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FIGURE LEGENDS

Figure 1

NPM1 interacts with MEF/ELF4. (A) 293T cells were transfected with the indicated expression plasmids. After 48 h, cell lysates were immunoprecipitated with anti-FLAG and anti-V5 antibodies. Immunoprecipitates were analyzed by 10% SDS-PAGE and subjected to immunoblotting with anti-V5 antibody (upper row) or anti-FLAG antibody (bottom row). (B) MEF/ELF4 interacts directly with NPM1 *in vitro*. *In vitro* association assays were undertaken by incubating His-MEF/ELF4 fusion protein immobilized by using a His-column with biotin-labeled MEF/ELF4 (lane 1). His alone was incubated with biotin-labeled NPM1 (lane 2) as a control. (C) NPM1 structure and the relative binding of MEF/ELF4 (schematic). HomoD, homodimerization domain, residues 1–117; AD/NLS, acidic domain/nuclear localization domain, residues 117–187; HeteroD, heterodimerization domain, residues 187–259; NBD, nucleic acid binding domain, residues 259–294. (D) The N-terminal portion of NPM1 is the MEF/ELF4-interacting domain. Bacterially expressed and purified GST, GST-NPM1, and GST-NPM1 mutants with deletions were mixed with bacterially expressed and purified His or His-MEF/ELF4 protein. Recombinant proteins were subjected to His or GST affinity columns, followed by immunoblotting with anti-GST or anti-His antibodies. (a): the reactive samples were subjected to analyses in His affinity column followed by immunoblotting with ant-His antibodies (left lower panel) or with anti-GST antibodies (left upper panel). (b): the reactive samples were subjected to GST affinity columns, followed by immunoblotting with anti-GST antibodies (right upper panel) or with anti-His antibodies (right lower panel).

Figure 2

EMSA with recombinant His-MEF/ELF4, His, GST, and GST-Wt-NPM1. His-MEF/ELF4 was incubated with GST and GST-Wt-NPM1 at room temperature prior to EMSA by using a biotin-conjugated APET probe (lanes 1-4). An excess amount of unlabeled APET competitor was added to the reaction mixtures (lanes 5 and 6).

Figure 3

Wt-NPM1 inhibits, whereas Mt-NPM1 enhances, MEF/ELF4-dependent APET promoter transactivation. (A) 293T human kidney cell lines, (B) COS7 monkey kidney cell lines, and (C) U937 human hematological cell lines were co-transfected with the luciferase reporter gene of an artificial MEF/ELF4 target promoter (APET) and effecter genes. Target promoter and effecter genes were as follows: lane 1: PGL4/APET; lane 2: PGL4/ETSm-APET; lane 3: PGL4/APET and PcDNA/MEF/ELF4; lane 4: PGL4/APET, pcDNA/MEF/ELF4, and pcDNA/Wt-NPM1; lane 5: PGL4/ETSm-APET and pcDNA/MEF/ELF4; and lane 6: PGL4/APET and pcDNA/Wt-NPM1. Luciferase activity by PGL4/APET alone was assigned a value of 1.0. The analysis was performed in triplicate assays, and the results were reproducible. The results are shown as the mean ± SD (*$P < 0.05$). (D) 293T cells transduced with siRNA encoding vector (siWt-NPM1) were harvested 72 h after transduction for Western blotting. Hsp90 is shown as a control. sicNPM1, control siRNA non-relevant to the expression of NPM1; Wild, without transduction. (E) 293T cells were co-transfected with the luciferase reporter plasmid (PGL4/APET), expression plasmid (pcDNA MEF/ELF4), and siWt-NPM1 gene (pcDNA/siRNA-Wt-NPM1) or control. Luciferase activity by PGL4/APET alone was assigned a value of 1.0. The analysis was performed in triplicate assays, and the results were reproducible. The results are shown as the mean ± SD (*$P < 0.05$). (F) 293T cells were co-transfected with the luciferase reporter gene of an artificial MEF/ELF4 target promoter and effector genes. Target promoter and effector genes were as follows: lane 1: PGL4/APET; lane 2: PGL4/APET and PcDNA/MEF/ELF4; lane 3: PGL4/APET, pcDNA/MEF/ELF4, and Wt-NPM1; lanes 4, 5 and 6: PGL4/APET, pcDNA/MEF/ELF4, and Mt-A-NPM1, Mt-I-NPM1 or Mt-J-NPM1; and lanes 7, 8, 9 and 10: PGL4/APET and pcDNA/Wt-NPM1, Mt-A-NPM1, Mt-I-NPM1 or Mt-J-NPM1. Luciferase activity by PGL4/APET alone was assigned a value of 1.0. The analysis was performed in triplicate assays, and the results were reproducible. The results are shown as the mean ± SD (*P < 0.05). (G) COS7 cells were co-transfected with the luciferase reporter gene of an artificial MEF/ELF4 target promoter, and effector genes. Target promoter and effector genes were as follows: lane 1: PGL4/APET; lane 2: PGL4/APET and PcDNA/MEF/ELF4; lane 3: PGL4/APET, pcDNA/MEF/ELF4, and Wt-NPM1; lanes 4 and 5: PGL4/APET, pcDNA/MEF/ELF4, and Mt-A-NPM1 or Mt-I-NPM1; and lanes 6, 7, and 8: PGL4/APET and pcDNA/Wt-NPM1, Mt-A-NPM1 or Mt-I-NPM1. Luciferase activity by pcDNA/APET alone was assigned a value of 1.0. The analysis was performed in triplicate assays, and the results were reproducible. The results are shown as the mean ± SD (*P < 0.05). (H) U937 cells were co-transfected with the luciferase reporter gene of an artificial MEF/ELF4 target promoter and effector genes. Target promoter and effector genes were as follows: lane 1: PGL4/APET; lane 2: PGL4/APET and PcDNA/MEF/ELF4; lanes 3: PGL4/APET, pcDNA/MEF/ELF4, and Wt-NPM1; lane 4: PGL4/APET, pcDNA/MEF/ELF4 and Mt-A-NPM1; and lanes 5 and 6: PGL4/APET and pcDNA/Wt-NPM1 or Mt-A-NPM1. Luciferase activity by pcDNA/APET alone was assigned a value of 1.0. The analysis was performed in triplicate assays, and the results were reproducible. The results are shown as the mean ± SD (*P < 0.05). (I) 293T cells were co-transfected with 0.1 μg of the luciferase reporter gene of an artificial MEF/ELF4 target promoter (lanes 1, 2, 3, 4, 5, 6, 7, 8 and 9) and 0.1 μg of effector genes (PcDNA/MEF/ELF4) (lanes 1, 2, 3, 4, 5 and 6). The effector genes were as follows: lane 1: 0.2 μg of Mt-A-NPM1; lane 2: 0.16 μg of Mt-A-NPM1 and 0.04 μg of Wt-NPM1; lanes 3: 0.1 μg of Mt-A-NPM1 and 0.1 μg of Wt-NPM1; lane 4: 0.04 μg of Mt-A-NPM1 and 0.16 μg of Wt-NPM1; and 5: 0.2 μg of Wt-NPM1; lane 6: none; lane 7: PGL4/APET and 0.2 μg of Mt-A-NPM1; lane 8: PGL4/APET and 0.2 μg of Wt-NPM1; lane 9: PGL4/APET. Luciferase activity by PGL4/APET alone was assigned a value of 1.0. The analysis was performed in triplicate assays, and the results were reproducible. The results are shown as the mean ± SD (*P < 0.05).

Figure 4

Mt-A-NPM1 does not interact with MEF/ELF4 in vivo. 293T cells were transfected with the indicated expression plasmids. After 48 h, cell lysates were immunoprecipitated with anti-FLAG and anti-V5 antibodies. Immunoprecipitates were analyzed by 10% SDS-PAGE and subjected to immunoblotting with anti-V5 antibody (upper row) or anti-FLAG antibody (bottom row).

Figure 5

Localization of MEF/ELF4 was unaffected by the mutation of NPM1. (A) 293T cells were transfected with GFP-MEF/ELF4 fusion protein expression vector and pcDNA/V-Wt-NPM1 (a) or pcDNA/V-Mt-A-NPM1 (b). Forty-eight hours after transfection, cells were fixed and immunofluorescence-stained with anti-V tag antibody. (B) Western blotting of Flag-MEF/ELF4 subcellular distribution in 293T cells co-transfected with pFlag-MEF/ELF4 and pcDNA/V-Wt-NPM1 or pcDNA/V-Mt-A-NPM1. Purity of the subcellular fractions was assessed by blotting with histone H1 (nuclear extraction) and Hsp70 (cytoplasmic extraction).

Figure 6

Mt-NPM1 stimulates MEF/ELF4-induced hyperproliferation and transformation. NIH3T3 cells transfected with various combinations of expression plasmids were plated in soft agar on 60-mm dishes and incubated for 2 weeks. (A) Microscopy of MEF/ELF4-transfected NIH3T3 cells with Wt-NPM1 or Mt-A-NPM1. (B) The average number of colonies of three independent experiments with standard deviation (*P < 0.05).

Figure 7

MEF/ELF4 transactivates the HDM2 promoter. (A) MEF/ELF4 binds to the HDM2 promoter *in vivo*. Flag-MEF/ELF4-bound DNA from 293T cells was immunoprecipitated with Flag antibody or normal mouse IgG. RQ-PCR amplification was performed on the corresponding templates by using primers for HDM2. (B) Structure of the HDM2 promoter region (-82 to -122) (schematic). (C) 293T cells were transfected with HDM2 promoter-driven luciferase reporter plasmid encoding wild-type [7B (a)] or mutant [7B (b)] protein. Luciferase activity by pcDNA alone was assigned a value of 1.0. The analysis was performed in triplicate assays, and the results were reproducible. The results are shown as the mean ± SD. (D) 293T cells were co-transfected with pFlag/MEF/ELF4 and pcDNA/Wt-NPM1 or pcDNA/Mt-A-NPM1. RQ-PCR amplification was undertaken on corresponding templates using primers for HDM2. The analysis was performed in triplicate assays, and the results were reproducible. The results are shown as the mean ± SD (*P < 0.05).

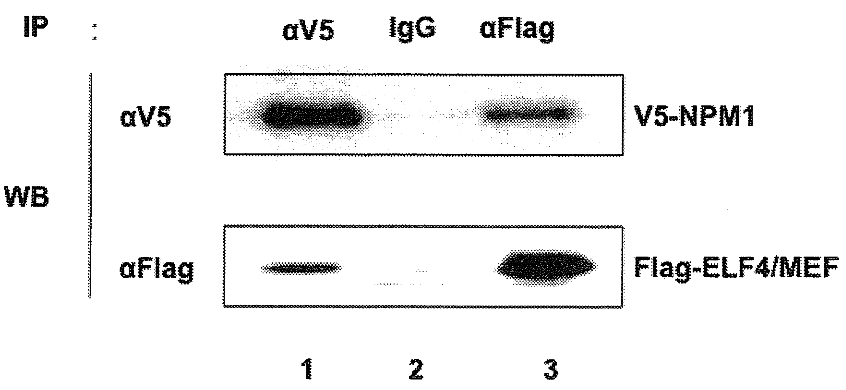
Figure 8

Expression of Mt-NPM1 and higher expression of MEF/ELF4 are associated with the elevated expression of HDM2 in CD34-positive AML cells. Total RNA isolated from 22 AML patients (CD34-positive leukemia cells) was analyzed for the expression of HDM2 by RQ-PCR. Stratified by the presence of the NPM1 mutation (A) and by the level of ELF4/MEF (B). *P < 0.009 against Wt -NPM1; **P < 0.03 against MEF/ELF4-L, assessed by ANOVA followed by Scheffe's multiple comparison test.

Table 1. Clinical and laboratory characteristics of patients

	Wt-NPM1	Mt-NPM1	P
No. of patients	14	8	
Sex			
Male	5	5	
Female	9	3	0.60
Median age, years	54.5	62	
(range)	(18–78)	(44–76)	
FAB classification			
M0	1	0	
M1	2	2	
M2	4	2	
M4	2	2	
M5	2	2	
M6	3	0	0.50
TLD+	6	4	0.50
Median WBC count, /μL	7,300	47,500	
(range)	(1300–556000)	(1700–114700)	0.10
Median LDH level	647	669	
(range)	(203–5325)	(270–2391)	0.07
Median BM count, /μL	337,000	475,000	
	(9000–738000)	(34,900–769000)	0.10

Figure 1A



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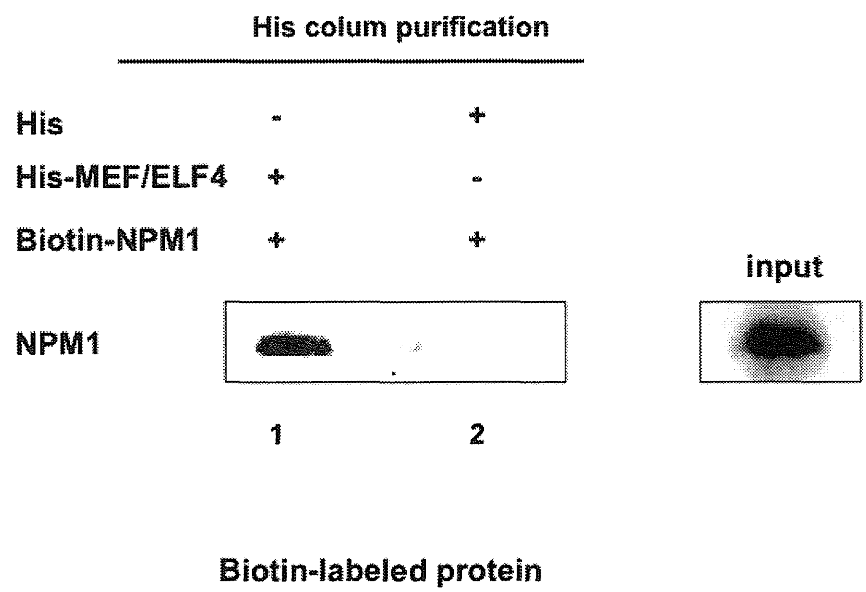


Figure 1C

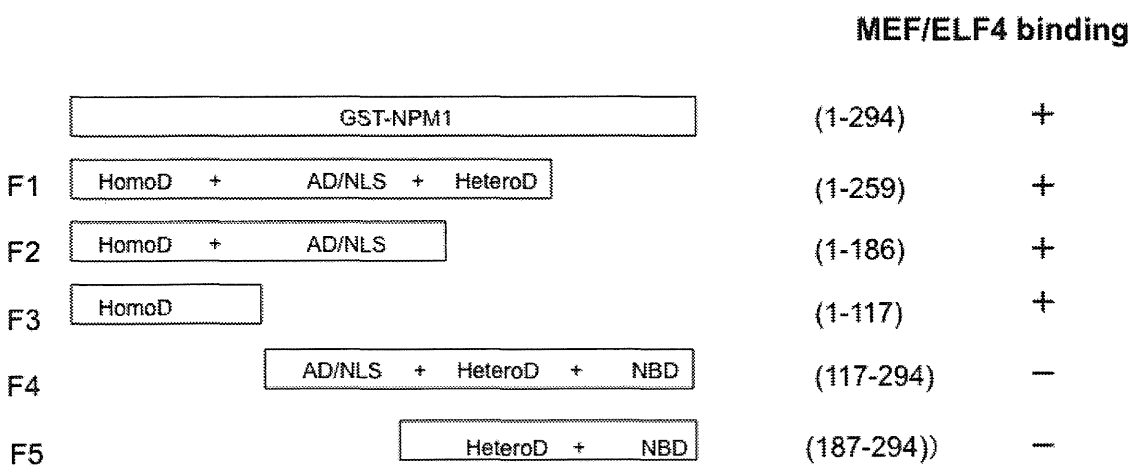
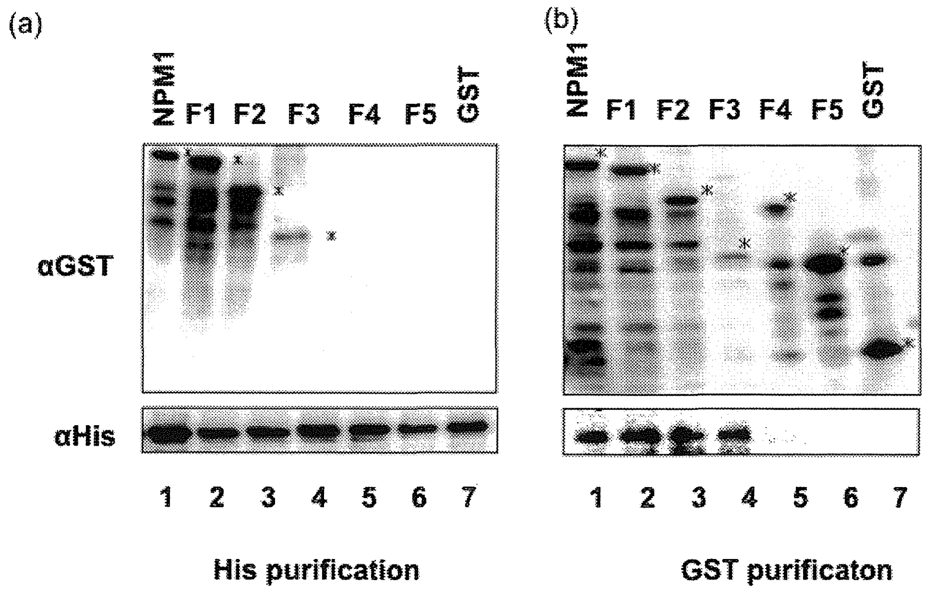


Figure 1D



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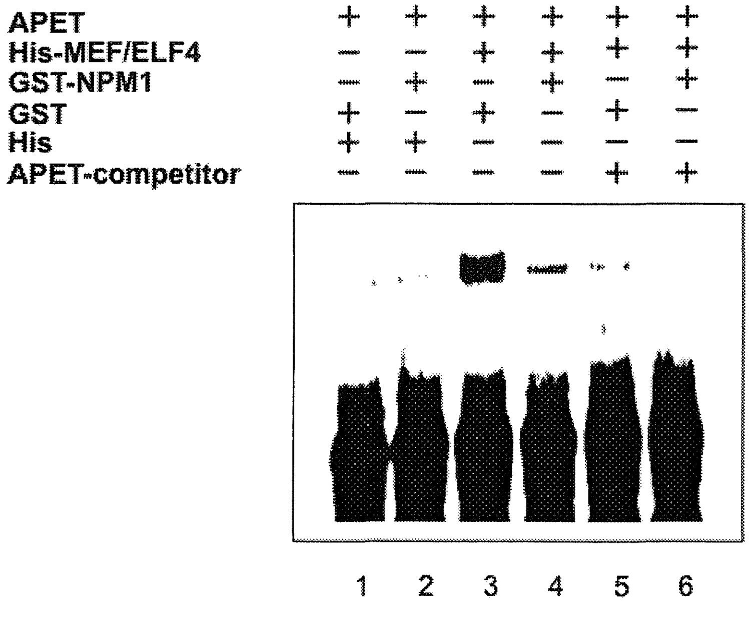

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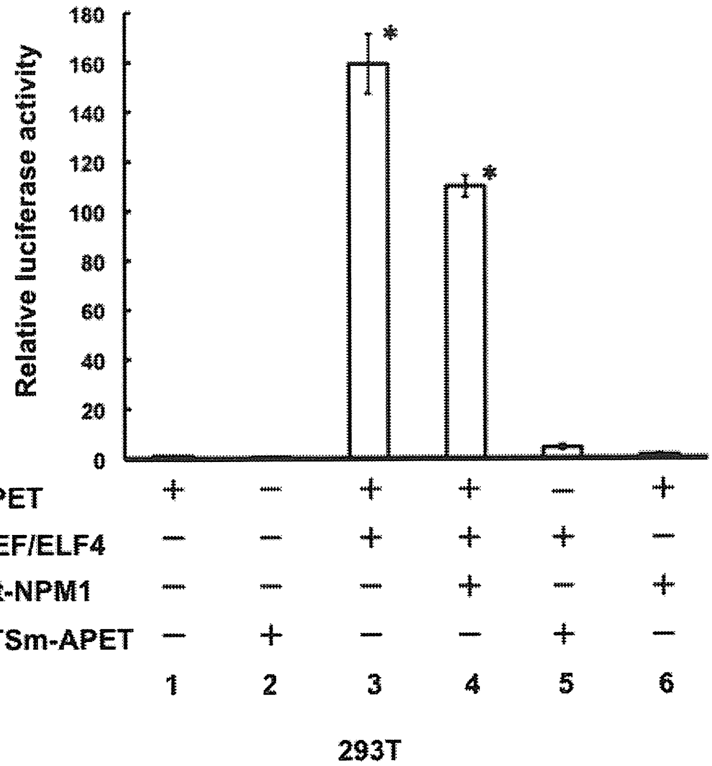

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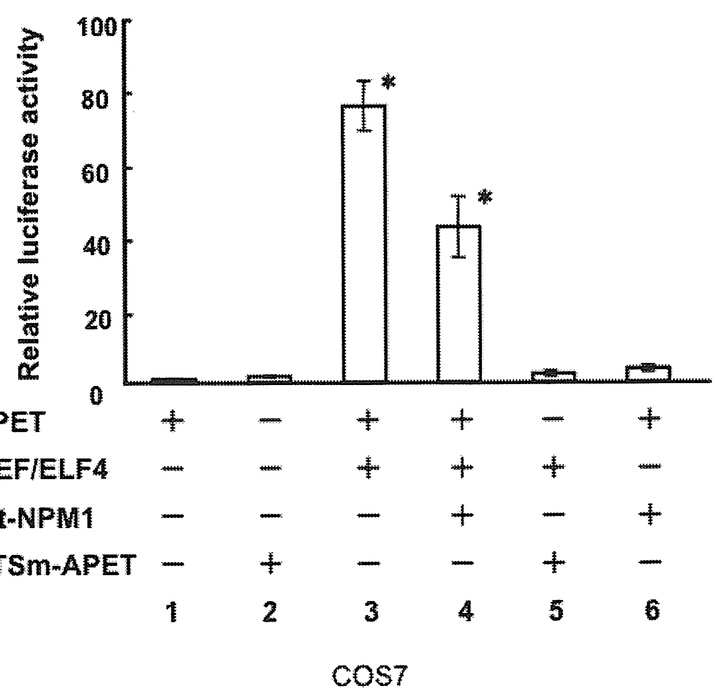

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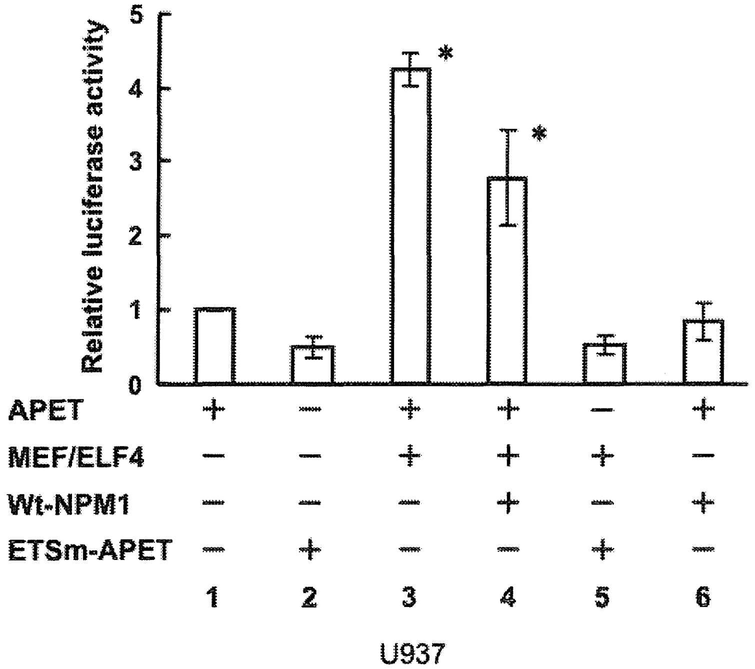

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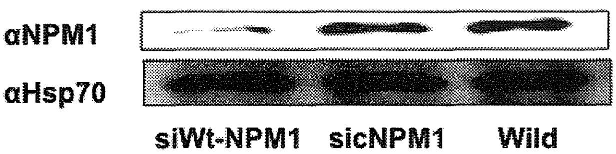

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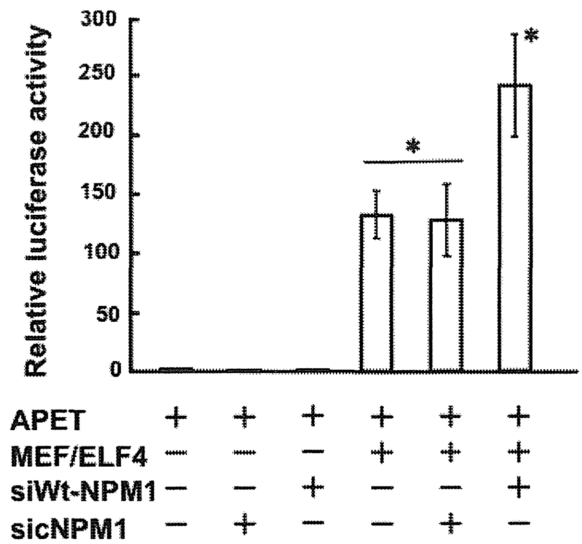

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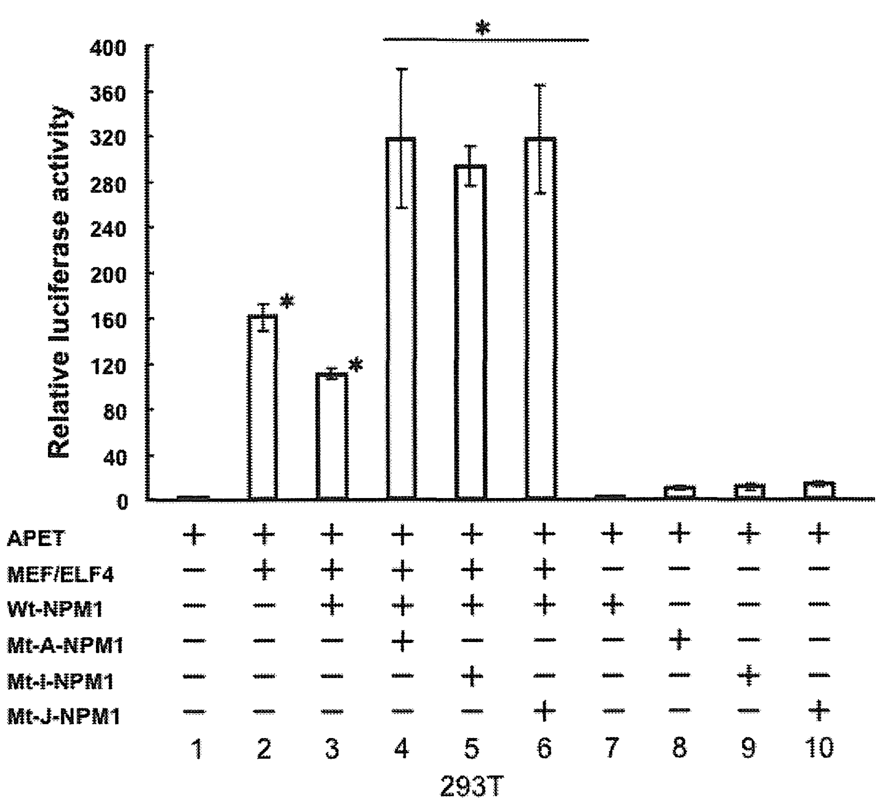

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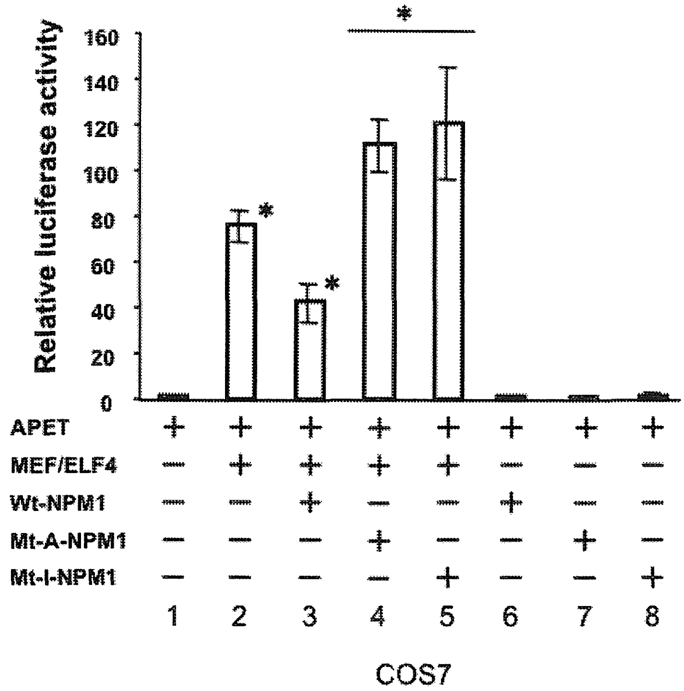

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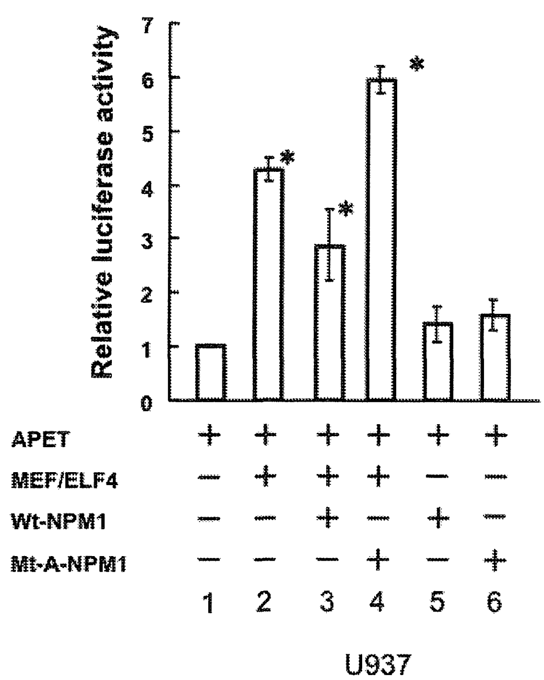

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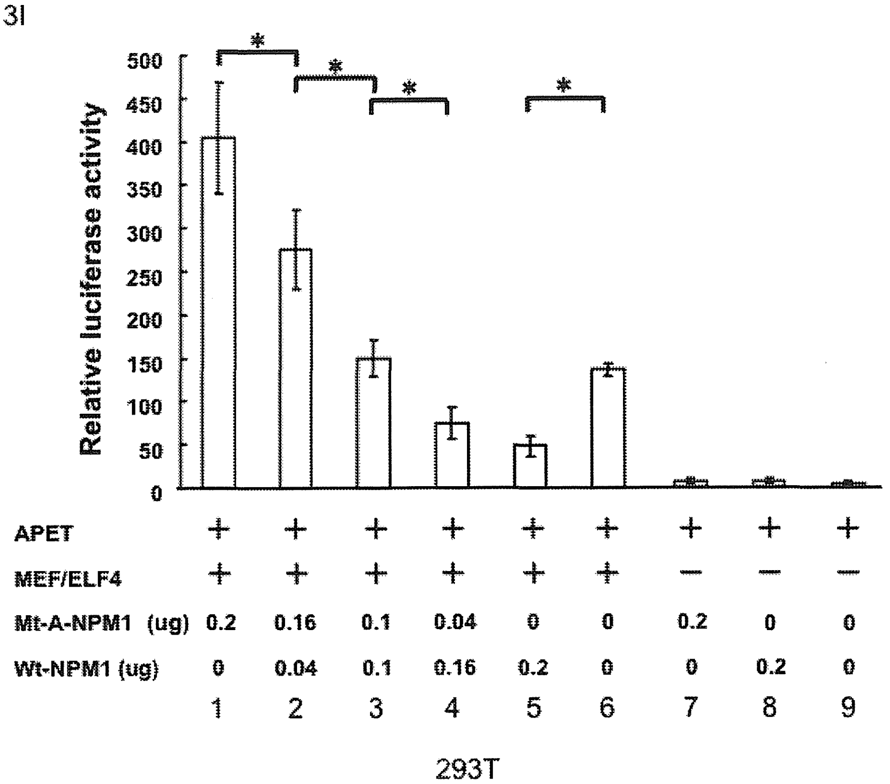

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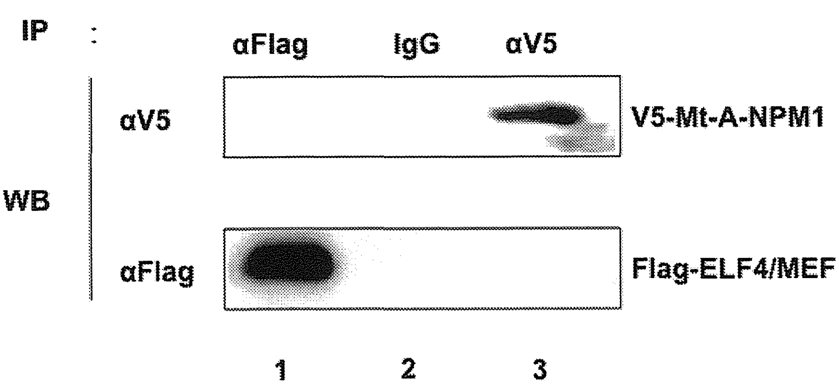

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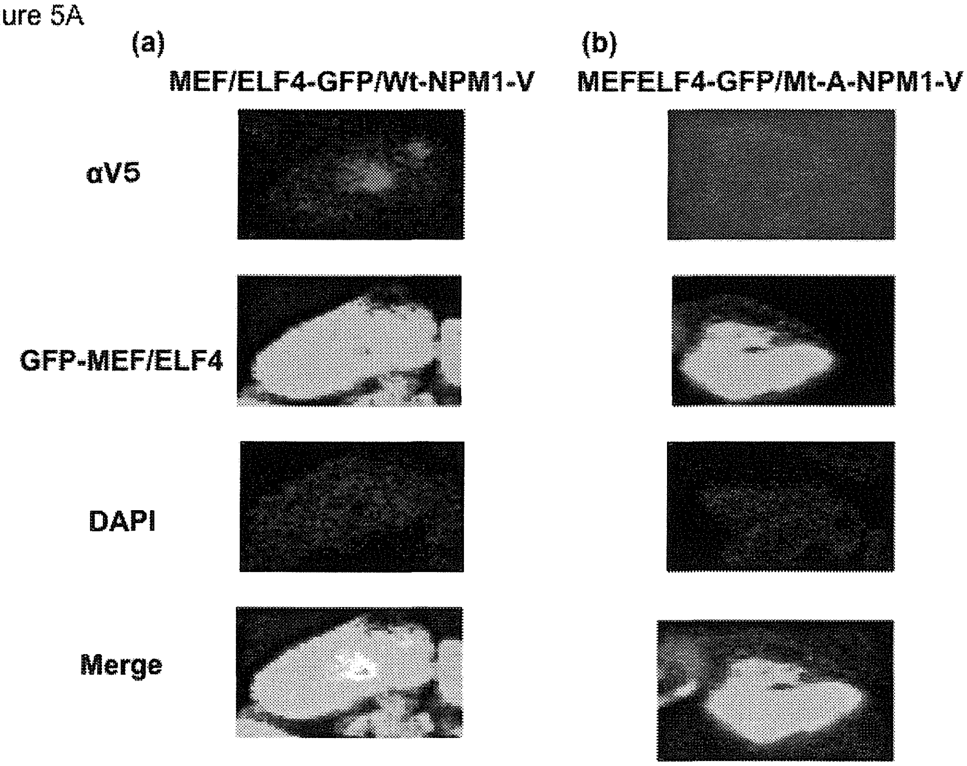

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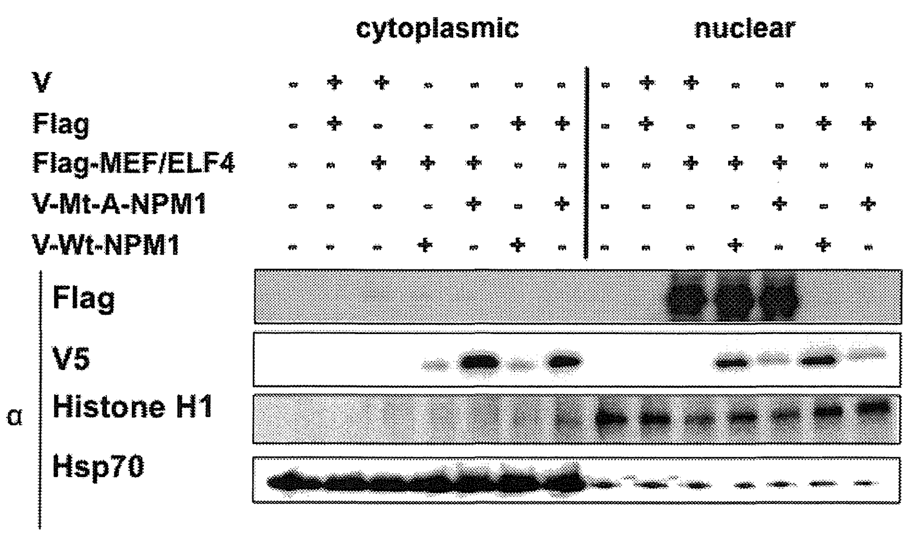

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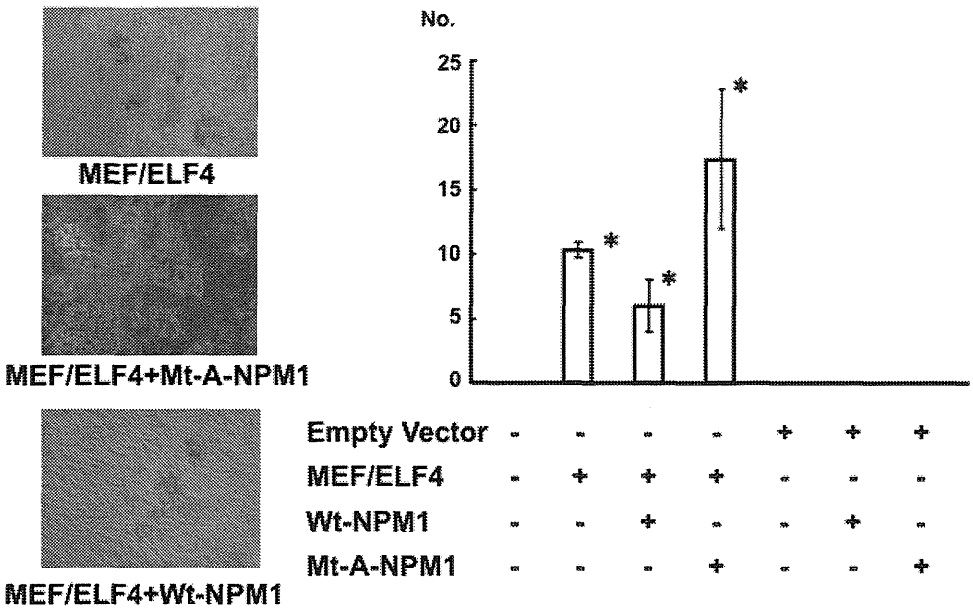

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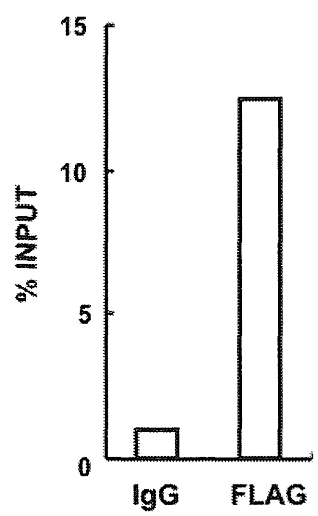

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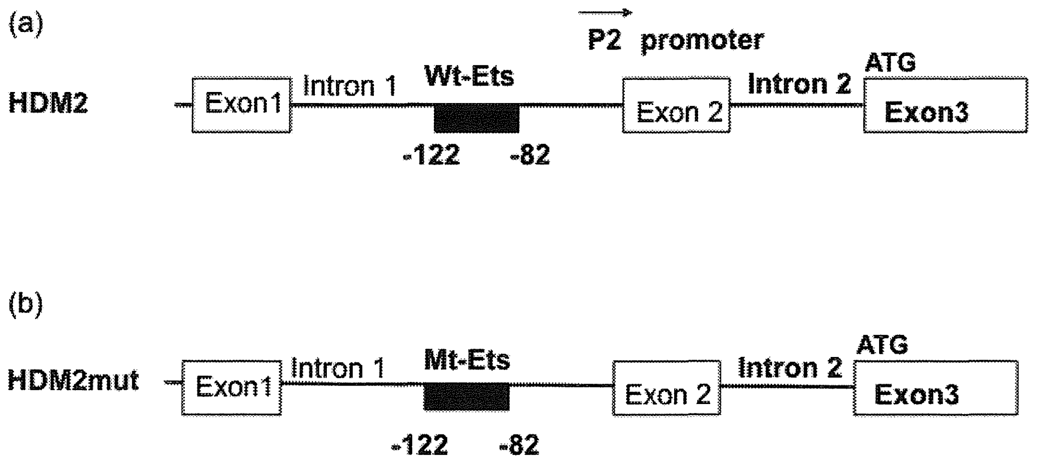


Figure 7C

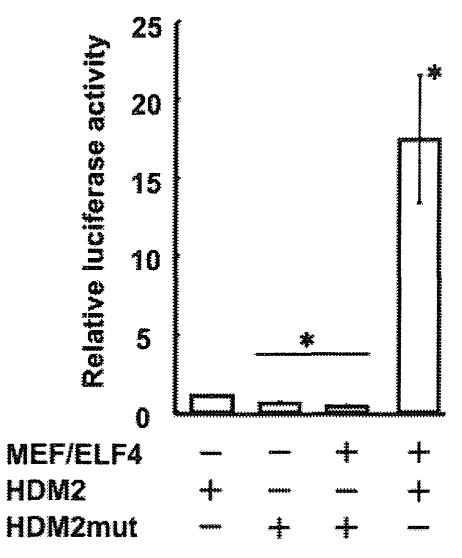
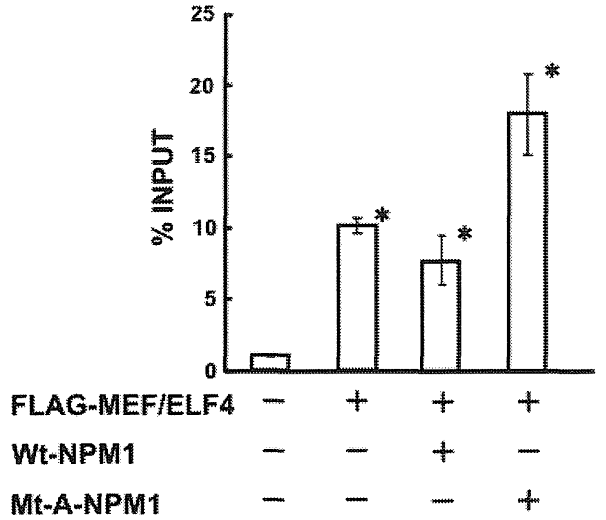


Figure 7D



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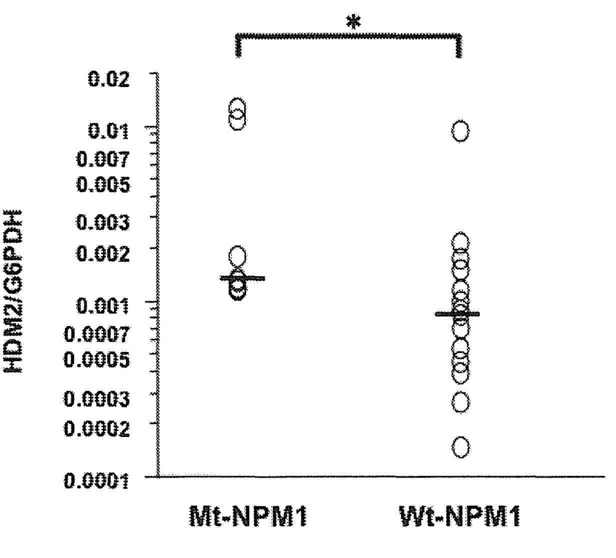

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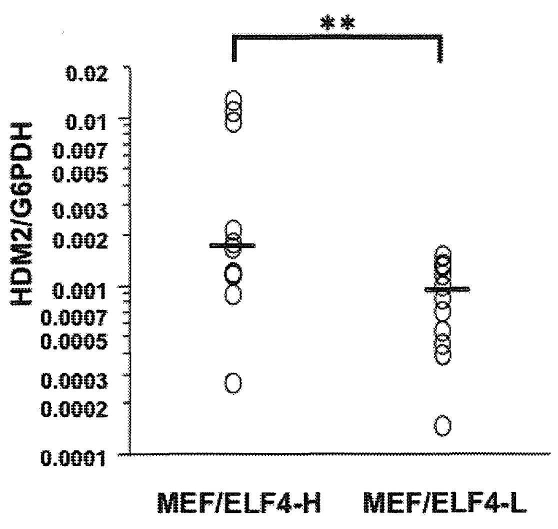
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HDM2/G6PDH
0.02
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MEF/ELF4-H
MEF/ELF4-L

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CASE REPORT

Successful treatment of a chronic-phase T-315I-mutated chronic myelogenous leukemia patient with a combination of imatinib and interferon-alfa

Hidehiro Itonaga · Hideki Tsushima · Tomoko Hata · Emi Matsuo · Daisuke Imanishi · Yoshitaka Imaizumi · Yasuhisa Kawaguchi · Takuya Fukushima · Yuko Doi · Sayaka Mori · Shimeru Kamihira · Masao Tomonaga · Yasushi Miyazaki

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Abstract The T315I BCR-ABL mutation in chronic myelogenous leukemia (CML) patients is responsible for up to 20% of all clinically observed resistance. This mutation confers resistance not only to imatinib, but also to second-generation BCR-ABL tyrosine kinases, such as nilotinib and dasatinib. A number of strategies have been implemented to overcome this resistance, but allogeneic stem cell transplantation remains the only established therapeutic option for a cure. A 61-year-old male was diagnosed with Philadelphia chromosome-positive chronic-phase CML in 2002. He was initially treated with imatinib and complete cytogenetic response (CCyR) was achieved 12 months later. However, after 18 months, a loss of CCyR was observed and a molecular study at 24 months revealed a T315I mutation of the BCR-ABL gene. At 30 months, imatinib/interferon-alfa (IFNα) combination therapy was initiated in an effort to overcome the resistance. Thirty months later, he re-achieved CCyR, and the T315I BCR-ABL mutation disappeared at 51 months. To our knowledge, this is the first case report showing the effectiveness of imatinib/IFNα combination therapy for CML patients bearing the T315I BCR-ABL mutation.

Keywords Chronic myelogenous leukemia · Imatinib · Interferon · T315I

H. Itonaga · Y. Miyazaki
Department of Hematology, Atomic Bomb Disease and Hibakusha Medicine Unit, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Nagasaki, Japan

H. Tsushima (✉) · T. Hata · D. Imanishi · Y. Imaizumi · T. Fukushima
Department of Hematology, Nagasaki University Hospital, 1-7-1 Sakamoto, Nagasaki 852-8501, Japan
e-mail: tsushima@nagasaki-u.ac.jp

E. Matsuo
Department of Internal Medicine, Nagasaki National Medical Center, Ohmura, Nagasaki, Japan

Y. Kawaguchi
Department of Hematology, Nagasaki Municipal Medical Center, Nagasaki, Nagasaki, Japan

Y. Doi · S. Mori · S. Kamihira
Department of Laboratory Medicine, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Nagasaki, Japan

M. Tomonaga
Department of Hematology, Japanese Red-Cross Nagasaki Atomic Bomb Hospital, Nagasaki, Nagasaki, Japan

Introduction

Chronic myelogenous leukemia (CML) is a clonal disease of the hematopoietic stem cell, which is characterized by an increased growth of predominantly myeloid cells in the bone marrow. The disease is associated with the Philadelphia chromosome, which arises by a reciprocal translocation between chromosomes 9 and 22 and harbors the BCR-ABL fusion oncogene [1]. Small molecules that specifically target the BCR-ABL gene product provide a successful treatment approach which can lead to a reduction in BCR-ABL transcripts below detectable levels. The drug imatinib, a rationally designed tyrosine kinase inhibitor (TKI), showed a superior response rate, improved progression-free survival, and overall survival, as compared with the previous standard therapy with IFNα [2–4].

Although high response rates are observed in patients who receive imatinib treatment, a small percentage of chronic-phase (CP) CML patients are refractory to the therapy [2]. Patients develop imatinib resistance via