA1 LOCUS TO MODEL t(8;21) AML IN MICE

Acute myelogenous leukemia (AML) accounts for 35% of adult leukemia cases.<sup>1</sup> A distinct subset of these leukemias is characterized by the (8;21) translocation.<sup>2</sup> The t(8;21) fuses the N-terminal 177 amino acids of the transcription factor AML1 (RUNX1) in frame with all but the first 30 amino acids of ETO.<sup>3</sup> AML1 is the DNA-binding subunit of core binding factor (CBF), a multimeric transcription factor complex that includes CBF $\beta$  and additional transcriptional coactivators. The AML1/ETO fusion protein retains the Runt homology domain of AML1 (responsible for DNA binding and interaction with CBF $\beta$ ) and lacks the C-terminal transactivation domain.<sup>4</sup> The role of AML1/ETO in the pathogenesis of AML is not yet well understood.

The t(8;21) is detectable years prior to the onset of AML and can persist for years in remission bone marrow samples.<sup>5–7</sup> Similarly, several independently generated murine transgenic strains in which AML1/ETO is expressed in adult bone marrow cells fail to develop spontaneous leukemias.<sup>8–12</sup> Taken together, these observations suggest that AML1/ETO is not sufficient to induce AML. The t(8;21) is likely an early event in the pathogenesis of AML. AML1/ETO appears to act on primitive stem/progenitor cells, promoting self-renewal over differentiation.<sup>10,13</sup> This may contribute to leukemogenesis by increasing the pool of cells available to acquire additional mutations.

The leukemia-initiating cell in t(8;21) AML has a primitive stem/progenitor phenotype.<sup>14,15</sup> We, therefore, hypothesized that targeting AML1/ETO expression to HSC might be important for experimental models of this disease. We chose to utilize the *Ly-6A* (*Sca1*) locus because we demonstrated previously that this strategy yields high-level transgene expression in the hematopoietic stem/progenitor compartment in adult mice.<sup>16</sup>

In contrast to germline *Aml1*<sup>+/AE</sup> mice, *Sca*<sup>+/AE</sup> mice are viable and develop normally.<sup>17</sup> Hematopoiesis is normal at baseline, apart from a modest elevation of the white blood cell count and a decrease in the frequency of myeloid progenitors. After a long latency, more than 80% of the mice develop a myeloproliferative disorder characterized by elevation of the neutrophil count, bone marrow myeloid hyperplasia, splenomegaly, and extramedullary hematopoiesis.<sup>17</sup> As noted with previous AML1/ETO mouse models, these animals do not develop AML spontaneously. Nevertheless, this model should provide a useful platform to study the biology of AML1/ETO and to evaluate the potential of additional mutations to cooperate with AML1/ETO in the induction of AML.

Inclusion of an *ires* EGFP reporter cassette in the targeting vector allowed us to monitor transgene expression in *Sca<sup>+/AE</sup>* mutant mice by flow cytometry using GFP as a surrogate for AML1/ETO. Identically targeted *Sca<sup>+/GFP</sup>* mice controlled for GFP expression and integration effects at the *Sca1* locus.<sup>16</sup> The transgene is highly expressed in mature myeloid cells from both strains.<sup>17</sup> Unexpectedly, transgene expression is significantly lower in B cells, immature thymocytes, and peripheral CD4<sup>+</sup> T cells in *Sca<sup>+/AE</sup>* mice compared to *Sca<sup>+/GFP</sup>* mice.<sup>17</sup>

## ROLE OF AML1 IN HEMATOPOIETIC DEVELOPMENT

Aml1 is expressed in functional HSCs<sup>18</sup> and is required for specification of adult definitive hematopoiesis.<sup>19,20</sup> In contrast to this early embryonic requirement, Aml1 is not necessary for maintenance of HSC in the adult mouse.<sup>21</sup> Interestingly, *Aml1* haploinsufficiency leads to a 50% reduction in long-term repopulating HSCs (LTR-HSCs), with a concomitant increase in multilineage committed progenitors, suggesting that this compartment is highly sensitive to *Aml1* gene dosage.<sup>22</sup> Conditional *Aml1* deletion revealed an absolute requirement for CBF function in normal lymphocyte and platelet development.<sup>21</sup> In contrast, Aml1 is apparently dispensable for myelomonocytic differentiation<sup>21</sup> despite high level expression in these lineages.<sup>23</sup> Overexpression of dominant Runx inhibitors can impair granulocytic differentiation,<sup>24-26</sup> raising the possibility that other Runx family members (Aml2 or Aml3) may compensate for the absence of Aml1 in the myeloid lineage.

Given the body of evidence indicating an important role for Aml1 in lymphocyte development, we hypothesized that the unexpectedly low level transgene expression we detected in lymphocytes from *Sca<sup>+/AE</sup>* mice might result from deleterious effects of AML1/ETO on hematopoietic lineages in which Runx factors are essential. Since our previous analysis relied on a reporter gene, we decided to measure AML1/ETO mRNA expression directly in purified cells from *Sca<sup>+/AE</sup>* mice using real-time quantitative RT-PCR. We found that AML1/ETO mRNA is expressed at relatively high levels in neutrophils, and at reduced levels in lymphocytes (Fig. 1A). This reduction in AML1/ETO mRNA is particularly apparent in B cells. These data mirror our flow cytometric analysis.<sup>17</sup> Since *Sca1* protein expression assessed by flow cytometry is not proportionately reduced in these cells (not shown), these results suggested that the *AML1/ETO* mutant allele might be selectively downregulated. To test this hypothesis, we analyzed *Sca1* mRNA expression by quantitative RT-PCR. In contrast to the results from flow cytometric analysis, we found that *Sca1* mRNA expression is significantly decreased in whole bone marrow, B cells, and T cells in *Sca<sup>+/AE</sup>* mice compared to *Sca<sup>+/GFP</sup>* mice (Fig. 1B). These results suggest that AML1/ETO is acting in *trans* to reduce expression of both the wildtype and targeted *Sca1* alleles.

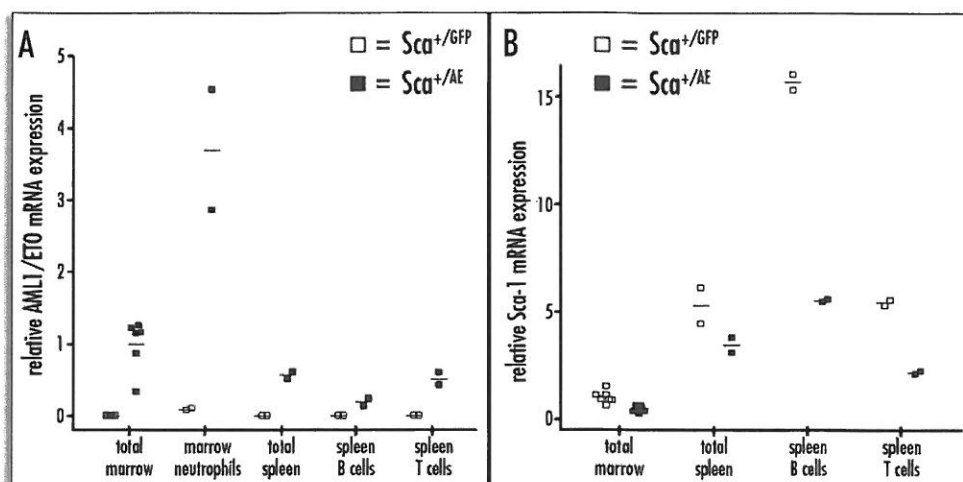


Figure 1. Real-time PCR analysis of gene expression. (A) AML1/ETO mRNA expression in cells from *Sca<sup>+/AE</sup>* mice (filled symbols) is shown. Expression is significantly higher in neutrophils compared to T and B cells. No background signal is detectable using mRNA from *Sca<sup>+/GFP</sup>* cells (open symbols). Each point represents mRNA expression in a sample from an individual animal measured in triplicate and averaged. (B) *Sca1* expression is shown for the indicated cellular populations. *Sca1* expression is significantly lower in bone marrow, B cells, and T cells from *Sca<sup>+/AE</sup>* mice compared to *Sca<sup>+/GFP</sup>* mice ( $p < 0.005$ ).

## AML1/ETO IS A DOMINANT INHIBITOR OF RUNX FUNCTION

Mice heterozygous for AML1/ETO targeted to the *Aml1* locus are phenotypically identical to Aml1 and Cbfb $\beta$  deficient mice,<sup>19,20,27,28</sup> providing genetic evidence that AML1/ETO is a dominant inhibitor of CBF function.<sup>29,30</sup> Direct biochemical evidence for a dominant negative effect of AML1/ETO came from cotransfection experiments showing that AML1/ETO can block AML1-dependent transcriptional activation,<sup>4</sup> and that this property of AML1/ETO is dependent on the C-terminus of ETO.<sup>31</sup> AML1/ETO recruits histone deacetylases and the corepressors mSin3A and N-CoR, providing the basis for the prevailing model of dominant inhibition in which the CBF transcriptional activator is converted into a repressor.<sup>32-35</sup> Tetramerization of AML1/ETO increases its affinity for DNA, thereby conferring a competitive advantage over the wildtype AML1 protein.<sup>36</sup> In an alternate model, changes in the pattern of transcription caused by AML1/ETO result from misdirection of the fusion protein to transcriptionally inactive subnuclear domains.<sup>37</sup>

We identified three consensus Runx sites in the 5' flanking region of the *Ly-6A* (*Sca1*) gene, located 0.8, 1.0 and 1.5 kb upstream of exon 1. We propose that *Sca1* transcriptional activity may be regulated by CBF, and that this provides a potential mechanism to explain the observed decrease in *Sca1* mRNA in *Sca<sup>+/AE</sup>* mice compared with *Sca<sup>+/GFP</sup>* mice. In this model, AML1/ETO represses transcription from both the wildtype and mutant *Sca1* alleles in *Sca<sup>+/AE</sup>* mice (Fig. 2).

## CONCLUSIONS AND IMPLICATIONS

*Sca1* transcriptional activity is strongly activated by types I and II interferon.<sup>38</sup> This may be an adaptive response to cytokines produced during an immune response, since *Sca1* is required for normal regulation of antigen-induced T cell proliferation.<sup>39</sup> We have shown that the targeted allele remains interferon-responsive in *Sca1* mutant mice.<sup>16</sup> The data presented here imply that *Sca1* may also be a Runx-regulated



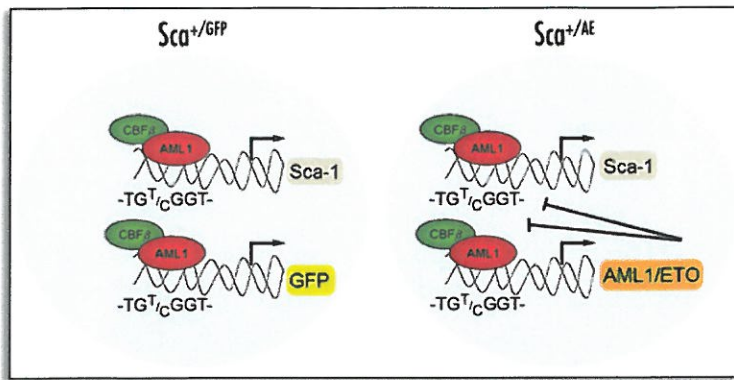


Figure 2. Model of dominant inhibition of *Sca1* by AML1/ETO. The wildtype and targeted *Sca1* alleles in heterozygous *Sca*<sup>+/GFP</sup> (left) and *Sca*<sup>+/AE</sup> (right) cells are depicted. There are three consensus Runx binding sites in the proximal *Sca1* promoter. Expression of GFP in *Sca*<sup>+/GFP</sup> mice does not affect transcription of either the wildtype or mutant alleles. In the proposed model, AML1/ETO expression in *Sca*<sup>+/AE</sup> mice represses transcription from both the wildtype and mutant alleles.

gene. Formal proof would require direct demonstration that Runx factors bind the *Sca1* promoter (e.g., by chromatin immunoprecipitation) and alter transcriptional activity (e.g., by promoter assays). An additional implication of these data is that the *Sca*<sup>+/AE</sup> mutation amounts to a *Sca1* knockdown (through the combined effects of haploinsufficiency and dominant inhibition of the remaining wild-type allele). This introduces a potential caveat in the interpretation of hematopoietic phenotypes observed using the *Sca*<sup>+/AE</sup> mice. We and others have found that resting hematopoiesis is intact in *Sca1* null mice (Ito et al.<sup>40</sup> and T.A.G., unpublished results). However, serial transplantation revealed a potential role for *Sca1* in hematopoietic stem cell self-renewal,<sup>40</sup> raising the possibility that AML1/ETO could have pleiotropic effects on this compartment in *Sca*<sup>+/AE</sup> mice.

The dominant negative effects of AML1/ETO are most evident in lymphocytes because Runx factors are specifically required for the development of these lineages. In conditional *Aml1*<sup>+/AE</sup> mice, AML1/ETO-expressing lymphocytes are selectively lost.<sup>8</sup> Since *Sca*<sup>+/AE</sup> mice are not mosaic, all lymphocytes contain the transgene and compensatory mechanisms must select for low AML1/ETO-expressing cells. Although transgene expression is lower in lymphocytes from *Sca*<sup>+/AE</sup> mice, we have shown that their T and B subsets are present in normal numbers.<sup>17</sup> None of the AML1/ETO mouse models are overtly immune deficient. It remains formally possible that subtle immune defect exist in patients and mice expressing AML1/ETO, although this has not been reported.

The germline *Aml1*<sup>+/AE</sup> mutation is embryonic lethal, apparently because Runx activity is lowered below a critical threshold through the combined effects of *Aml1* haploinsufficiency and dominant inhibition by AML1/ETO. Using *Sca1* as a reporter gene, we found that AML1/ETO represses Runx activity between 54% (bone marrow) and 65% (B cells) in *Sca*<sup>+/AE</sup> mice (Fig. 1B). Since these mice are wildtype at the *Aml1* locus, this may explain in part why this is not an embryonic lethal mutation. Although our model captures the dominant negative effects of AML1/ETO, to fully model the milieu in t(8;21) leukemic cells, it may be necessary to lower Aml1 (and perhaps Eto) activity further.

Although AML1/ETO expression is not sufficient to induce AML, continued expression may be required for persistence of leukemia. Even in genetically complex tumors, inactivation of a critical

oncogene can cause regression by inducing proliferation arrest, differentiation, or apoptosis.<sup>41</sup> Therefore, AML1/ETO may provide a relevant target for novel AML therapeutics. Proposed strategies include redirecting transcriptional repression mediated by AML1/ETO,<sup>42</sup> inhibition of histone deacetylases recruited by the fusion protein,<sup>43</sup> or targeting the AML1/ETO fusion directly via RNA interference.<sup>44</sup>

## MATERIALS AND METHODS

**Cell sorting.** Bone marrow cells and splenocytes were harvested from 6–10 week old *Sca*<sup>+/GFP</sup> and *Sca*<sup>+/AE</sup> mice. After red cell lysis in hypotonic buffer (150 mM NH<sub>4</sub>Cl, 100 mM KHCO<sub>3</sub>, 0.1 mM EDTA), cells were washed and resuspended in FACS buffer (PBS with 0.2% BSA and 0.01% sodium azide). Cells were counted and viability assessed by trypan blue exclusion. Bone marrow cells and splenocytes were stained with directly conjugated anti-B220, CD3, CD11b, or Gr-1 antibodies (eBioscience). Approximately 2–3 × 10<sup>6</sup> B220 + CD3<sup>−</sup> (B cells), 2–3 × 10<sup>6</sup> B220 + CD3<sup>+</sup> (T cells), and 4–6 × 10<sup>6</sup> CD11b + Gr-1<sup>+</sup> (neutrophils) were sorted using a MoFlo cell sorter (DAKO Cytomation). Post-sort analysis confirmed high purity (>99%) and viability (>90%).

**RNA preparation and cDNA synthesis.** Total RNA was isolated from freshly sorted cells using the High Pure RNA Isolation kit (Roche). RNA was quantitated using either the Ribogreen reagent (Molecular Probes), or a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies). Random hexamer-primed cDNA was synthesized using AMV reverse transcriptase (Promega).

**Real-time PCR.** Mouse *GAPDH*, human *AML1/ETO*, and *Sca1* cDNAs were amplified and quantitated using dual-labeled fluorescent internal probes. Between 1–10% of the cDNA preparation was added to a 25  $\mu$ L reaction containing 1X JumpStart Taq ReadyMix (Sigma), 200 nM each primer, 280 nM probe and 1X ROX reference dye (Sigma). Thermal cycling and data collection was performed using a GeneAmp 5700 system (Applied Biosystems). Thermal cycling was as follows: 50°C for 2 minutes, 95°C for 10 minutes, followed by 40 cycles of 95°C denaturation (15 seconds) and 60°C annealing/extension (60 seconds). The primer and probe sequences used were as follows: *hAML1/ETO* forward primer 5'-AATCACAGTG-GATGGGCCCC, *hAML1/ETO* reverse primer 5'-TGCGTCTTCACATC-CACAGG, *hAML1/ETO* probe 5'-6FAM-CTGAGAAGCACTCCACAA-TGCCAGACT-MGBNFQ, *mGAPDH* forward primer 5'-TGCACCAC-CAACTGCTTAG, *mGAPDH* reverse primer 5'-GGATGCAGGGATGA-TGTTC, *mGAPDH* probe 5'-HEX-CAGAAGACTGTGGATGGCCC-CTC-TAMRA, *Sca1* forward primer 5'-TGGAGATCCTGGGTACTAAGG, *Sca1* reverse primer 5'-AGCTCAGGCTGAACAGAAGCA, and *Sca1* probe 5'-6FAM-CACCTGGACCATGGC-MGBNFQ. The *Sca1* primer set is specific for this gene since no signal was obtained using cDNAs from the other highly homologous Ly-6 family members Ly-6C, Ly-6G or Ly-6M (data not shown). Serial dilutions of calibrator cDNA samples were included in each experiment to allow for quantitation using the relative standard curve method. To correct for variability in mRNA content, mRNA quality, and efficiency of cDNA synthesis, relative GAPDH expression was measured for each sample. The relative mRNA levels for AML1/ETO and *Sca1* were then corrected by dividing by the relative GAPDH expression for each sample. Differences between the means were assessed using a 2-tailed t-test.

## References

- Xie Y, Davies SM, Xiang Y, Robison LL, Ross JA. Trends in leukemia incidence and survival in the United States (1973–1998). *Cancer* 2003; 97:2229–35.
- Rowley JD. Identification of a translocation with quinacrine fluorescence in a patient with acute leukemia. *Ann Genet* 1973; 16:109–12.
- Miyoshi H, Kozu T, Shimizu K, Enomoto K, Maseki N, Kaneko Y, Kamada N, Ohki M. The t(8;21) translocation in acute myeloid leukemia results in production of an AML1-MTG8 fusion transcript. *EMBO J* 1993; 12:2715–21.
- Meyers S, Lenny N, Hiebert SW. The t(8;21) fusion protein interferes with AML1-1B-dependent transcriptional activation. *Mol Cell Biol* 1995; 15:1974–82.

5. Wiemels JL, Xiao Z, Buffler PA, Maia AT, Ma X, Dicks BM, Smith MT, Zhang L, Feusner J, Wiencke J, Pritchard-Jones K, Kempinski H, Greaves M. In utero origin of t(8;21) AML1-ETO translocations in childhood acute myeloid leukemia. *Blood* 2002; 99:3801-5.
6. Kusec R, Laczika K, Knobl P, Friedl J, Greinix H, Kahls P, Linkesch W, Schwarzenberger I, Mitterbauer G, Purtscher B, et al. AML1/ETO fusion mRNA can be detected in remission blood samples of all patients with t(8;21) acute myeloid leukemia after chemotherapy or autologous bone marrow transplantation. *Leukemia* 1994; 8:735-9.
7. Nucifora G, Larson RA, Rowley JD. Persistence of the 8;21 translocation in patients with acute myeloid leukemia type M2 in long-term remission. *Blood* 1993; 82:712-5.
8. Higuchi M, O'Brien D, Kumaravelu P, Lenny N, Yeoh EJ, Downing JR. Expression of a conditional AML1-ETO oncogene bypasses embryonic lethality and establishes a murine model of human t(8;21) acute myeloid leukemia. *Cancer Cell* 2002; 1:63-74.
9. Yuan Y, Zhou L, Miyamoto T, Iwasaki H, Harakawa N, Hetherington CJ, Burel SA, Lagasse E, Weissman IL, Akashi K, Zhang DE. AML1-ETO expression is directly involved in the development of acute myeloid leukemia in the presence of additional mutations. *Proc Natl Acad Sci USA* 2001; 98:10398-403.
10. de Guzman CG, Warren AJ, Zhang Z, Gartland L, Erickson P, Drabkin H, Hiebert SW, Klug CA. Hematopoietic stem cell expansion and distinct myeloid developmental abnormalities in a murine model of the AML1-ETO translocation. *Mol Cell Biol* 2002; 22:5506-17.
11. Rhodes KL, Hetherington CJ, Harakawa N, Yergeau DA, Zhou L, Liu LQ, Little MT, Tenen DG, Zhang DE. Analysis of the role of AML1-ETO in leukemogenesis, using an inducible transgenic mouse model. *Blood* 2000; 96:2108-15.
12. Schwiager M, Lohler J, Friel J, Scheller M, Horak I, Stocking C. AML1-ETO inhibits maturation of multiple lymphohematopoietic lineages and induces myeloblast transformation in synergy with ICSP deficiency. *J Exp Med* 2002; 196:1227-40.
13. Mulloy JC, Cammenga J, Berguido FJ, Wu K, Zhou P, Comenzo RL, Jhanwar S, Moore MA, Nimer SD. Maintaining the self-renewal and differentiation potential of human CD34+ hematopoietic cells using a single genetic element. *Blood* 2003; 102:4369-76.
14. Bonnet D, Dick JE. Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. *Nat Med* 1997; 3:730-7.
15. Miyamoto T, Weissman IL, Akashi K. AML1/ETO-expressing nonleukemic stem cells in acute myelogenous leukemia with 8;21 chromosomal translocation. *Proc Natl Acad Sci USA* 2000; 97:7521-6.
16. Hanson P, Mathews V, Marrus SH, Graubert TA. Enhanced green fluorescent protein targeted to the Sca-1 (Ly-6A) locus in transgenic mice results in efficient marking of hematopoietic stem cells in vivo. *Exp Hematol* 2003; 31:159-67.
17. Fenske TS, Pengue G, Mathews V, Hanson PT, Hamm SE, Riaz N, Graubert TA. Stem cell expression of the AML1/ETO fusion protein induces a myeloproliferative disorder in mice. *Proc Natl Acad Sci USA* 2004; 101:15184-9.
18. North TE, Stacy T, Matheny CJ, Speck NA, de Bruijn MF. Runx1 is expressed in adult mouse hematopoietic stem cells and differentiating myeloid and lymphoid cells, but not in maturing erythroid cells. *Stem Cells* 2004; 22:158-68.
19. Okuda T, van Deursen J, Hiebert SW, Grosfeld G, Downing JR. AML1, the target of multiple chromosomal translocations in human leukemia, is essential for normal fetal liver hematopoiesis. *Cell* 1996; 84:321-30.
20. Wang Q, Stacy T, Binder M, Marin-Padilla M, Sharpe AH, Speck NA. Disruption of the *Cbfa2* gene causes necrosis and hemorrhaging in the central nervous system and blocks definitive hematopoiesis. *Proc Natl Acad Sci USA* 1996; 93:3444-9.
21. Ichikawa M, Asai T, Saito T, Yamamoto G, Seo S, Yamazaki I, Yamagata T, Mitani K, Chiba S, Hirai H, Ogawa S, Kurokawa M. AML-1 is required for megakaryocytic maturation and lymphocytic differentiation, but not for maintenance of hematopoietic stem cells in adult hematopoiesis. *Nat Med* 2004; 10:299-304.
22. Sun W, Downing JR. Haploinsufficiency of AML1 results in a decrease in the number of LTR-HSCs while simultaneously inducing an increase in more mature progenitors. *Blood* 2004; 104:3565-72.
23. Lorschach RB, Moore J, Ang SO, Sun W, Lenny N, Downing JR. Role of RUNX1 in adult hematopoiesis: Analysis of RUNX1-IRES-GFP knock-in mice reveals differential lineage expression. *Blood* 2004; 103:2522-9.
24. Castilla LH, Garrett L, Adya N, Orlic D, Dutra A, Anderson S, Owens J, Eckhaus M, Bodine D, Liu PP. The fusion gene *Cbfb-MYH11* blocks myeloid differentiation and predisposes mice to acute myelomonocytic leukaemia. *Nat Genet* 1999; 23:144-6.
25. Kohzaki H, Ito K, Huang G, Wice HJ, Murakami Y, Ito Y. Block of granulocytic differentiation of 32Dcl3 cells by AML1/ETO(MTG8) but not by highly expressed Bcl-2. *Oncogene* 1999; 18:4055-62.
26. Burel SA, Harakawa N, Zhou L, Pabst T, Tenen DG, Zhang DE. Dichotomy of AML1-ETO functions: Growth arrest versus block of differentiation. *Mol Cell Biol* 2001; 21:5577-90.
27. Wang Q, Stacy T, Miller JD, Lewis AF, Gu TL, Huang X, Bushweller JH, Bories JC, Alt FW, Ryan G, Liu PP, Wynshaw-Boris A, Binder M, Marin-Padilla M, Sharpe AH, Speck NA. The CBFbeta subunit is essential for CBFalpha2 (AML1) function in vivo. *Cell* 1996; 87:697-708.
28. Sasaki K, Yagi H, Bronson RT, Tominaga K, Matsunashi T, Deguchi K, Tani Y, Kishimoto T, Komori T. Absence of fetal liver hematopoiesis in mice deficient in transcriptional coactivator core binding factor beta. *Proc Natl Acad Sci USA* 1996; 93:12359-63.
29. Yergeau DA, Hetherington CJ, Wang Q, Zhang P, Sharpe AH, Binder M, Marin-Padilla M, Tenen DG, Speck NA, Zhang DE. Embryonic lethality and impairment of haematopoiesis in mice heterozygous for an AML1-ETO fusion gene. *Nat Genet* 1997; 15:303-6.
30. Okuda T, Cai Z, Yang S, Lenny N, Lyu CJ, van Deursen JM, Harada H, Downing JR. Expression of a knocked-in AML1-ETO leukemia gene inhibits the establishment of normal definitive hematopoiesis and directly generates dysplastic hematopoietic progenitors. *Blood* 1998; 91:3134-43.
31. Lenny N, Meyers S, Hiebert SW. Functional domains of the t(8;21) fusion protein, AML1-ETO. *Oncogene* 1995; 11:1761-9.
32. Amann JM, Nip J, Strom DK, Lutterbach B, Harada H, Lenny N, Downing JR, Meyers S, Hiebert SW. ETO, a target of t(8;21) in acute leukemia, makes distinct contacts with multiple histone deacetylases and binds mSin3A through its oligomerization domain. *Mol Cell Biol* 2001; 21:6470-83.
33. Wang J, Hoshino T, Redner RL, Kajigaya S, Liu JM. ETO, fusion partner in t(8;21) acute myeloid leukemia, represses transcription by interaction with the human N-CoR/mSin3/HDAC1 complex. *Proc Natl Acad Sci USA* 1998; 95:10860-5.
34. Lutterbach B, Westendorf JJ, Linggi B, Patten A, Moniwa M, Davie JR, Huynh KD, Bardwell VJ, Lavinsky RM, Rosenfeld MG, Glass C, Seto E, Hiebert SW. ETO, a target of t(8;21) in acute leukemia, interacts with the N-CoR and mSin3 corepressors. *Mol Cell Biol* 1998; 18:176-84.
35. Gelmetti V, Zhang J, Fanelli M, Minucci S, Pelicci PG, Lazar MA. Aberrant recruitment of the nuclear receptor corepressor-histone deacetylase complex by the acute myeloid leukemia fusion partner ETO. *Mol Cell Biol* 1998; 18:185-91.
36. Bushweller JH, Liu Y, Chruszcz M, Minor W, Speck NA. Structural and functional basis of the dominant negative phenotype of AML1-ETO, product of the t(8;21). *Blood abstract* 2003; 104(11 suppl).
37. McNeil S, Zeng C, Harrington KS, Hiebert S, Lian JB, Stein JL, van Wijnen AJ, Stein GS. The t(8;21) chromosomal translocation in acute myelogenous leukemia modifies intranuclear targeting of the AML1/CBFalpha2 transcription factor. *Proc Natl Acad Sci USA* 1999; 96:14882-87.
38. Khan KD, Lindvall G, Maher SE, Bothwell AL. Characterization of promoter elements of an interferon-inducible Ly-6E/A differentiation antigen, which is expressed on activated T cells and hematopoietic stem cells. *Mol Cell Biol* 1990; 10:5150-9.
39. Stanford WL, Haque S, Alexander R, Liu X, Latour AM, Snodgrass HR, Koller BH, Flood PM. Altered proliferative response by T lymphocytes of Ly-6A (Sca-1) null mice. *J Exp Med* 1997; 186:705-17.
40. Ito CY, Li CY, Bernstein A, Dick JE, Stanford WL. Hematopoietic stem cell and progenitor defects in Sca-1/Ly-6A-null mice. *Blood* 2003; 101:517-23.
41. Felsner DW. Cancer revoked: Oncogenes as therapeutic targets. *Nat Rev Cancer* 2003; 3:375-80.
42. Steffen B, Serve H, Berdel WE, Agrawal S, Linggi B, Buchner T, Hiebert SW, Muller-Tidow C. Specific protein redirection as a transcriptional therapy approach for t(8;21) leukemia. *PNAS* 2003; 100:8448-53.
43. Minucci S, Nervi C, Lo Coco F, Pelicci PG. Histone deacetylases: A common molecular target for differentiation treatment of acute myeloid leukemias? *Oncogene* 2001; 20:3110-5.
44. Heidenreich O, Krauter J, Riehle H, Hadwiger P, John M, Heil G, Vornlocher HP, Nordheim A. AML1/MTG8 oncogene suppression by small interfering RNAs supports myeloid differentiation of t(8;21)-positive leukemic cells. *Blood* 2003; 101:3157-63.