

gene was amplified by polymerase chain reaction (PCR) using the primers 5'CTGCAGGTAAATGAAGAAGG3' and 5'GGAA-GAGGTGCAAAGAATAAG3'; digestion by *PvuII* (New England Biolabs, Hitchin, United Kingdom) led to changes in size in the amplified wild-type DNA only. Confirmation of the presence of the 384s allele was performed by sequencing.

A total of 2 (0.4%) of the 473 patients (95% CI, 0%-1%) were heterozygous for the AT Cambridge II variant, which was therefore significantly less frequent in PATHROS than in the Spanish patients (1.7%; 95% CI, 0.9%-2.4%; $P = .02$). Both were women who developed only 1 VT, at ages 32 and 75, respectively. Both had normal AT activity.

These results suggest a possible heterogeneity in the geographic distribution of the Cambridge II variant, possibly due to a founder effect. Moreover, AT A384S was present in only 10 of 192 probands with AT deficiency who had AT gene sequencing in our laboratory since the year 2000. Altogether, these data do not argue for the AT Cambridge II being a prevalent genetic risk factor for thrombosis or a very frequent cause of AT deficiency in France.

Véronique Picard, Isabelle Présot, Pierre-Yves Scarabin, Martine Aiach, Joseph Emmerich, and Martine Alhenc-Gelas

The participating members of the Paris Thrombosis Study are as follows: M. Alhenc-Gelas, J. Emmerich, E. Arnaud, J. N. Fiessinger, M. Aiach, AP-HP Hôpital Broussais and INSERM U428, Paris, France; V. Nicaud, INSERM U258, Paris, France.

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Correspondence: Martine Alhenc-Gelas, Service d'Hématologie Biologique A, Hôpital Européen Georges Pompidou, 20 rue Leblanc, F75908 Paris cedex 15, France; e-mail: martine.alhenc-gelas@egp.aphp.fr.

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To the editor:

Wnt signaling and phosphorylation status of β -catenin: importance of the correct antibody tools

Two recent papers in *Blood* provide the first indications that deregulated Wnt signaling plays a role in the development of acute lymphoblastic leukemias (ALLs).^{1,2} Activating mutations in the critical Wnt mediator β -catenin lead to the development of thymic lymphomas, the murine counterpart of T-ALL.¹ The Wnt signaling pathway plays a key role in the development of various cell types and is subject to strict molecular regulation. The localization and consequently the function of β -catenin is regulated by Ser/Thr and possibly Tyr phosphorylation. Nuclear β -catenin serves as a coactivator for Tcf-mediated transcription of target genes that play important roles in leukemic progression.

Given the importance of the phosphorylation status of β -catenin, we have previously generated an antibody specific for β -catenin dephosphorylated at residues Ser37 and Thr41. Wnt signals specifically increase the levels of dephosphorylated β -catenin as detected with this antibody.^{3,4} The antibody, named inactive β -catenin (ABC), is produced by hybridoma clone 8E7. Another antibody claimed to recognize the dephosphorylated form is termed 8E4 (www.upstate.com).

Using 8E4 instead of 8E7, various investigators have studied activation of the Wnt pathway. Gottardi and Gumbiner⁵ report that dephosphorylated β -catenin readily interacts with both cytoplasmic cadherins and nuclear Tcf. Derksen et al⁶ and Diks et al⁷ report very similar patterns of bands in Western blots using 8E4 and a pan- β -catenin antibody, whereas Jamieson et al⁸ using 8E7 antibody show that Wnt signaling is activated in blast crises of chronic myelogenous leukemia (CML).

These conclusions critically depend on the specificity of the antibodies. We therefore mapped the antigenic epitope recognized by the 8E4 and 8E7 antibodies using a set of N-terminal deletion clones of β -catenin covering the regulatory region that is the target of the Wnt pathway kinases (Ser33 to Ser45; Figure 1A). Using these constructs, 8E4 was found to recognize all deleted forms (Figure 1B), implying that it does not recognize the regulatory region of β -catenin. These results

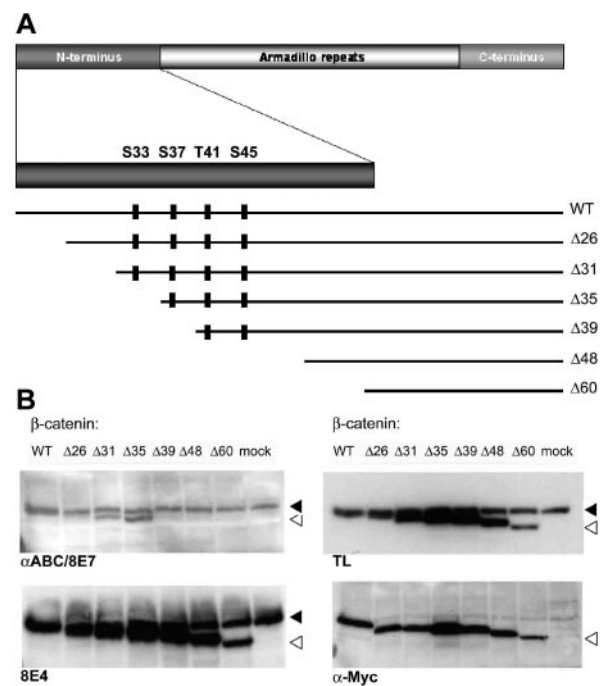


Figure 1. 8E4 recognizes all deleted forms of β -catenin. (A) Schematic overview of the β -catenin molecule, with emphasis on the N-terminus. The 4 well-characterized Ser and Thr amino acids that are regulated by phosphorylation are shown as well as the various deletion constructs used to express myc-tagged truncated forms of β -catenin. (B) The 293 cells were transfected with myc-tagged β -catenin deletion constructs to identify the epitope recognized by 8E7 and 8E4. The endogenous β -catenin band is indicated by a black arrow, the open arrow indicates the transfected, N-terminal deleted form. Similar to the pan- β -catenin antibody (TL), 8E4 recognizes all deletion constructs, whereas 8E7 (α ABC) only recognizes deleted β -catenin forms up to Δ 35. The following antibodies were used: α ABC/8E7 (Upstate Biotech, Lake Placid, NY; catalog no. 05-665), 8E4 (Upstate Biotech; catalog no. 05-601), TL (BD Transduction Laboratories, San Jose, CA; anti- β -catenin; catalog no. 610154), α -Myc (clone 9E10; supernatant of hybridoma used).

are at odds with the claims on the company's website (<http://www.upstate.com>) that this antibody was raised against amino acids 27-37 of human β -catenin. As published previously, the 8E7 epitope mapped directly C-terminal to amino acid 35.³

It is unfortunate that these antibodies with such similar names, commercialized by the same company, have strikingly different specificities. Our observations imply that the 8E4 antibody does not possess the advertised specificity for the dephosphorylated regulatory region of β -catenin and should not be used to study activity of the Wnt pathway, for instance, on cytospin preparations of cells suspected for hematologic malignancies.

Mascha van Noort, Floor Weerkamp, Hans C. Clevers, and Frank J. T. Staal

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Correspondence: Frank J. T. Staal, Department of Immunology, Erasmus MC, University Medical Center Rotterdam, Dr. Molewaterplein 50, 3015GE, Netherlands; e-mail: f.staal@erasmusmc.nl.

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To the editor:

Interferon- α or homoharringtonine as salvage treatment for chronic myeloid leukemia patients who acquire the T315I BCR-ABL mutation

We report here the clinical outcome for 2 chronic myeloid leukemia (CML) patients who acquired the T315I mutation while on imatinib, both of whom were treated successfully, one with recombinant interferon-alpha (rIFN) and the other with semisynthetic homoharringtonine (HHT).

Patient 1 was a 75-year-old man with CML diagnosed in October 2003 who started imatinib 400 mg daily. After 4 months he achieved a complete cytogenetic remission (CCyR) with low BCR-ABL transcript numbers (0.3%) quantified by real-time quantitative polymerase chain reaction (RQ-PCR).¹ He maintained a good response for 12 months but then BCR-ABL transcripts started to increase progressively and a T315I mutation was identified. The earlier samples were then analyzed retrospectively. Thirty months after starting imatinib, cytogenetics revealed loss of CCyR (2% Philadelphia chromosome [Ph]-positive cells) and the patient started rIFN 9 MU/week. Ten months later the patient was still in complete hematologic remission (CHR) with low BCR-ABL transcripts (3%), a level that is just consistent with CCyR. Interestingly, on rIFN, the percentage of mutant transcripts measured by quantitative single-nucleotide polymorphism pyrosequencing¹ decreased from 100% to 30% (Figure 1A).

The second patient was a 60-year-old woman diagnosed with chronic-phase CML in 1997. She received rIFN and thereafter imatinib 400 mg daily from October 2002. She achieved a major cytogenetic response (15% Ph-positive cells) at 12 months but lost her response 2 years later. Bone marrow examination then revealed myelofibrotic transformation with 100% Ph positivity. Imatinib was increased to 600 mg/day, but she went into accelerated phase at 40 months, and thus received dasatinib 70 mg twice a day. Four months later a bone marrow aspirate still showed accelerated-phase leukemia, and a T315I mutation was identified. The earlier samples were then analyzed and the mutated clone was detected before starting dasatinib. The patient started on HHT at 1.25 mg/m² subcutaneously twice daily for 5 consecutive days every month. Five months later she achieved a CHR. The marrow showed a

minor cytogenetic response, and at 10 months the percentage of the mutant clone had decreased from 100% to 27% (Figure 1B).

The finding of a T315I mutation in the BCR-ABL gene characteristic of CML is associated with clinical resistance to imatinib, nilotinib, and dasatinib.²⁻⁴ The only established salvage option for patients harboring this mutation is allogeneic stem cell transplantation (allo-SCT)⁵; the role of the aurora kinase inhibitor MK-0457 is not yet clear.⁶ Because the mechanisms of action of rIFN and HHT differ from that of tyrosine kinase inhibitors (TKIs), we evaluated their effect in these 2 patients with a predominant T315I clone.^{2,7,8} Although longer follow-up is needed, both patients

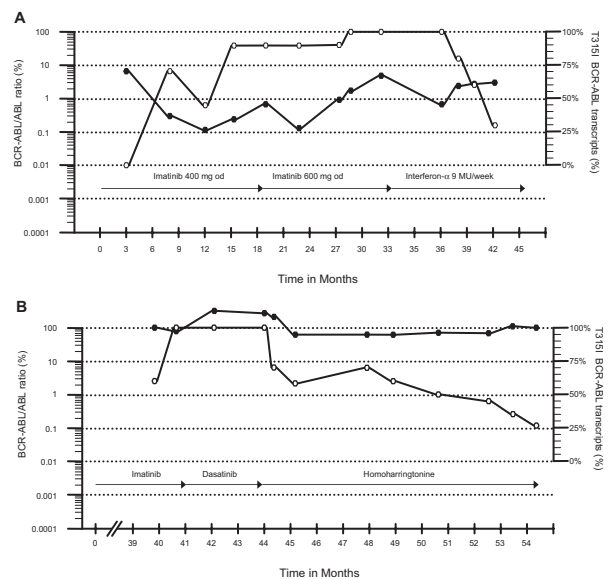


Figure 1. Total and T315I BCR-ABL in vivo kinetics. The figure shows BCR-ABL transcript levels measured by RQ-PCR and the relative size of the mutant clone. ● and ○ represent the total BCR-ABL transcripts and the percentage of the mutant clone, respectively. The type and duration of therapy are represented by the arrows.