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 (*$P < 0.05$).

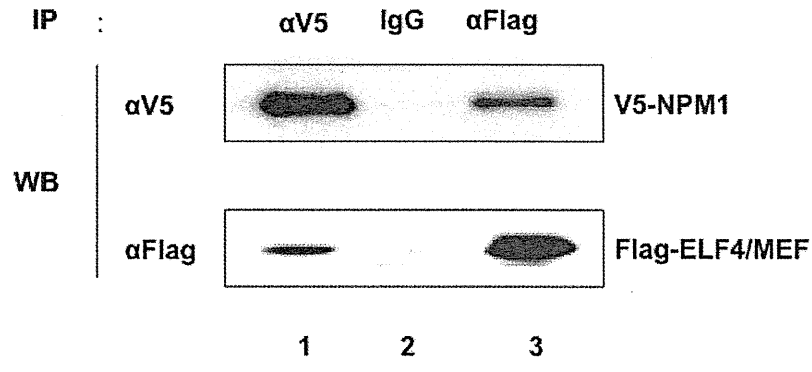
Figure 8

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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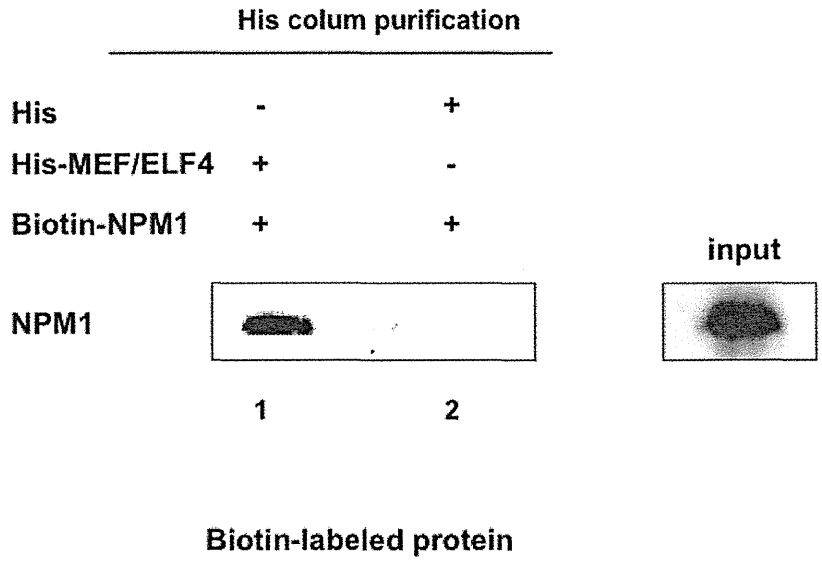


Figure 1C

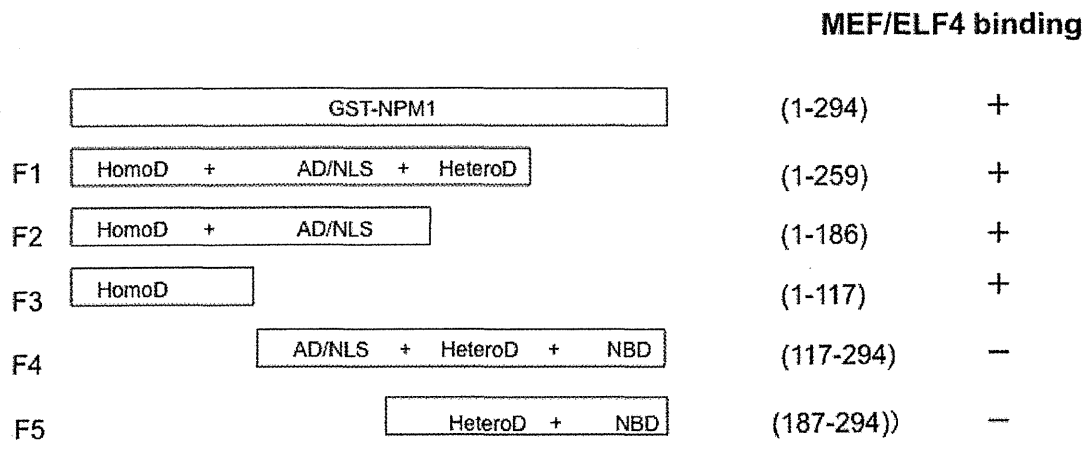
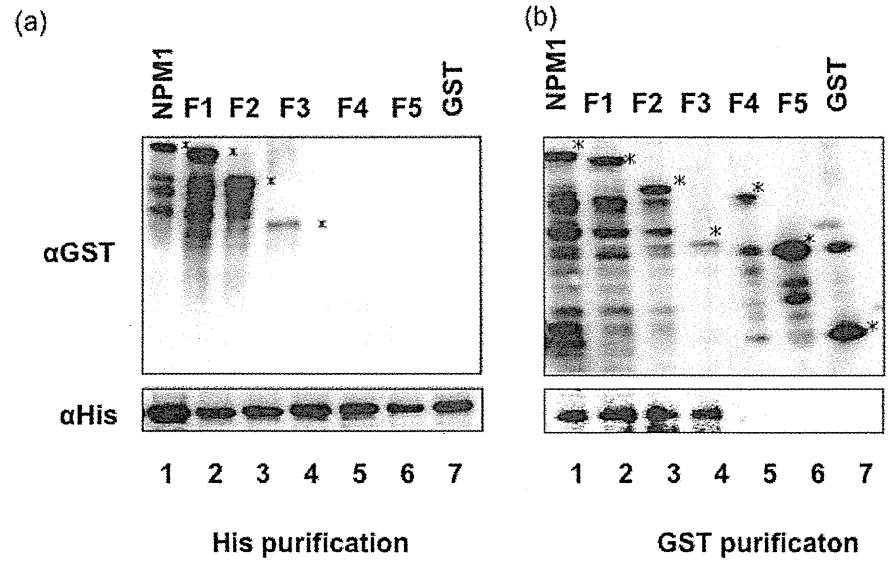


Figure 1D



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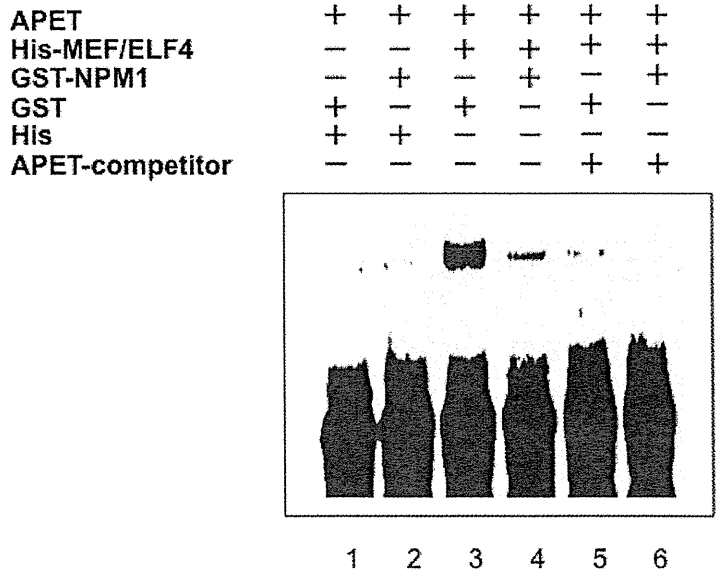
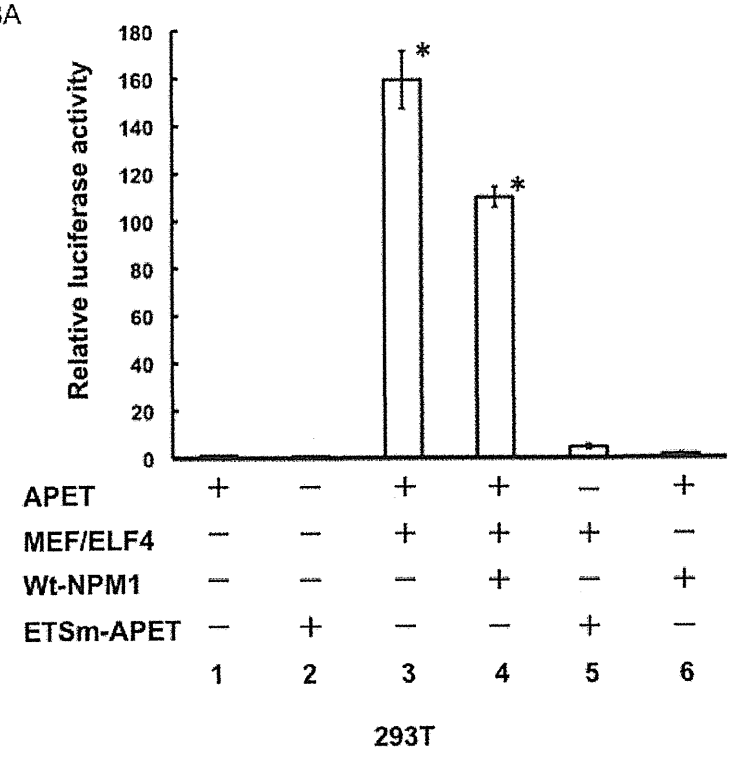
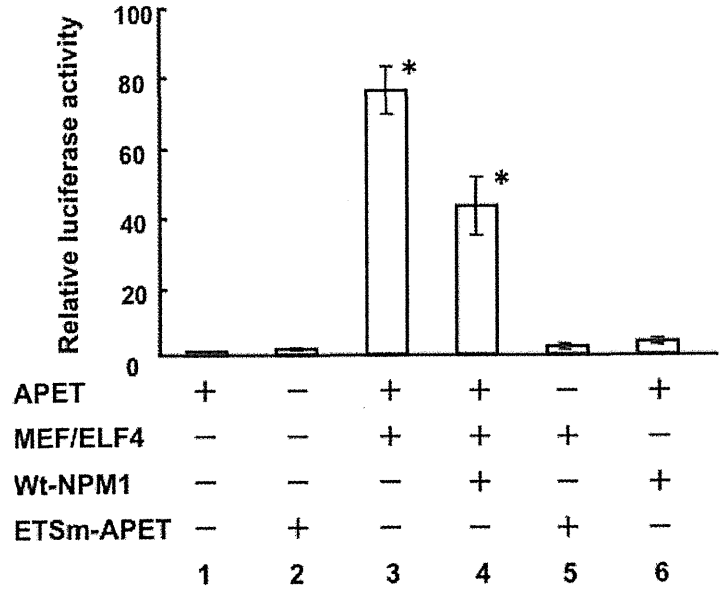


Figure 3A



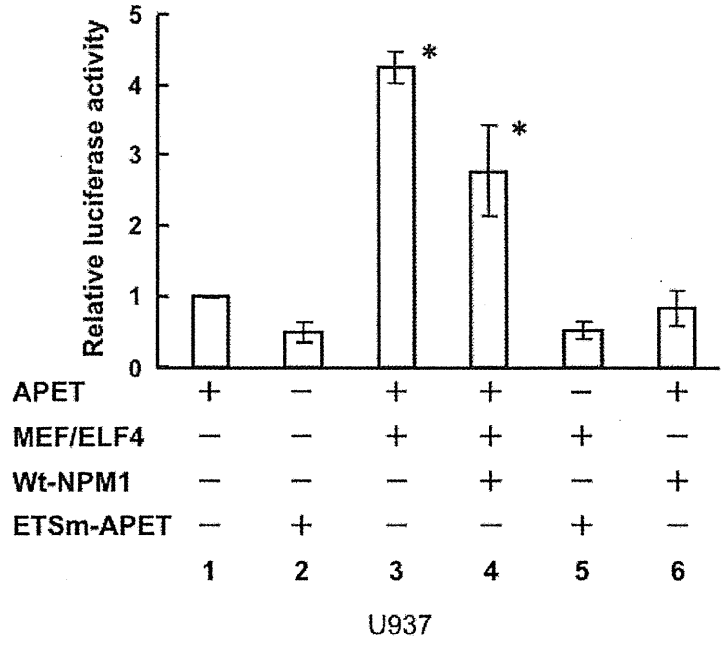
293T

Figure 3B



COS7

Figure 3C



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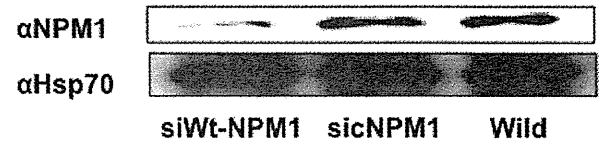

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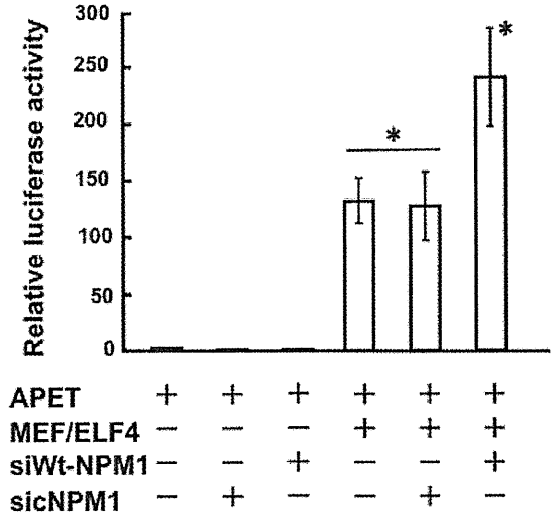

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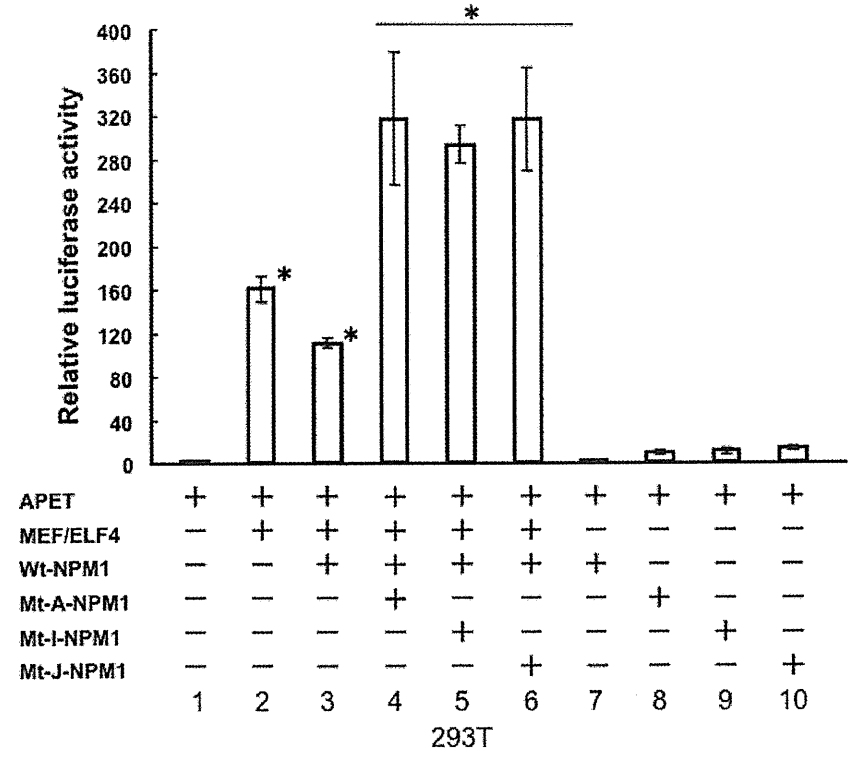

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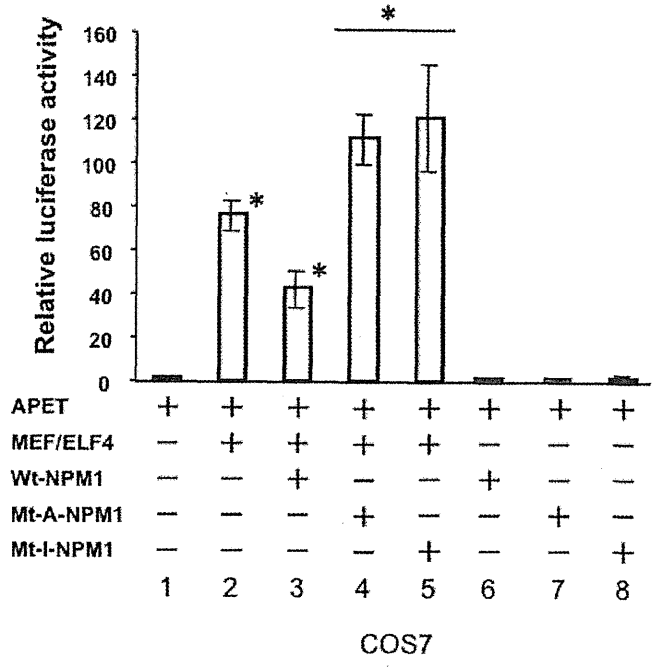

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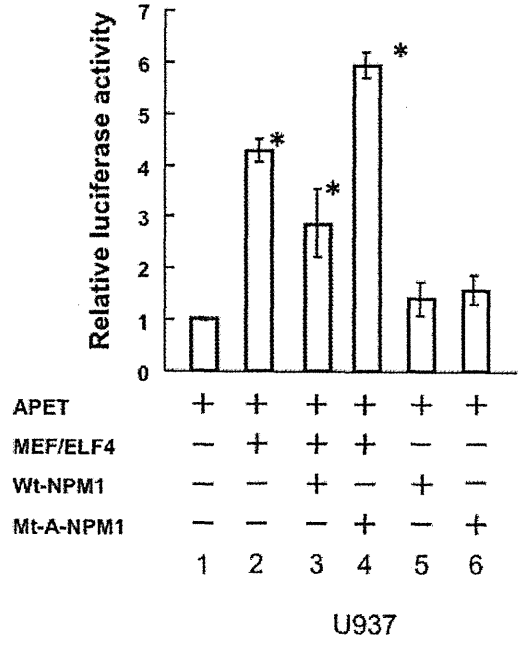

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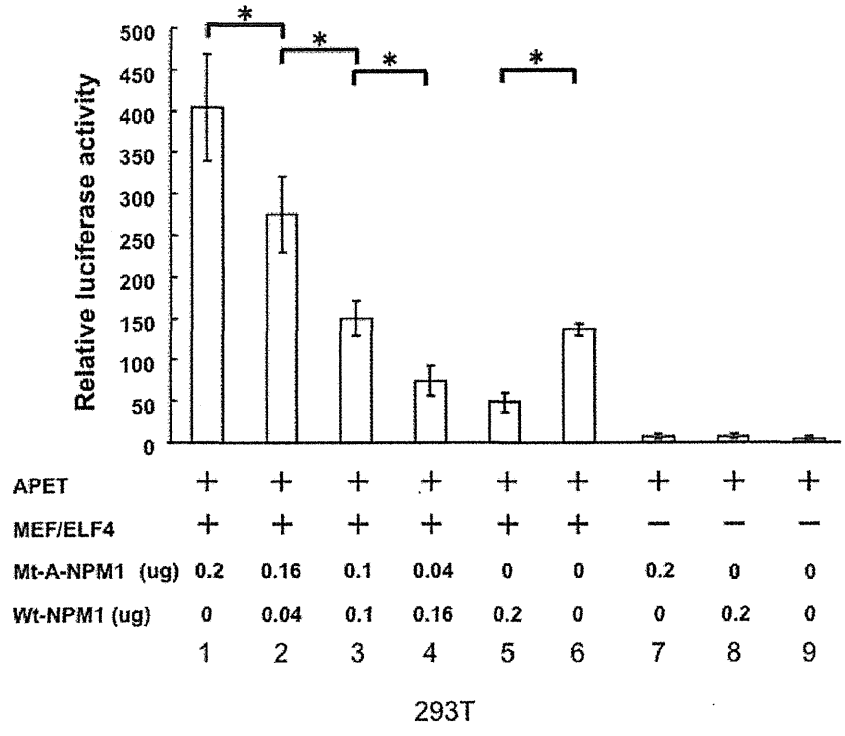

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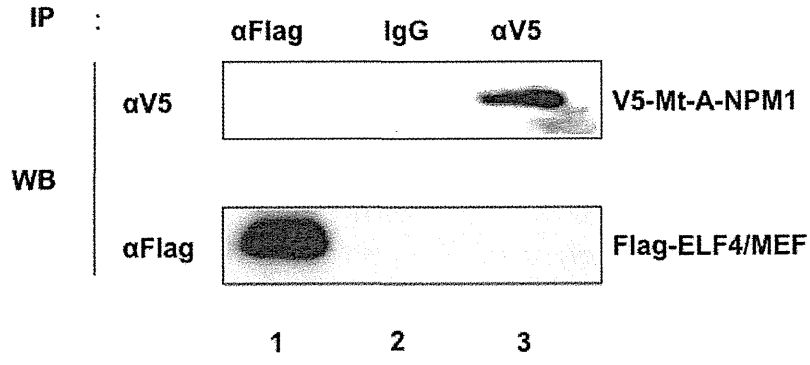

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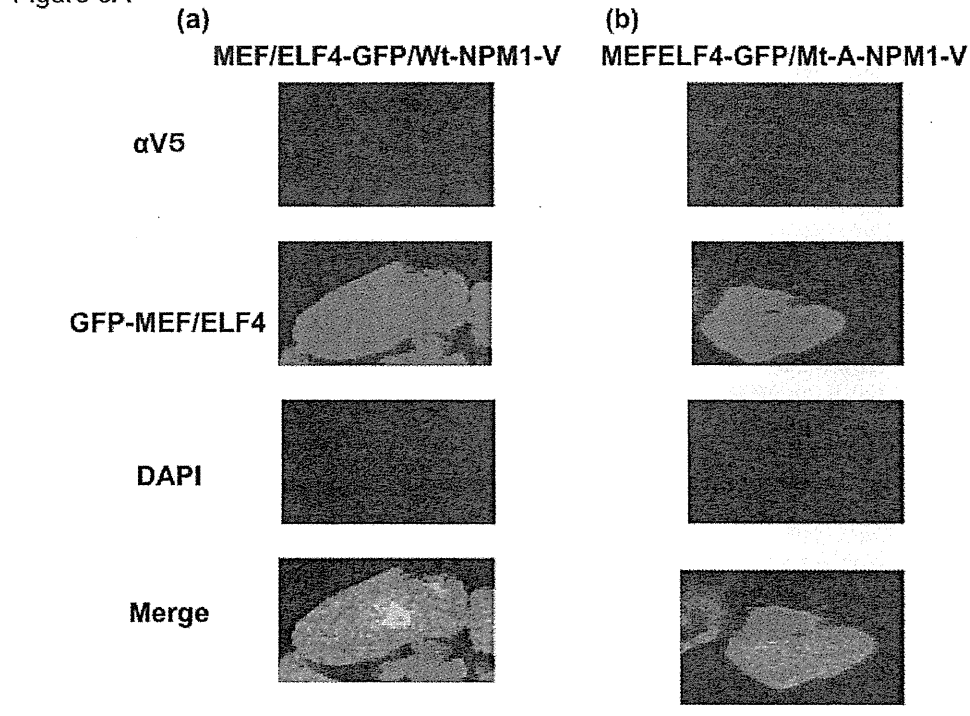


Figure 5B

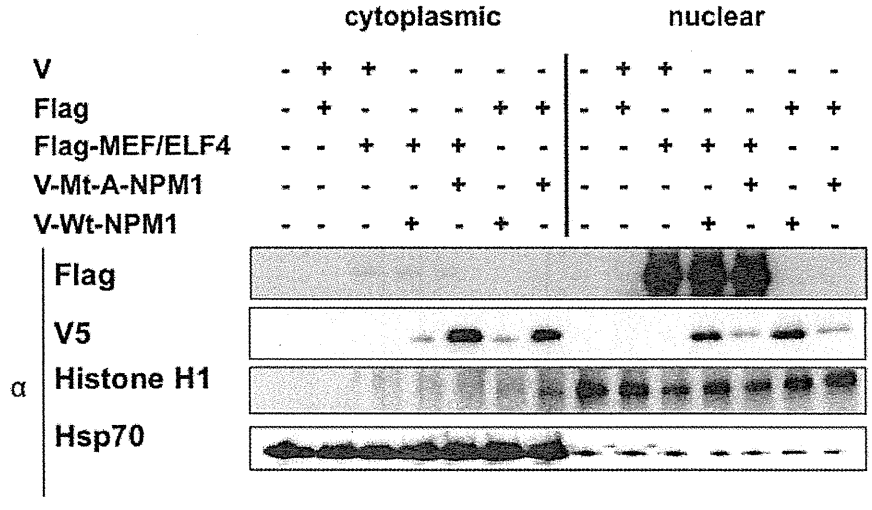
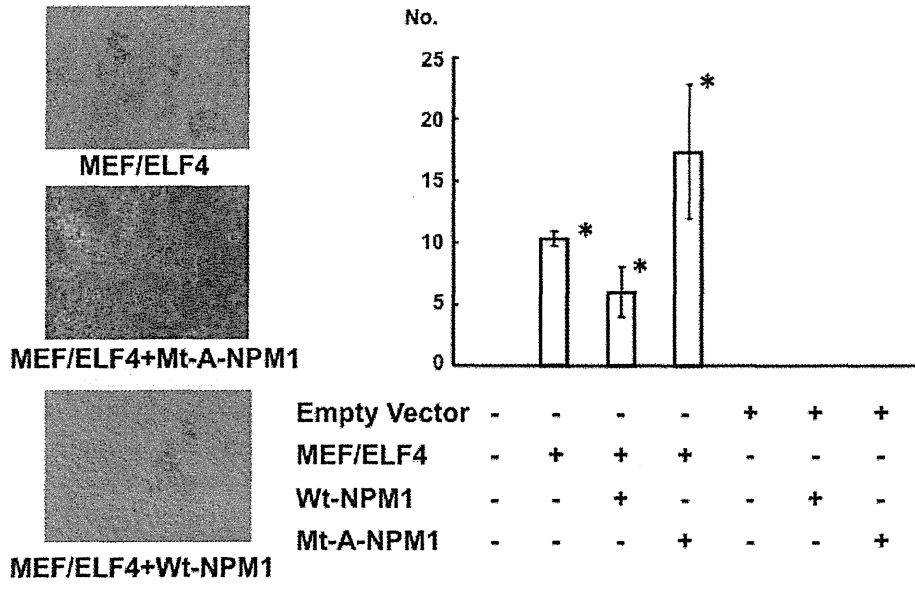


Figure 6



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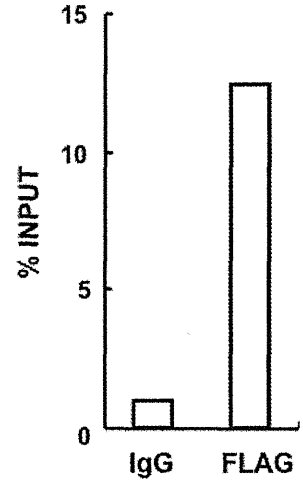


Figure 7B

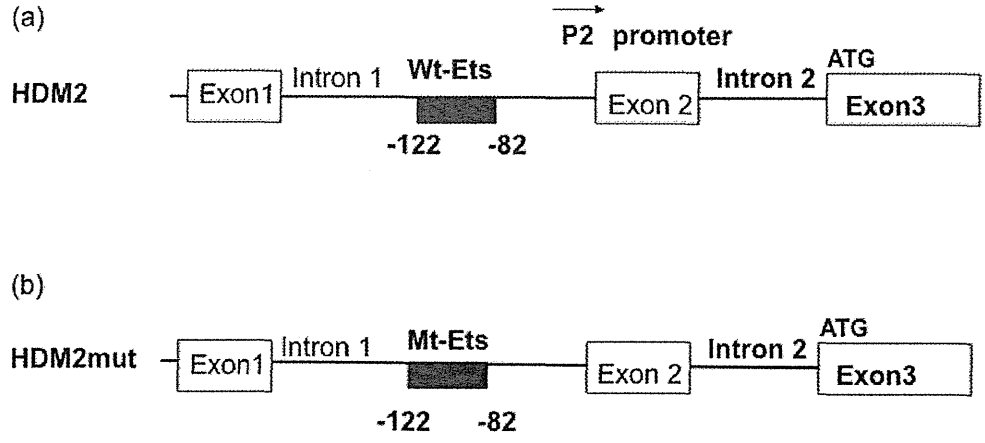


Figure 7C

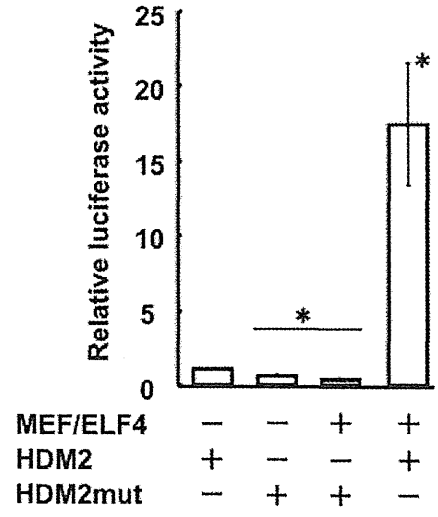


Figure 7D

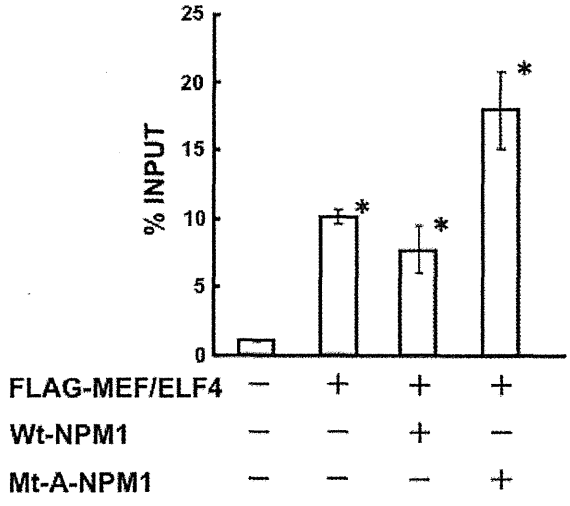


Figure 8A

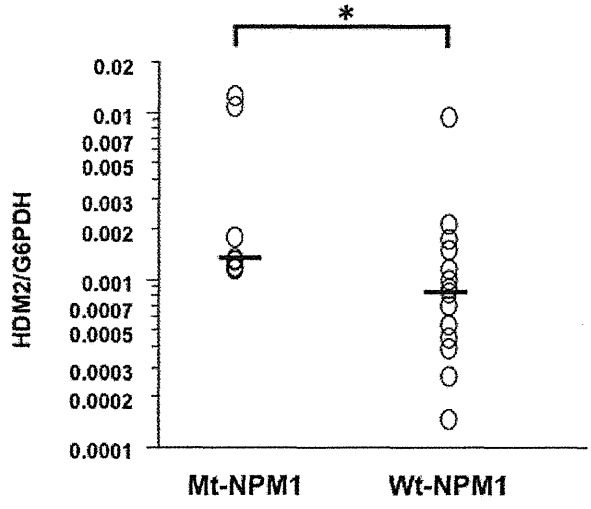
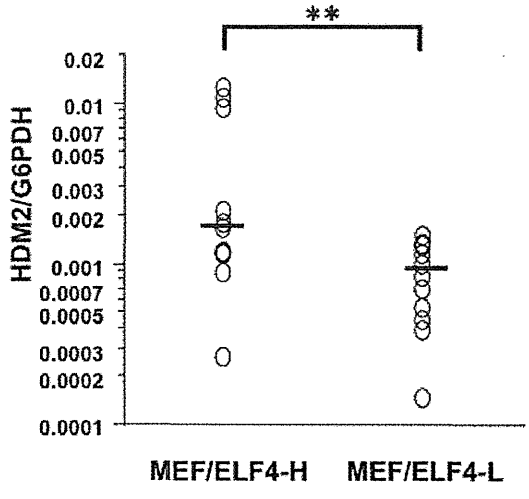


Figure 8B



Biol Blood Marrow Transplant xxx (2013) 1–9

Distinct Clinical Features of Infectious Complications in Adult T Cell Leukemia/Lymphoma Patients after Allogeneic Hematopoietic Stem Cell Transplantation: A Retrospective Analysis in the Nagasaki Transplant Group

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A B S T R A C T

Although allogeneic hematopoietic stem cell transplantation (allo-SCT) is performed as a curative option in adult T cell leukemia-lymphoma (ATL) patients, its high transplantation-related mortality raises a serious issue. The clinical features of infectious complications after transplantation are not well known. To analyze the impact of infections after allo-SCT for ATL, we retrospectively compared infectious complications in 210 patients at 3 institutions in Nagasaki prefecture between 1997 and 2009. There were 91 patients with acute myeloid leukemia (AML), 51 with acute lymphoblastic leukemia/lymphoblastic lymphoma (ALL/LBL), and 68 with ATL. No patient received ganciclovir or foscarvir as prophylaxis, and most patients received antifungal prophylaxis with fluconazole or micafungin. The cumulative incidence of cytomegalovirus (CMV) infection at 3 years was 69.2% in ATL patients versus 54.4% in AML patients ($P = .0255$). Cumulative infection-related mortality was significantly higher in ATL patients than in the 2 other groups (ATL versus AML, $P = .0496$; ATL versus ALL/LBL, $P = .0075$), and most death-causing pathogens were bacteria and fungus. The appearance of CMV infection was negatively associated with infectious mortality in ATL patients, but the P value for this association was near the borderline of significance ($P = .0569$). In multivariate analysis, transplantation using unrelated bone marrow and episodes of CMV infection were associated with worse overall survival in ATL patients, but were not in either AML or ALL/LBL patients. Collectively, the impact of infectious complications after transplantation in ATL patients was different from that in AML and ALL/LBL patients, suggesting that a more intensive strategy for infection control in ATL patients is required to reduce infectious mortality.

INTRODUCTION

Adult T cell leukemia-lymphoma (ATL) is a peripheral T cell neoplasm caused by human T cell lymphotropic virus type I (HTLV-I) [1-4]. The clinical features of ATL are heterogeneous and are characterized by various degrees of lymphadenopathy, abnormal lymphocytosis, hepatosplenomegaly, skin lesions, and hypercalcemia dividing the disease into 4 subtypes: acute, lymphoma, chronic, and smoldering [5]. Over the past decade, allogeneic hematopoietic stem cell transplantation (allo-SCT) was performed in young patients with aggressive ATL (acute, lymphoma, unfavorable chronic type) in Japan because aggressive ATL shows resistance to a variety of cytotoxic agents and has a poor outcome [6,7]. Several reports demonstrated that allo-SCT provided apparent long-term remission in some patients, along with the graft-versus-ATL effect [8-17]. However, transplantation-related mortality was higher than that observed for acute leukemia (acute myeloid leukemia [AML] and acute lymphoblastic leukemia/lymphoblastic lymphoma [ALL/LBL]), especially within 6 months of allo-SCT [10,14].

In general, ATL patients are susceptible to various opportunistic infections, including *Pneumocystis jirovecii* pneumonia (PJP), invasive fungal infections, and herpes virus diseases because of defective cellular immunity. Suzumiya et al. reported that cytomegalovirus (CMV) was involved in 35 of 47 autopsied cases of ATL and that CMV pneumonia was a significant cause of death [18]. Furthermore, it has been reported that development of PJP, invasive fungal infection, and herpes virus disease are more frequent in patients with ATL [19-22]. Collectively, infectious complications in patients with ATL may be different from those in patients with acute leukemia or malignant lymphoma, during allo-SCT. Recently,

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a nationwide retrospective study in Japan pointed out that infectious mortality was a main cause of transplantation-related mortality after transplantation for ATL [14]. However, there are no differences in prophylaxis and treatment for infection between ATL and other hematological diseases during the allo-SCT procedure. It remains unclear whether the current strategy for infection is sufficient or not for post-transplant patients with ATL because of the lack of detailed information for infectious complications after allo-SCT in these patients.

In the present report, we retrospectively analyzed 210 post-transplant patients with ATL, AML, or ALL/LBL to clarify differences in the clinical features of infectious complications after allo-SCT for patients with ATL.

PATIENTS AND METHODS

Patient Population

In this study, adult patients aged 16 years or older who received allo-SCT at 3 hospitals in Nagasaki prefecture with the diagnosis of AML, ALL/LBL, or ATL were included. These patients underwent allo-SCT between September 1997 and December 2009. Of 228 patients whose data were available, 18 were excluded because of death without neutrophil engraftment. The remaining 210 patients were included in the analysis. This study was approved by the Ethical Committees of the participating hospitals.

Definition of Clinical Endpoints and Responses

Neutrophil engraftment was defined by the recovery of an absolute neutrophil count of at least 0.5×10^9/L for 3 consecutive points; platelet recovery was defined by the recovery of a count of at least 500×10^9/L without transfusion support. Diagnosis and clinical grading of acute and chronic graft-versus-host diseases (aGVHD and cGVHD) were performed according to established criteria [23,24].

For ATL patients, response to treatment was divided into 4 categories: complete remission (CR), partial remission (PR), stable disease, and progressive disease. Responses were defined as follows: CR, disappearance of all disease; PR, ≥ 50% reduction in measurable disease; stable disease, failure to attain CR, PR, and no progressive disease; progressive disease, any new lesions or lesions with proliferation (increases in the size and number of abnormal cells). For acute leukemia patients, CR was defined as the presence of all of the following: fewer than 5% blasts in bone marrow, no leukemia blasts in peripheral blood, recovery of peripheral neutrophil counts to more than 1.0×10^9/L, and platelet counts to more than 100×10^9/L, and no evidence of extramedullary leukemia.

Prophylaxis of Infection, and Monitoring and Preemptive Therapy for CMV diseases

During the allo-SCT procedure, each patient was treated in a reverse isolation room, which was ventilated with a high-efficiency particulate air filtration system. As prophylaxis of fungal infection, itraconazole (200 mg/day) was administered to most patients from the start of conditioning [25,26]. In the cases of itraconasole intolerance because of its adverse effects, in principle, fluconazole (100 mg/day) was administered before the year of 2004, and micafungin (50 mg/day) was used after 2005 when micafungin became available for the prophylaxis of fungal infection after allo-SCT in Japan. If these drugs also could not be tolerated, either amphotericin B or voriconazole was administrated in accordance with the institutional strategy. Prophylaxis against PJP was performed primarily with oral trimethoprim-sulfamethoxazole [25]. All patients received prophylactic antibodies (ceftazidime or ciprofloxacin) after absolute neutrophil counts had become less than 0.5×10^9/L [25,27]. Acyclovir (1000 mg/day) was used for the prevention of diseases by the herpes simplex virus and varicella-zoster virus until day 100 after transplantation [25,28] regardless of seropositivity. After the recovery of neutrophils, CMV pp65 antigen in peripheral blood was monitored weekly through day 100 after transplantation to detect CMV antigenemia [29]. The CMV antigenemia test was considered positive if at least one positive-stained cell per 5.0×10^4 cells was detected on the slides. Preemptive therapy was initiated when CMV antigenemia became positive. In most cases, ganciclovir (GCV) was administrated at an induction dose of 5 mg/kg intravenously every 12 hours as preemptive therapy [30,31]. After antigenemia had been eliminated, GCV was either discontinued immediately or continued for a short time at a maintenance dose, in accordance with the institutional strategy. Based on the high positivity of CMV antibody among the Japanese population [32,33], the evaluation of CMV-serostatus before transplantation was depended on the institutional strategy. Instead, for prophylaxis of CMV, all patients received irradiated blood products, which were depleted of leukocytes by filters [34]. No patient was transfused with CMV-seronegative blood components.

Fungal Infections

Invasive fungal infections were divided into candidemia, invasive aspergillosis, and apparent organ damage by other fungi or molds [35]. Invasive aspergillosis was defined as possible (based on clinical signs and symptoms plus a compatible chest computed tomography scan or X-ray), probable (based on clinical signs and symptoms, compatible imaging test results, plus a positive respiratory tract culture for *Aspergillus spp.* or positive galactomannan assay), and definite (based on histology for an invasive mold infection by *Aspergillus*) infections. Candida infection was defined by the positive results of fungal cultures in blood or urine samples, or by evidence of infectious lesions in any organ system demonstrated by radiographic or histological evaluation.

CMV Infection and CMV Disease

Diagnosis of CMV infection and CMV disease was made based on previously described criteria [36]. In brief, CMV disease was defined by the presence of clinical signs and/or symptoms of end-organ disease combined with the detection of CMV infection in a biopsy specimen or bronchoalveolar lavage fluid in case of pneumonia. CMV infection was defined as the isolation of a virus or detection of viral protein or nucleic acids in any body fluid or tissue specimen. CMV infection included both CMV antigenemia and CMV disease.

Viral Infections Other Than CMV

Patients who were culture- or polymerase chain reaction (PCR)-positive for adenovirus with corresponding clinical signs and symptoms were considered to have an adenovirus infection involving those sites [37]. BK virus infection was defined as hemorrhagic cystitis with positive PCR results in urine samples [38], and for human herpes virus-6 (HHV-6) infection, detection of the virus genome by PCR from blood and/or cerebrospinal fluid [39].

Statistical Analysis

The Kaplan–Meier method was used to estimate overall survival (OS) after allo-SCT. The 95% confidence interval of 3-year OS was calculated. To illustrate the effects of cGVHD on OS, OS was measured from a predefined landmark time of 100 days after allo-SCT when analyzing the effect of this factor. Probabilities between subgroups were compared by means of the log-rank test. Cumulative incidences of infectious complications and infection-related death were calculated using Gray's method, considering deaths related to relapse or other complications than infection as a competing risk.

Furthermore, simultaneous effects of prognostic factors on OS were analyzed using multivariate regression analysis based on the Cox's proportional hazards model and linear logistic model, respectively. Variables considered were age of the patient at transplantation, sex of the patient, donor type, disease status at conditioning, and the t(9;22) chromosome abnormality or others for ALL/LBL, cytogenetic risk group defined by the Medical Research Council group [40,41] for AML and French-American-British classification of M0/M6/M7 or others for AML, clinical subtypes according to criteria of the Japanese Lymphoma Study Group for ATL [5], the incidence of CMV infection, disease and antigenemia, bacterial infection, fungal infection, viral infection other than CMV, aGVHD, the conditioning regimen, and institution where patients received allo-SCT. The most appropriate models were selected based on Akaike's information criteria. All analyses were performed using SAS version 9.2 software (SAS Institute, Cary, NC). Values of $P < .05$ were considered significant in all analyses.

RESULTS

Patient Characteristics and Transplantation Conditions

In 228 patients who received allo-SCT in our study, 7 AML, 2 ALL/LBL, and 9 ATL patients experienced graft failure and/or rejection. The rate of graft failure and/or rejection was not statically difference among the 3 groups.

The characteristics of remaining 210 patients are shown in Table 1. All ATL and ALL/LBL patients received standard-dose chemotherapy before the procedure of transplantation, but 10 AML patients did not receive any chemotherapy before allo-SCT. Sixteen related donors for ATL patients showed a positive result for the anti-HTLV-1 antibody. Peripheral blood mononuclear cells of these donors were subjected to Southern blot analysis to examine the monoclonal integration of the HTLV-1 provirus into the genome, and all 16 donors were confirmed as carriers of HTLV-1.

Table 1
Patient Characteristics

	AML	ALL/LBL	ATL
No. of patients	91	51	68
Median age at allo-SCT (range)	42 (17-70)	32 (16-60)	51 (30-67)
Male sex	41	21	42
Disease classification			
AML (French-America-British)			
M0	6		
M1	9		
M2	36		
M3	5		
M4	19		
M5	6		
M6	7		
M7	1		
Others	2		
AML cytogenetic risk group*			
Favorable	18		
Intermediate	46		
Adverse	21		
Unknown	6		
ALL cytogenetic			
t(9;22)		17	
Normal		13	
Others		17	
Unknown		4	
ATL subtype			
Acute			50
Lymphoma			17
Chronic			1
Disease status at allo-SCT			
CR	39	38	15
PR	-	-	21
Relapse/Induction failure	42	13	32
Untreated	10	0	0
Donor			
HLA-matched related donor	46	21	37
Alternative donor	45	30	31
HLA matching			
0 mismatched loci	73	35	47
1 mismatched locus	5	7	6
2 mismatched locus	13	9	15
Source of stem cells			
Bone marrow	56	31	30
Peripheral blood stem cell	23	14	20
Cord blood	12	6	18
Conditioning regimen			
Myeloabrative	74	45	32
Reduced-intensity Myeloabrative	17	6	36
GVHD prophylaxis			
Cyclosporine A + sMTX	45	22	33
Cyclosporine A ± others	3	1	10
Tacrolimus + sMTX	31	25	15
Tacrolimus ± others	12	3	10
Prophylaxis for fungal infection			
Itraconazole	51	29	40
Fluconazole	22	12	20
Micafungin	13	7	5
Voriconazole	3	1	3
Amphotericin	2	2	0
Acute GVHD			
Grade 0	47	18	40
Grade I	17	9	5
Grade II-IV	27	24	23
Chronic GVHD			
Absent	40	19	34
Limited type	15	10	5
Extensive type	29	18	16
Anti-HTLV-1 antibody of donor			
Positive	0	0	16
Negative	91	51	52
CMV serostatus of recipient			
Positive	52	30	37
Negative	7	5	0
Unknown	32	16	31

ATL patients were older and were more likely to receive reduced intensity conditioning regimens than patients with AML or ALL/LBL. Reduced-intensity conditioning using anti-thymocyte globulin was administered to 2 ATL patients. In general, HLA matched unrelated bone marrow recipients were more likely to receive tacrolimus-based GVHD prophylaxis than those who received allo-SCT using other stem cell sources. No patients received in vitro T cell-depleted transplantation. The procedure of prophylaxis for fungal infection was similar among 3 groups.

The median time to engraftment was 16 days (range, 7 to 29 days), 16 days (range, 10 to 45 days), and 16 days (range, 9 to 32 days) in AML, ALL/LBL, ATL groups, respectively. Acute GVHD developed in 44 AML (48.4%), 33 ALL/LBL (64.7%), and 28 ATL patients (41.2%). Severe acute GVHD (grade II-IV) was observed in 27 AML (29.7%), 24 ALL/LBL (47.1%), 23 ATL patients (33.8%). In 84 AML, 47 ALL/LBL, and 55 ATL patients who were alive for over 100 days after transplantation, chronic GVHD developed in 44 AML (52.4%), 28 ALL/LBL (59.6%), and 21 ATL patients (38.2%). Extensive chronic GVHD was observed in 29 AML (34.5%), 18 ALL/LBL (38.3%), 16 ATL patients (29.1%).

CMV Antigenemia, Disease, and Infection

The characteristics of CMV infection (CMV antigenemia and CMV disease) are shown in Table 2. The incidence of CMV antigenemia without CMV disease in the ATL group was similar to that in AML and ALL/LBL groups. One AML patient and 3 ALL/LBL patients with CMV antigenemia improved spontaneously, but all ATL patients received GCV and/or foscarvir with or without intravenous gamma globulin. CMV disease was documented in 13 of 91 AML patients (14.3%), 9 of 51 ALL/LBL patients (17.6%), and 15 of 68 ATL patients (22.1%). In patients who developed CMV disease, 2 AML and 4 ATL patients developed CMV diseases despite preemptive therapy for prior CMV antigenemia. Cumulative incidence of CMV infection at 100 days and 1 year were followed: 51.7% (95% CI: 40.7% to 61.5%) and 54.4% (95% CI: 43.2% to 64.3%) in AML groups, 63.9% (95% CI: 48.5% to 75.8%) and 63.9% (95% CI: 48.5% to 75.8%) in ALL/LBL groups, and 67.5% (95% CI: 54.3% to 77.7%) and 69.2% (95% CI: 56.0% to 79.2%) in ATL groups. There was a significant difference in the cumulative incidence of CMV infection between AML and ATL groups (AML versus ATL, $P = .0255$; ALL/LBL versus ATL, $P = .7011$). Among the patients with episodes of CMV infection, although all 32 ALL/LBL patient experienced the first CMV infection from engraftment to 100 days after allo-SCT (post-engraftment phase), 2 of 48 AML (4.2%) and 1 of 45 ATL patients (2.2%) experienced CMV antigenemia as first CMV infection after 100 days (late phase). In patients who experienced the improvement of CMV infection once, 12 of 46 AML (26.1%), 11 of 32 ALL/LBL (34.4%), and 15 of 43 ATL patients (34.9%) developed recurrent CMV infection.

All 12 CMV-seronegative patients received transplantation form CMV-seropositive donors. In these CMV-seronegative patients, 3 of 7 AML and 2 of 5 ALL/LBL

AML indicates acute myeloid leukemia; ALL, acute lymphoblastic leukemia; LBL, lymphoblastic lymphoma; ATL, adult T cell leukemia/lymphoma; allo-SCT, allogeneic hematopoietic stem cell transplantation; CR, complete remission; PR, partial remission; sMTX, short-term methotrexate; GVHD, graft versus host disease; CMV, cytomegalovirus.

* Based on the karyotype of leukemia cells, patients were classified into either the favorable, intermediate, or adverse risk group, defined by the Medical Research Council group.

Table 2
CMV Infection

	AML (n = 91)	ALL/LBL (n = 51)	ATL (n = 68)
CMV infection			
Antigenemia without CMV disease	36 (39.6%)	23 (45.1%)	30 (44.1%)
Pneumonia	5 (5.5%)	3 (5.9%)	8 (11.8%)
Gastroenteritis	7 (7.7%)	3 (5.9%)	4 (5.9%)
Hepatitis	0	3 (5.9%)	0
Pneumonia and Gastritis	1 (1.1%)	0	2 (2.9%)
Gastroenteritis and Hepatitis	0	0	1 (1.5%)

AML indicates acute myeloid leukemia; ALL, acute lymphoblastic leukemia; LBL, lymphoblastic lymphoma; ATL, adult T cell leukemia/lymphoma.

patients developed CMV antigenemia, but none had CMV disease.

Pathogens Other Than CMV That Caused Clinical Infection after Transplantation

Table 3 shows infectious agents other than CMV. Cumulative incidence of bacterial infection at 100 days and 1 year were followed: 13.4% (95% CI: 7.3% to 21.3%) and 16.0% (95% CI: 9.2% to 24.5%) in AML groups, 11.8% (95% CI: 4.7% to 22.3%) and 11.8% (95% CI: 4.7% to 22.3%) in ALL/LBL groups, and 20.9% (95% CI: 12.1% to 31.4%) and 23.0% (95% CI: 13.5% to 34.0%) in ATL groups. The ATL group showed the highest cumulative incidence of bacterial infection, but there was no significant difference among the 3 groups (data not shown). Serious bacterial infections were documented among 37 patients (sepsis, n = 27; pneumonia, n = 9; meningitis, n = 1). Three ATL patients exhibited *Pseudomonas aeruginosa* infection (pneumonia, n = 2; sepsis, n = 1), but none in AML and ALL/LBL groups did.

Cumulative incidence of fungal infection at 100 days and 1 year were followed: 2.2% (95% CI: 0.4% to 7.0%) and 5.8% (95% CI: 2.1% to 12.2%) in AML groups, 2.0% (95% CI: 0.2% to 9.3%) and 6.4% (95% CI: 1.6% to 16.1%) in ALL/LBL groups, and 7.5% (95% CI: 2.7% to 15.5%) and 9.4% (95% CI: 3.8% to 18.2%) in ATL groups. The ATL group showed the highest cumulative incidence of invasive fungal infection, without a significant difference among the three groups (data not shown). In total, 3 patients (2 with ATL and 1 with ALL/LBL) developed PJP without prophylactic oral trimethoprim-sulfamethoxazole, but none did in the AML group, even without the prophylaxis. Two ATL patients developed PJP with CR, at day 18 and 137 after allo-SCT, respectively. One ALL patient also developed PJP coincident with the relapse of ALL at day 392 after allo-SCT.

Cumulative incidence of viral infection other than CMV at 100 days and 1 year were followed: 15.9% (95% CI: 9.3% to 24.1%) and 29.1% (95% CI: 19.6% to 39.3%) in AML groups, 15.9% (95% CI: 7.4% to 27.3%) and 22.3% (95% CI: 11.8% to 34.9%) in ALL/LBL groups, and 24.5% (95% CI: 14.8% to 35.5%) and 33.1% (95% CI: 21.2% to 45.5%) in ATL groups. There was no significant difference among the 3 groups in the cumulative incidence of viral infections other than CMV (AML versus ALL/LBL versus ATL = 31.2% versus 25.0% versus 33.1% at 3 years) (data not shown).

Death Caused by Infections

Cumulative infection-related mortalities at 100 days and 1 year were followed: 2.3% (95% CI: 0.4% to 7.2%) and 6.1% (95% CI: 2.2% to 12.7%) in AML groups, 0.0% (95% CI: 0.2% to 9.5%) and 2.0% (95% CI: 0.2% to 9.5%) in ALL/LBL groups, and 9.4% (95% CI: 3.8% to 18.2%) and 14.7% (95% CI: 7.1% to 24.8%) in ATL groups. The ATL group showed the highest mortality, which was significantly greater than that of the other 2 groups (AML versus ATL, $P = .0496$; ALL/LBL versus ATL, $P = .0075$) (Figure 1A). However, in all 210 patients, ATL was not a significant factor despite the high hazard rate for infection-related mortality on multivariate analysis (Hazard rate (HR) 2.283; 95% confidence interval (CI): 0.834 to 6.251,

Table 3
Infectious Agents Other than CMV

	Pathogen	Pre-engraftment (Days 0 to Engraftment)	Post-engraftment (Engraftment to Days +100)	Late Phase (Days 100 to >365)
AML group (n = 91)	Bacteria	Gram-positive organism (n = 4) Gram-negative organism (n = 2)	Gram-positive organism (n = 6)	Gram-positive organism (n = 3) Gram-negative organism (n = 1)
	Fungus	*Aspergillus spp* (n = 1)	*Aspergillus spp* (n = 1)	*Candida spp* (n = 2) *Aspergillus spp* (n = 2)
	Virus*	None	*Human herpes virus 6* (n = 5) *Adenovirus* (n = 1) *Herpes simplex virus* (n = 3) *Varicella-zoster virus* (n = 2) *BK virus* (n = 2) *Epstein-Barr virus* (n = 1)	*Human herpes virus 6* (n = 1) *Adenovirus* (n = 1) *Herpes simplex virus* (n = 1) *Varicella-zoster virus* (n = 7) *Epstein-Barr virus* (n = 1)
ALL/LBL group (n = 51)	Bacteria	Gram-positive organism (n = 3)	Gram-positive organism (n = 1)	None
	Fungus	None	*Candida spp* (n = 1)	*Mucor spp* (n = 1) *Aspergillus spp* (n = 2) *Pneumocystic jirovecii* (n = 1)
	Virus*	*BK virus* (n = 1)	*Human herpes virus 6* (n = 3) *Adenovirus* (n = 3) *Herpes simplex virus* (n = 1) *Influenza virus* (n = 1)	*Varicella-zoster virus* (n = 4)
ATL group (n = 68)	Bacteria	Gram-positive organism (n = 6) Gram-negative organism (n = 5)	Gram-positive organism (n = 1) Gram-negative organism (n = 3)	Gram-positive organism (n = 3) Gram-negative organism (n = 2)
	Fungus	*Aspergillus spp* (n = 1)	*Candida spp* (n = 2) *Aspergillus spp* (n = 3)	*Aspergillus spp* (n = 2) *Pneumocystic jirovecii* (n = 1)
	Virus*	*Human herpes virus 6* (n = 2) *Adenovirus* (n = 1) *BK virus* (n = 2)	*Human herpes virus 6* (n = 2) *Adenovirus* (n = 5) *Varicella-zoster virus* (n = 3) *BK virus* (n = 1)	*Herpes simplex virus* (n = 1) *Varicella-zoster virus* (n = 3)

AML indicates acute myeloid leukemia; ALL, acute lymphoblastic leukemia; LBL, lymphoblastic lymphoma; ATL, adult T cell leukemia/lymphoma.
* Virus did not include CMV infection.