second is the initiation of new clinical trials to obtain more data on the safety and efficacy of rFIX. One clinical trial will study rFIX in children younger than 6 years of age with severe hemophilia B, including both previously treated patients and previously untreated patients. The other trial will study rFIX in previously treated patients with severe or moderately severe hemophilia B who are 12 years and older. Both trials were designed taking into consideration the CPMP Guidelines for the Investigation of Recombinant Factor VIII and Factor IX (CPMP/BPWG/1561/99 [October 2000]).

Wyeth Pharmaceuticals and Baxter Healthcare—the exclusive distributor of BeneFIX in the European Union—encourage physicians to support efforts to collect additional safety and efficacy information.

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Response:

The recombinant factor IX clinical investigator group’s response to Dr Haase

As academic hematologists and clinical investigators, we depend on the integrity and validity of clinical research data to guide our decisions and are committed to generating reliable data of the highest quality. Furthermore, we are committed to publishing the outcomes of our research, especially when they significantly contribute to advancing our clinical knowledge and improving the quality of health care and medical practice. Regulatory agencies, such as the European Agency for the Evaluation of Medicinal Products (EMEA), are also dependent on the results of well-conducted studies to make their decisions. We thank this agency for scrutinizing data to ensure that its objectives can be achieved.

We congratulate Wyeth Pharmaceuticals for its courage to sponsor expensive clinical research in rare diseases, especially in hemophilia. These investigations have required a multifaceted effort and have included intricate and complex clinical protocols in both the inpatient and outpatient settings. The investigators who participated in these trials believe that the administrative oversights uncovered by the EMEA-commissioned Good Clinical Practice (GCP) inspection do not compromise the scientific integrity or robustness of the data that were generated by these studies.

We are reassured by independent audits of the data, as cited in Dr Haase’s letter, because these audits confirm that the published data are real and representative of the population studied. Dr Haase’s letter also indicates that the postmarketing data continue to support our published conclusions regarding the safety and efficacy profile of human recombinant factor IX (rFIX).

Lastly, we are reassured by the fact that in light of the findings of the GCP inspection, the Committee for Proprietary Medicinal Products (CPMP) still considers that the benefit/risk balance of rFIX for the treatment and prophylaxis of bleeding in previously treated patients (PTPs) with hemophilia B is positive. We directly acknowledged in our publications that additional studies are required to define the true risk of immune responses to rFIX, including the development of inhibitors and allergic reactions, and we support the need for enhanced surveillance of new patients receiving rFIX. We must emphasize to the readership of Blood that the publications generated by these clinical trials1,2 were based on valid data generated and interpreted in an objective manner. These reports accurately reflect the investigators’ favorable clinical experiences with the study patient populations as described.


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References


To the editor:

ETO sequence may be dispensable in some AML1-ETO leukemias

Acute myeloid leukemia (AML) is a heterogeneous disease, with individual cases showing variability in clinical presentation, blast-cell morphology, therapeutic response, and long-term prognosis. One of the most common cytogenetic abnormalities described in AML is t(8;21)(q22;q22) found in 10% to 15% of cases.1 As a consequence of the chromosomal translocation, 5 exons of the AML-1 gene are fused to nearly the entire coding sequence of the ETO gene, generating an easily detectable polymerase chain reaction (PCR) product of a constant size (260 bp), corresponding to an in-frame fusion of AML1 exon 5 to ETO exon 2. This novel chimeric gene, AML/ETO, encodes a fusion protein with a primary inhibitory role in the normal hematopoietic differentiation program.

Adult patients with de novo–diagnosed AML enrolled in the Spanish Estudi i Tractament de Leucèmies Agudes i Mielodisplàssies (CETLAM) 99 protocol were tested for the presence of chimeric AML1-ETO and CBFβ-MYH11 mRNA using the BIOMED-1 protocols. Briefly, 1 µg of RNA obtained from fresh leukemic bone marrow was retrotranscribed into cDNA using random hexamers. RNA quality assessment was carried out with 5 µL of cDNA in a 1-step PCR amplification of the normal ABL.
cDNA (3 μL) was used for the first-step amplification using specific primers for the AML1-ETO fusion gene transcript, followed by a second-step (nested) amplification. Amplified PCR products from PCR I and II were separated by electrophoresis on a 2% agarose gel.

In one patient with a typical M2 leukemia with the t(8;21)(q22;q22), a different-sized PCR amplifier was observed. This faster migrating band was cut from the gel and used for reamplification by PCR. The sequence of this purified product revealed a 50-bp frameshift deletion in exon 2 of ETO. The loss of 50 bp originated a disruption of the reading frame of this transcript resulting in the introduction of a premature stop codon 41 amino acid downstream (Figure 1). The encoded truncated protein would only conserve 31 residues from the ETO coding sequence that mediate homo- and heterodimerization with ETO.

In the case under discussion, the reverse transcriptase (RT)–PCR showed a single band, which indicates the presence in this patient of a unique transcript.

The expression of this abnormal transcript was investigated through the evolution of the disease by means of real-time quantitative PCR following the recommendations of the European SANCO (Health and Consumer Protection, European Commission) Concerted Action using the ABI PRISM 7700 Sequence Detector (Applied Biosystems, Foster City, CA).3 Based on these findings, fusion transcript values obtained were normalized with respect to the number of ABL transcripts and expressed as fusion-gene copy number per 10⁴ copies of ABL. At the time of diagnosis, the real-time PCR showed 28 000 copy number of AML1-ETO/abl1)/X 10⁴, and after therapy the transcript levels rapidly decreased to 1.3 copies. This parallels with the results obtained in typical AML1-ETO fusion cases.

The AML1-ETO fusion protein retains many of the important functional domains of both AML1 and ETO, including the RHD (Runt homology DNA-binding domain) of AML1, and the ETO sequences that mediate homo- and heterodimerization with ETO/MTG family members and interaction with nuclear corepressors. The ability of AML1-ETO to repress transcription is dependent on both the RHD of AML1, and the hydrophobic heptad repeat (HHD) and zinc fingers of ETO.3,7 In the patient described here, the fusion-truncated protein would not have any of the most important ETO domains, and thus it shows that most of the ETO sequence may be dispensable in this AML1-ETO leukemia.

The fact that the patient had a typical M2 leukemia may be explained by the determinant effect on the leukemic phenotype of cooperative and yet unidentified mutations associated with the AML1-ETO rearrangement.5

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