bortezomib and dexamethasone are warranted. The most common toxicities were gastrointestinal symptoms, thrombocytopenia, neutropenia, fatigue, and peripheral neuropathy (Figure 2). The majority of the side effects were low grade. The thrombocytopenia was transient, with recovery within the 10-day rest period in each cycle, and was not associated with bleeding complications. Six patients had herpes zoster. The dose of bortezomib was reduced in 18 patients and the drug was discontinued in 12 patients because of side effects: peripheral neuropathy (n=6), gastrointestinal symptoms (n=4), skin rash (n=1) and orthostatic hypotension (n=1).

In our observational analysis, the response rate to bortezomib was comparable to those in two prospective clinical studies, CREST and SUMMIT, which reported response rates of 35-50%. The median duration of response was 9 months. In the CREST study, the median duration of response was 9.5 months and 13.7 months in the 1.0 mg/m² and 1.3 mg/m² groups, respectively, while in the SUMMIT study the median response duration was 12 months. A possible explanation for the shorter duration of response in our series may be that dose reduction was performed more frequently by local physicians in our study than in CREST and SUMMIT, which were conducted in expert myeloma centers. Therefore, the clinical practice in community hospitals of reducing doses in patients with treatment-related toxicity without adherence to well-defined guidelines may jeopardize the outcome of the efficacy of bortezomib in multiple myeloma.

A remarkable observation in our series was the high incidence of herpes zoster. Six patients developed a herpes zoster infection during treatment with bortezomib. The transcription factor NF-κB has been demonstrated to play a pivotal role in cytokine signaling and the generation of cell-mediated immune response in numerous models. Therefore, inhibition of NF-κB may increase the risk of reactivation of the varicella zoster virus. Prophylactic antiviral medication should be considered in predisposed patients who receive bortezomib.

In conclusion, bortezomib can induce marked and durable response in advanced multiple myeloma. Overall, bortezomib was well tolerated and the toxicity was acceptable.

**References**


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**Letters to the Editor**

**Disorders of Hemostasis**

**Exon skipping partially restores factor VIII coagulant activity in patients with mild hemophilia A with exon 13 duplication**

Ectopic mRNA was analyzed by reverse transcriptase polymerase chain reaction (RT-PCR) in patients with duplication of F8 gene exon 13, a mutation which has been demonstrated to be a cause of mild hemophilia A in 32% of Northern Italian subjects. Two different transcripts originate from mutated genomic DNA, due to alternative splicing processes. The larger-sized transcript contains both duplicated exons 13, the smaller one contains only one exon 13. The residual FVIII:C activity which accounts for the mild hemophilia A phenotype derives from the latter transcript.

We described the duplication of exon 13 as a disease causative mutation in a cohort of Northern Italian patients with hemophilia A (HA). The association of the alteration with a residual FVIII coagulant activity >8% suggested that a certain amount of normal protein was being produced. We could, therefore, hypothesize: (i) that an elongated form of mRNA is generated and that it somehow ends up in the translation of a partially functioning normal protein; or (ii) that alternative splicing processes produce two forms of RNA, but that only one of them is able to translate a correct protein.

To verify these working hypotheses, we undertook a study on ectopic mRNA obtained from the only available tissue, peripheral blood. mRNA was isolated from the lymphocytes of 6/10 patients and from 40 healthy controls, following informed consent. cDNA, reverse transcribed (Reverse Transcription kit - Clontech, Palo Alto, CA),
CA, USA) from total RNA, was amplified under standard conditions with 50 pmol primers located at positions 1814 to 1835 (11F: 5’-TGACCCGCTATTACTCT-GAAAAATGCTCATATTTGGC-3′) sited at positions 2314 to 2333 (GenBank, accession number M14113, http://www.ncbi.nlm.nih.gov). The results showed that besides the 521 bp product, which was the only mRNA fragment present in the healthy controls (Figure 1A, lane 1) an additional 731 bp fragment was detected in the RNA of HA patients (Figure 1A, lanes 2 and 3). Long and short fragments from the mRNA of HA patients, excised from the 2% agarose gel, purified (Ultrasfree-DA Centrifugal Device- Millipore Corporation, Bedford, MA, USA), and sequenced using a fluorescent ABI Prism Big Dye terminator kit (Applied Biosystem, UK), showed that the aberrant-sized fragment (731 bp) contained two exons 13, duplicated in frame. Following the duplication, a new sequence was created whereby the first amino acid of the duplicated exon 13 was a glycine instead of the native serine. Sequencing of the first amino acid of the duplicated exon 13 was a non-functional. To better understand the causes of the detrimental effects that the mutation has on the protein’s function, we inspected the molecular model of the FVIII protein using the O’ program on a Silicon Graphics Octane R12K biprocessor and by secondary structure prediction run on the Network Protein Sequence Analysis. The extra 70 amino acid residues may cause incorrect folding of the secondary structure of the FVIII protein. The addition of seven β strands may significantly change the physico-chemical properties of the protein’s surface and, therefore, possible interactions of FVIII with other proteins could be affected.

To our knowledge, this is the first example of a mutation which interferes positively in HA with the splicing mechanism, since one of the two induced alternative splicing processes (exon skipping) repairs a genomic error.

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Letters to the Editor

Figure 1. mRNA analysis and schematic representation of alternative splicing processes which lead to differently sized fragments. A. Analysis of ectopic mRNA amplified by RT-PCR. Five microliters from the second PCR amplification products from both normal and patient RNA were electrophoresed through a 2% ethidium bromide stained gel. Lane M: 100 bp DNA ladder; lane 1, mRNA from a normal control; lanes 2 and 3, mRNA from two different patients carrying the exon 13 duplication. Lengths of the normal (521 bp) and mutated (731 bp) fragments are shown. B. Partial schematic representation of the mRNA of the patients. C. box: exon; triangle: intron; / indicates the junction of part of intron 13 with a part of intron 12 as the result of the duplication. Schematic drawing representing the theoretical splicing processes which could lead to the elongated (731 bp) and normal-sized (521 bp) fragments. Upper part: splicing occurs within the newly created intron sequence (prediction score 0.89). Middle and lower parts: alternative splicing involving different donor and acceptor sites (see text) leads to exon 13 skipping.

The extra 70 amino acid residues may cause significant changes in protein structure and function. The detrimental effects that the mutation has on the FVIII protein’s function can be assessed by analyzing the computational and structural aspects of the protein using software tools such as O’ and Network Protein Sequence Analysis. The extra 70 amino acid residues may cause incorrect folding of the secondary structure of the FVIII protein. The addition of seven β strands may significantly change the physico-chemical properties of the protein’s surface, leading to possible interactions of FVIII with other proteins that could be affected.

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We identified a novel mutation in an asymptomatic 72-year-old Japanese woman with severe factor XI (FXI) deficiency. Sequence analysis showed a homozygous missense mutation Trp599Arg (g.234T>A) according to Genbank accession number M20218. This residue belongs to a region conserved in human FXI and the FXI of several animals. Molecular modeling showed that the Trp599 residue is positioned in an alpha helix in the C-terminal region of the FXI molecule.

The factor XI gene (F11) (23 Kb in size) is located on chromosome 4q35, and contains 15 exons plus 14 introns. To date, more than 40 different FXI gene lesions have been documented in the factor XI mutation database (http://uwcmml1s.uwcm.ac.uk/uwcm/mg/search/119891.htm). We identified a novel causative homozygotic mutation in a Japanese woman with severe factor XI (FXI) deficiency, which resulted in a Trp599 to Arg substitution near the carboxyl-terminal region of FXI. The proband was a 72-year-old Japanese woman who consulted Iki public hospital after she had been bitten by a mamushi pit viper. She was immediately treated by serotherapy and coagulation tests were performed. Her platelet count was decreased for a time, but was within the normal range after a few hours. She had no bleeding tendency in that time. While results showed a remarkably prolonged activated partial thromboplastin time (89 s, reference range; 28-32 s), her bleeding time, prothrombin time and fibrinogen levels were within normal ranges. No inhibitor of the intrinsic coagulation pathway was found in her plasma. Her parents were first cousins. After obtaining signed documents of informed consent, blood specimens were drawn from the proband and her son. FXI coagulant activity (FXI:C) was assayed by a one-stage method based on the activated partial thromboplastin time using FXI-deficient plasma. FXI antigen (FXI:Ag) concentrations were determined using goat anti-FXI-IgG (Cedarlane, Ontario, Canada), biotin labeled anti-FXI-IgG and peroxidase-conjugated streptavidin (DAKO A/S, Glostrup, Denmark) by an in-house enzyme-linked immunosorbent assay (ELISA). Both FXI:C and FXI:Ag levels of the proband were below 1% of normal control values. Coagulation factors, apart from FXI, were within normal ranges. FXI:C and FXI:Ag for her son were 56.6% and 62.0%, respectively. Polymerase chain reaction (PCR) amplification of exons 1 through 15 of the F11 gene was performed with the use of genomic DNA as a template, with primer and conditions as described previously. Purified PCR products were directly sequenced using a Taq Dye Deoxy Terminator Sequencing Kit (Applied Biosystems, Foster City, USA). Sequence analysis showed a T→C mutation at codon 599 in exon 15 (g.234T→C according to Genbank accession number M20218), resulting in a substitution of Trp599 (TGG) by Arg (CGG) in the catalytic domain. This point mutation created an Msp I restriction endonuclease recognition site in the exon 15 nucleotide sequences. Results of an Msp I digestion indicated that the proband was homozygous for the mutation, while her son was heterozygous for the mutation (Figure 1). No Msp 1 restriction endonuclease sites were detected in any of exon 15 sequences from 50 normal Japanese individuals.

Trp599 belongs to a structurally conserved region present in human FXI (Genbank accession number NM-000128), rabbit FXI (AF395821), bovine FXI (NM028066) and mouse FXI sequences (BC019485) (Figure 2A). It is...