(16.5% *vs.* 12.5% at 2 years, p=0.546). Variables that showed a significant effect on RFS in the univariate Cox model included additional chromosome aberrations (p=0.005), peripheral blood blasts % (p=0.024) and sex (p=0.03). Results of multivariate analysis are shown in Table 1. The presence of additional chromosome aberrations was identified as the only independent prognostic factor for RFS (p=0.027). These updated data strongly support recent reports showing the feasibility and remarkable efficacy of imatinib-combined chemotherapy for newly diagnosed Ph- ALL.[3-9,14,15] The main objective of this report was to identify factors affecting RFS, an issue of rapidly increasing importance given the development of novel tyrosine kinase inhibitors which are expected to further expand the treatment options for this disease. Our data indicated that additional chromosome aberrations, particularly +der(22)t(9;22) and abn(9p), were associated with shorter RFS. It is well known that additional chromosome aberrations are seen frequently in Ph+ ALL. Before the imatinib era, some groups reported the prognostic relevance of additional aberrations.[16-18] By contrast, from a large series of 204 patients, Moorman *et al.*[19] recently showed no significant effect of specific additional aberrations, including +der(22)t(9;22) and del(9p), on survival. In this study, analyzing patients treated with imatinib-combined chemotherapy, the 2-year RFS rate exceeded 80% for those without additional aberrations, whereas outcomes for those with additional aberrations were relatively unfavorable.

Acquisition of resistance to imatinib is an emerging problem in the treatment of chronic myeloid leukemia. One of the most common mechanisms of resistance is the mutation involving the ABL kinase domain. Although it has not been confirmed whether such mutations compromise the clinical outcome of Ph+ ALL patients treated with imatinib-combined chemotherapy, our observation that most of the early relapses occurred during the consolidation courses consisting of imatinib alone implies possible imatinib resistance. If that is the case, switching from imatinib to other novel tyrosine kinase inhibitors based on the pre-treatment cytogenetic results soon after achieving CR or even earlier could be an alternative treatment approach for further improving outcome in Ph+ ALL. Lack of mutation analysis is a major limitation of this study. Recently, Pfeifer *et al.*[20] studied the ABL kinase domain mutation status in newly diagnosed Ph+ ALL patients who were treated with imatinib-combined chemotherapy, and showed that even before exposure to imatinib, mutations were detected in 38% of patients. Importantly, the frequency of the mutant allele was low in such patients. However, at the time of relapse, the same mutation was present as the dominant clone in 90% of the relapsing cases.[20] Altogether, further insights will be provided by investigating the association between karyotype and mutation status at diagnosis.

Despite such limitations, the analysis of 80 patients entered into a single trial identified karyotype at diagnosis as a significant prognostic factor for RFS in newly diagnosed Ph+ ALL patients treated with imatinib-combined chemotherapy. Although our results need to be confirmed regarding kinase domain mutation status, these findings may play a critical role in the future treatment of Ph+ ALL.

Table 1. Multivariate analysis of factors associated with relapse-free survival.

P-value	*HR (95% CI)**	*Factors*	
Additional [chromosome aberrations	0.027	2.84 (1.12-7.19) 1.00	Present Absent
Peripheral blood blasts%	0.051	1.12 (1.00-1.22)	Per 10% increase
Sex	0.148 1.00	1.73 (0.82-3.64) Female	Male

*HR, hazard ratio; 95% CI, 95% confidence interval. *Values higher than unity indicate higher risk for failure.*

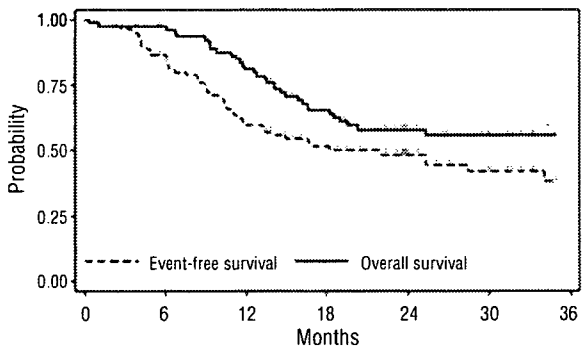

Figure 1. Kaplan-Meier curves for event-free and overall survival. The probabilities of event-free and overall survival at 2 years were 48.5% and 58.1% respectively (n=80).

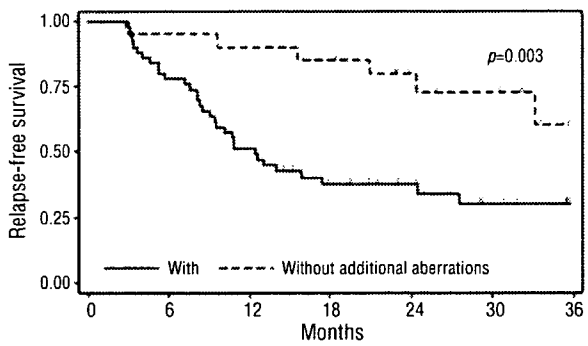

Figure 2. Relapse-free survival for patients with and without additional cytogenetic aberrations. Patients with additional cytogenetic aberrations (n=50) had significantly shorter relapse-free survival than those without (n=20).

Authorship and Disclosures

MY designed and co-ordinated the study, analyzed the data, and wrote the paper; JT, NU, FY, SM, and IJ designed the study, and provided patient sample and clinical data; IS, HA, KN, YU, MT, and AM provided patient sample and clinical data; HN co-ordinated the study, and revised the paper. YM provided patient sample and clinical data, and engaged in data management. SO designed the study, provided patient sample and clinical data, and engaged in data management; KM designed the study, and analyzed the data; TN chaired the study group, co-ordinated the study, and revised the paper; RO served as the principal investigator, chaired the study group, and revised the paper. All authors reviewed the paper, interpreted the results, and approved the final version. The authors reported no potential conflicts of interest.

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bjh research paper

Clinical significance of nuclear non-phosphorylated beta-catenin in acute myeloid leukaemia and myelodysplastic syndrome

Jinglan Xu,[1] Momoko Suzuki,[1] Yousuke Niwa,[1] Junji Hiraga,[1] Tetsuro Nagasaka,[2] Masafumi Ito,[3] Shigeo Nakamura,[2] Akihiro Tomita,[1] Akihiro Abe,[1] Hitoshi Kiyoi,[4] Tomohiro Kinoshita[1] and Tomoki Naoe[1]

[1]Department of Haematology and Oncology, Nagoya University Graduate School of Medicine, Tsurumai-cho, Showa-ku, Nagoya, [2]Department of Clinical Pathophysiology, Nagoya University Graduate School of Medicine, Tsurumai-cho, Showa-ku, Nagoya, [3]Department of Pathology, Japanese Red Cross Nagoya 1st Hospital, Michishita-cho, Nakamura-ku, Nagoya, and [4]Department of Infectious Diseases, Nagoya University Hospital, Tsurumai-cho, Showa-ku, Nagoya, Japan

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Correspondence: Jinglan Xu, Department of Haematology and Oncology, Nagoya University School of Medicine, 65 Tsurumai-cho, Showa-ku, Nagoya 466-8550, Japan. E-mail: xuj@med.nagoya-u.ac.jp

Summary

Wnt signaling activates the canonical pathway and induces the accumulation of non-phosphorylated beta-catenin (NPBC) in the nucleus. Although this pathway plays an important role in the maintenance of haematopoietic stem cells as well as in oncogenesis, the significance of nuclear NPBC remains unclear in malignant haematopoiesis. This study examined the expression of nuclear NPBC in bone marrow specimens from 54 and 44 patients with *de novo* acute myeloid leukaemia (AML) and myelodysplastic syndrome (MDS), respectively. On immunohistochemistry with an anti-NPBC antibody, the nuclei were positively stained in 22 and 18 of AML and MDS specimens, respectively. Staining of nuclear NPBC was associated with AML subtypes (M6 and M7), low complete remission (CR) rate, and poor prognosis. Nuclear NPBC was also associated with a high score when using the International Prognostic Scoring System (IPSS) for MDS and with −7/−7q and complex karyotypes. These findings suggest that *in situ* detection of nuclear NPBC by immunohistochemistry could provide new insights into the pathogenesis and prognosis of AML and MDS.

Keywords: beta-catenin, non-phosphorylated beta-catenin, acute myeloid leukaemia, myelodysplastic syndrome, immunohistochemistry.

The Wnt/beta-catenin pathway is involved in the self-renewal and proliferation of haematopoietic stem cells (Reya *et al*, 2003; Willert *et al*, 2003). Signaling is initiated by binding of Wnt proteins to transmembrane receptors of the Frizzeled family (Giles *et al*, 2003). In the absence of Wnt signals, a dedicated complex of proteins that includes the tumor suppressor gene product APC, axin, and glycogen synthase kinase-3-beta (GSK3-beta) phosphorylates specific serine and threonine residues within the N-terminal region of beta-catenin, which leads to the ubiquitination of beta-catenin and its degradation by proteasomes (Conacci-Sorrell *et al*, 2002; Noort *et al*, 2002; Staal *et al*, 2002; Giles *et al*, 2003). Wnt signals block GSK3beta activity, resulting in the accumulation of non-phosphorylated beta-catenin (NPBC), which is finally translocated to the nucleus (Noort *et al*, 2002; Staal *et al*, 2002). Nuclear NPBC interacts with T-cell transcription factor (TCF) and lymphoid enhancer factor (LEF), and it activates target genes such as *MYC* and *CCND1* (He *et al*, 1998; Tetsu & Maccormick, 1999). Therefore, nuclear NPBC is known to be oncogenic in many solid tumors (Bienz & Clevers, 2000; Polakis, 2000). Mutations of APC, beta-catenin, or axin, which are observed in various tumors, lead to stabilization of NPBC (Morin *et al*, 1997; Barker & Clevers, 2000).

In the bone marrow (BM), Wnt proteins activate the beta-catenin pathway and the non-obese severe combined immunodeficient (NOD-SCID)-repopulating capacity of normal haematopoietic stem cells. They lead to increased expression of *HOXB4* and *NOTCH1* implicated in the self-renewal of haematopoietic stem cells (Reya *et al*, 2003). Up-regulation of the beta-catenin pathway has been suggested in chronic myeloid leukaemia (CML)-derived granulocyte-macrophage progenitor cells (GMPs) and multiple myeloma (MM) cells (Derksen *et al*, 2004; Jamieson *et al*, 2004). Furthermore, beta-catenin reportedly plays a significant role in promoting cell proliferation, adhesion, and survival *in vitro* (Chung *et al*, 2002). The expression of beta-catenin is also enhanced by oncogenic FLT3 signals and associated with poor prognosis (Tickenbrock *et al*, 2005; Ysebaert *et al*, 2006). However, there are some contradictory reports. Studies of conditional knockout mice with a beta-catenin gene (*Ctnnb1*) deletion indicated that *Ctnnb1* is not indispensable for haematopoiesis (Cobas *et al*, 2004). Furthermore, an active form of *Ctnnb1* compromised haematopoietic stem cell maintenance and blocked differentiation in transgenic mice experiments (Simon *et al*, 2005). The role of the Wnt/beta-catenin pathway in malignant haematopoiesis therefore needs to be further elucidated.

According to previous studies, the expression of beta-catenin is associated with activation of the Wnt pathway as well as poor prognosis (Tickenbrock *et al*, 2005; Ysebaert *et al*, 2006). However, beta-catenin is associated not only with Wnt signaling but also with adherence junctions (Conacci-Sorrell *et al*, 2002). It is anchored to the cell inner surface membrane via cadherins. In normal bone marrow (BM), the vascular endothelium expresses a significantly higher amount of beta-catenin relative to the level in haematopoietic cells. Accordingly, immunohistochemical detection of nuclear NPBC would enable a better understanding of the role of beta-catenin in leukaemia.

This study investigated the subcellular localization of beta-catenin in BM specimens from acute myeloid leukaemia (AML) and myelodysplastic syndrome (MDS) patients using two anti-beta-catenin antibodies: one against C-terminal peptides and another against N-terminal non-phosphorylated peptides. The latter antibody detected nuclear NPBC, and positive staining for nuclear NPBC was associated with particular clinical characteristics of AML and MDS.

Materials and methods

Patient samples

For clinical samples, BM clots were obtained during routine diagnostic procedures. Beta-catenin expression was analyzed in BM specimens from patients newly diagnosed at the Nagoya University Hospital between 2000 and 2006 (Table I). The *de novo* AML patients consisted of 35 men and 19 women with a median age of 53 years (range, 20–81 years), and *FLT3* mutations were detected in seven of 22 patients with AML (31·8%). The MDS patients consisted of 28 men and 16 women with a median age of 57 years (range, 22–89 years). BM mononuclear cells were harvested by standard Ficoll/Paque density gradient centrifugation (Amersham Pharmacia Biosciences, Roozendaal, the Netherlands), and were suspended in RPMI 1640 medium supplemented with 10% fetal bovine serum, 100 IU/ml of penicillin G and 100 μg/ml of streptomycin.

Table I. Clinical characteristics of AML and MDS patients according to nuclear NPBC expression.

	Nuclear NPBC$^+$	Nuclear NPBC$^-$	*P*-value
Patients with *de novo* AML	22	32	
Age (median)	54 (20–81)	53 (18–71)	NS
Sex/male/female	18/4	17/15	0·005
Laboratory data			
WBC (×10^9/l; median)	3·5 (0·8–92·5)	5·3 (0·7–202·1)	NS
Hb (g/l; median)	74 (43–134)	98 (36–141)	0·01
PLT (×10^9/l; median)	7·6 (0·3–170)	4·2 (1·1–27·3)	NS
PB blasts (%; median)	24 (0–82)	39 (0–99)	NS
BM blasts (%; median)	46·2 (20–86·5)	77·5 (29–98)	0·02
CR rate	13/22 (59·1%)	24/27 (88·9%)	0·01
Relapse rate	16/21 (76·2%)	14/24 (58·3%)	0·03
Patients with MDS	18	26	
Age	59 (26–76)	57 (22–89)	
Sex/male/female	12/6	16/10	0·05
Laboratory data			
WBC (×10^9/l; median)	2·9 (1·2–9·0)	2·6 (1·6–5·9)	NS
Hb (g/l; median)	75 (46–151)	85 (47–127)	NS
PLT (×10^9/l; median)	79 (7–122)	44 (7–400)	NS
BM blasts (%; median)	3 (0–30)	5 (0–30)	NS
IPSS score*			
Low risk	0	4	NS
Intermediate-1	4	12	NS
Intermediate-2	4	4	NS
High risk	3	1	0·04

NPBC, non-phosphorylated beta-catenin; AML, acute myeloid leukaemia; MDS, myelodysplastic syndrome; WBC, white blood cell count; Hb, hemoglobin concentration; PLT, platelet count; PB, peripheral blood; BM, bone marrow; CR, complete remission; IPSS, International Prognostic Scoring System.

*Full data was available in 32 of the 44 patients with MDS.

Antibodies

For immunohistochemical and immunoblot studies, two monoclonal antibodies were used; one was against C-terminal peptides (clone14, IgG1; BD Transduction Laboratories/Life Science Research, Heidelberg, Germany), enabling recognition of pan beta-catenin (PBC), and the other was against

N-terminal-peptides (clone 8E4, IgG1; Alexis Biochemicals, Lausanne, Switzerland), enabling recognition of NPBC.

Immunohistochemical staining

Samples were fixed with ice-cold 4% paraformaldehyde for 16–24 h, embedded in paraffin, sectioned transversely (thickness, 3 μm), and processed for immunohistochemistry to determine the localization of beta-catenin. After removal of paraffin with xylene and dehydration with a series of ethanol solutions, the tissue sections were subjected to microwave irradiation (750 W) for 15 min in 0·01 mol/l citrate buffer (pH 6·0). The sections were then placed in an automated immunostainer (Ventana Medical Systems, Tucson, AZ, USA) as described (Xu *et al*, 2002). For negative controls, primary antibodies were replaced with mouse IgG. The subcellular distribution of beta-catenin (i.e. restriction to the nucleus or presence in the membrane) was assessed without knowledge of the French-America-British (FAB) subtypes, *FLT3* mutations or karyotypes. We investigated a single case twice for NPBC expression. The entire section was screened to find the region with the highest immunostaining. The score was determined in each case after counting at least 500 nuclei in 3–5 randomly selected regions. When 20% or more of the BM mononuclear cells were positive for nuclear staining of NPBC, they were classified as nuclear NPBC$^+$. The cut-off value of 20% was determined by the median distribution of the percentage of BM mononuclear cells stained by NPBC. As described in the results, some erythroblasts were positive for NPBC but the number was <20% except in the case of M6 patients. On the other hand, almost all blasts in M7 and other cases tested positive. The discrimination of cell types based on the 20% criterion therefore enabled a clear delineation.

Immunoblotting

Cell lysates from AML cells were extracted as previously described (Ozeki *et al*, 2004). A total of 1×10^6 cells were directly lysed in sample buffer and then subjected to sodium dodecyl sulphate-polyacrylamide gel electrophoresis on a 10% gel, and the separated proteins were transferred to a polyvinylidene difluoride membrane (Bio-Rad, Hercules, CA, USA). The membrane was initially incubated at room temperature for 1 h with 5% nonfat milk and 0·1% Tween-20 in Tris-buffered saline and then overnight with mouse monoclonal antibodies at a 1:2000 dilution in the same solution. After washing, the membrane was incubated for 1 h with a 1:5000 dilution of horseradish peroxidase-conjugated mouse antibodies to mouse IgG (MBL, Amersham, Bucks, UK), and immune complexes were then detected with enhanced chemiluminescence (ECL) reagents (Amersham).

Statistical analysis

The χ^2 test was used to calculate the difference of frequencies between nuclear NPBC$^+$ and NPBC$^-$ groups. The Mann–Whitney *U*-test was used to compare continuous variables. Kaplan–Meier curves were drawn using STATVIEW software (Macintosh; SAS Institute, Cary, NC, USA). *P*-values <0·05 were considered significant.

Results

Using an anti-beta-catenin C-terminal peptide antibody, beta-catenin was stained in the membrane and cytoplasm of erythroid cells from normal BM. In *de novo* AML specimens, significant staining was observed only in M6. This antibody also detected BM vessels whose density was increased in AML specimens as previously reported (Serinsöz *et al*, 2004; Fig 1A). On the other hand, an anti-N-terminal nonphosphorylated peptide antibody gave no significant staining in the normal BM. In AML specimens, nuclear NPBC was detected in erythroid blasts, megakaryoblasts and some myeloblasts (Fig 1A). In M6 specimens, nuclear NPBC was detected in 30–80% of myelomonocytic cells and nearly 100% of erythroblastic cells (Fig 1A). In M7 specimens, megakaryocytes were also strongly positive for nuclear NPBC (Fig 1A). In total, 20% or more of the BM mononuclear cells were positive for nuclear NPBC in 22 (40·7%) of 54 AML patients (Table I, Fig 1B). There was a strong male predominance of nuclear NPBC$^+$ cases, comprising 81·8% (18/22) in AML patients (Table I). However, the reason for this is unclear. In our cohort study, the karyotypes of female patients correlated to t(8;21)/t(15;17), which did not express nuclear NPBC. Thus the small numbers of studied patients seem to give some bias to the male predominance. A large-scale study is necessary to confirm this association. Nuclear NPBC$^+$ staining was closely associated with AML subtype: it occurred frequently (8/9) in M6 and M7 and rarely (0/7) in M3 (Fig 1B), and nuclear NPBC$^+$ was preferentially detected in erythroid and megakaryoblastic leukaemia compared to other myeloid leukaemias (M6–M7 vs. M0–M5, $P < 0·001$).

In MDS specimens, erythroid cells and endothelial cells were stained with the anti-beta-catenin C-terminal peptide antibody (Fig 2A). As observed for AML specimens, the cytoplasm and inner membrane were stained by this antibody. The anti-beta-catenin N-terminal nonphosphorylated peptide antibody detected nuclear staining in myeloblasts and erythroblasts that was similar to the pattern seen in AML cases (Fig 2A). Nuclear NPBC was found in 18 (40·9%) of 44 MDS patients, and was related to the FAB classification of MDS (Table I, Fig 2B). Nuclear NPBC$^+$ was preferentially detected in refractory anaemia with excess blasts in transformation (RAEBT) compared to other MDS subtypes [RAEBT *versus* refractory anaemia (RA)/refractory anaemia with ringed sideroblasts (RARS)/RAEB, $P = 0·01$].

To confirm whether these two antibodies recognized beta-catenin, a total of 41 samples from AML and MDS patients were subjected to immunoblot analysis. The anti-beta-catenin C-terminal peptide antibody detected bands at a molecular weight of 95 kDa, corresponding to beta-catenin, in most

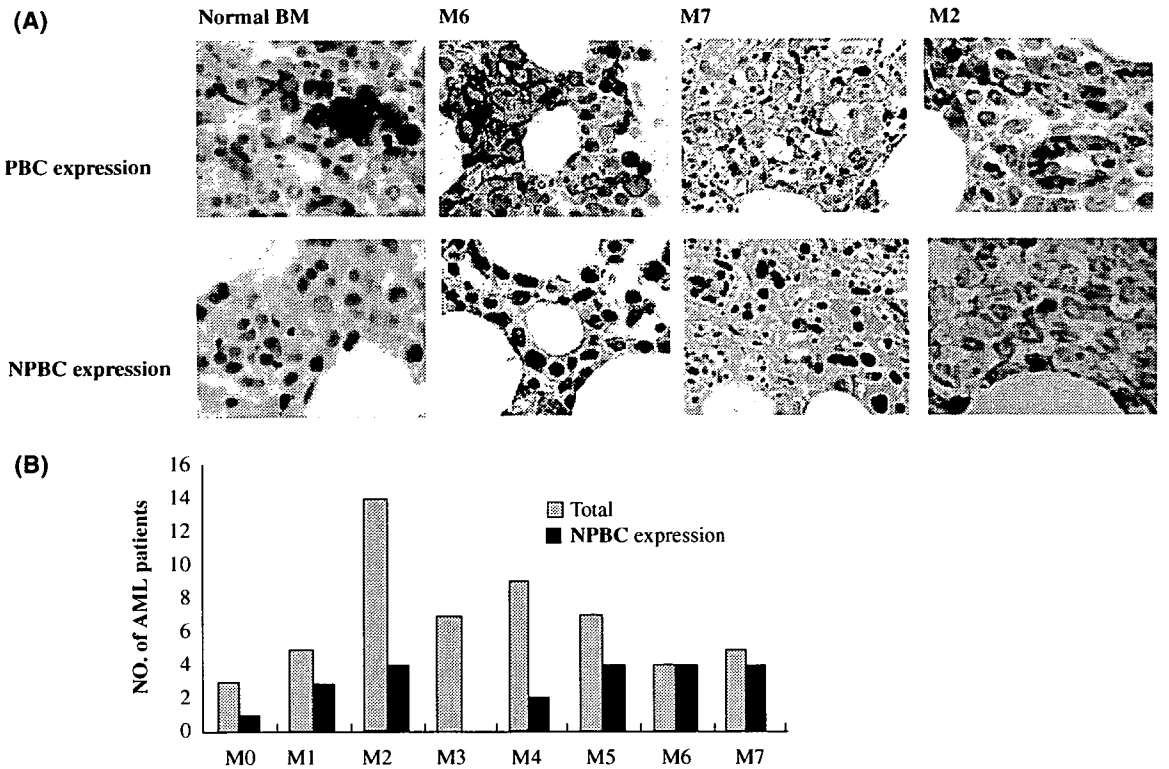

Fig 1. Specific association of NPBC expression with FAB subtypes of AML specimens. (A) In normal BM clots, PBC is expressed on the membranes of erythroid cells as well as endothelial cells. NPBC was not detected in normal BM clots. Most erythroid cells and endothelial cells showed cell membrane expression of PBC without expression in the nucleus or cytoplasm. NPBC was expressed in leukemia cells and was always restricted to the nucleus, especially in M6 and M7 specimens. Prominent staining of endothelial cells was seen in the vascular tissue in BM derived from an M2 patient with, though NPBC staining was negative in the same specimen. Original magnification ×40. (B) The graph presents data based on nuclear NPBC staining in paraffin sections from 54 patients with AML.

samples except for M3 (Fig 3A). The anti-N-terminal nonphosphorylated peptide antibody gave bands of the same size in only a few samples of AML and MDS (Fig 3B). The results of immunoblotting corresponded to those of immunostaining, although the latter was more sensitive than the former.

The above findings suggest that expression of nuclear NPBC could be used to identify some subsets of AML and MDS. Next we studied whether nuclear NPBC was associated with chromosomal abnormalities or genetic alterations. Previous studies suggested that AML-associated translocations, such as t(8;21) and t(15;17), contributed to the activation of gamma-catenin, or that *FLT3* mutation might be associated with the stabilization of beta-catenin. In this study, however, nuclear NPBC was never detected in AML with t(8;21) or t(15;17). In AML/MDS with −7/−7q and a complex karyotype, nuclear NPBC was frequently detected ($P = 0{\cdot}007$ and $P = 0{\cdot}02$, respectively; Table II). Moreover, detection was not related to *FLT3* internal tandem duplication (ITD; Table II).

Finally, we studied whether clinical characteristics and outcome were different between nuclear NPBC$^+$ and NPBC$^-$ AML patients. NPBC$^+$ AML patients showed significantly lower hemoglobin levels, lower blast percentages in the BM, and lower CR rates (Table I). There were no significant differences between the NPBC$^+$ and NPBC$^-$ groups in the MDS patients (Table I). However, nuclear NPBC was associated with a high International Prognostic Scoring System (IPSS) score (Table I). Of note, nuclear NPBC$^+$ AML patients had worse overall survival than NPBC$^-$ AML patients (Fig 4A). Even if the M6/M7 subtype and/or M3 subtype was excluded from the analysis, there was still a significant association between nuclear NPBC$^+$ and survival (Fig 4B–D).

Discussion

This study used nuclear NPBC as a biomarker for the activated Wnt/beta-catenin signaling pathway. The anti-beta-catenin C-terminal peptide antibody detected total beta-catenin in immunoblots but mainly cytoplasmic and membrane-associated beta-catenin in immunohistological analysis, whereas the anti-beta-catenin N-terminal nonphospholylated peptide antibody detected only nuclear beta-catenin in both analyses (data not shown). Accordingly, it was concluded that the nuclear staining with the latter antibody identified nuclear nonphosphorylated beta-catenin. Nuclear NPBC was detected in 22 (40·7%) of 54 AML patients and 18 (40·9%) of 44 MDS patients. Positive staining of nuclear NPBC was associated with particular AML subtypes (M6 and M7), a low complete remission rate, and poor prognosis. The presence of nuclear

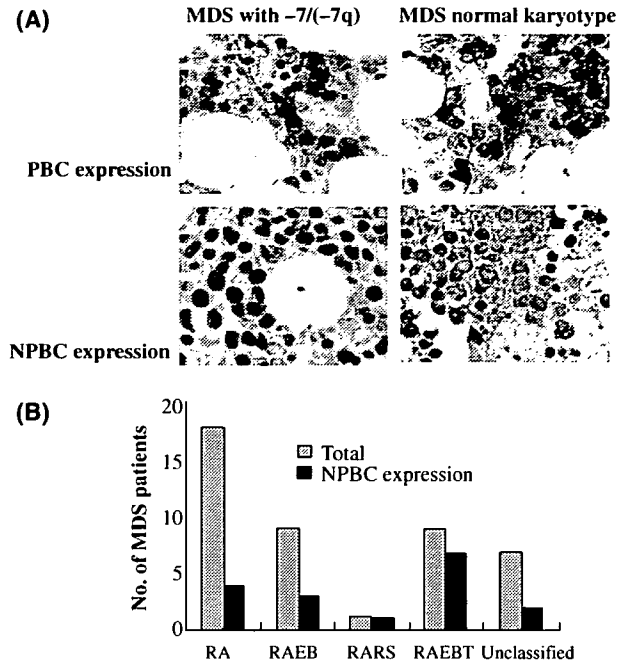

Fig 2. Specific association of NPBC expression with FAB subtypes of MDS specimens. (A) Most erythroid cells and endothelial cells showed cell membrane expression of PBC without expression in the nucleus or cytoplasm. NPBC was expressed in erythroid cells and was always restricted to the nucleus, especially in refractory anaemia with excess blasts in transformation (RAEBT) and MDS specimens with −7/−7(q). Original magnification ×40. (B) The graph presents data obtained for nuclear NPBC staining in paraffin sections from 44 patients with MDS. RA, refractory anaemia; RARS, RA with ringed sideroblasts; RAEB, RA with excess blasts.

NPBC was also associated with a high IPSS score of MDS and with −7/−7q and complex karyotypes.

Previous reports indicated that a significant proportion of AML samples expressed beta-catenin on immunoblot analysis.

Table II. Cytogenetic abnormalities and *FLT3* mutation according to nuclear NPBC expression.

	Nuclear NPBC$^+$ (*N*)	Nuclear NPBC$^-$ (*N*)	*P*-value
Karyotypes			
t(8;21)	0	3	NS
t(15;17)	0	4	NS
−5/−5q	4	1	NS
−7/−7q	12	6	0·007
Complex	13	7	0·02
Others	19	2	NS
Normal	10	31	0·0003
Unknown	7	1	0·02
FLT3 mutation			
Wild type	3	12	0·006
ITD	2	5	NS

Patients are counted more than once due to the coexistence of more than one cytogenetic abnormality. Complex: patients had three or more cytogenetic abnormalities.
NPBC, non-phosphorylated beta-catenin; ITD, internal tandem repeat; NS, not significant.

The expression of beta-catenin is related to CD34 expression, poor prognosis and clonogenic capacity *ex vivo* (Ysebaert *et al*, 2006). Furthermore, beta-catenin is expressed in normal CD34$^+$ progenitor cells and the expression level is reduced upon differentiation (Simon *et al*, 2005). In these studies, however, the total beta-catenin level was analyzed only by immunoblot analysis. Beta-catenin is expressed not only as nuclear NPBC but also as a cadherin-associated protein in the inner cytomembrane (Conacci-Sorrell *et al*, 2002). The present study found that normal erythroblasts expressed cytoplasmic or membrane-associated beta-catenin but not nuclear NPBC (Fig 1A). Both membrane-associated and nuclear beta-catenin was expressed in malignant erythroblasts in subtype M6

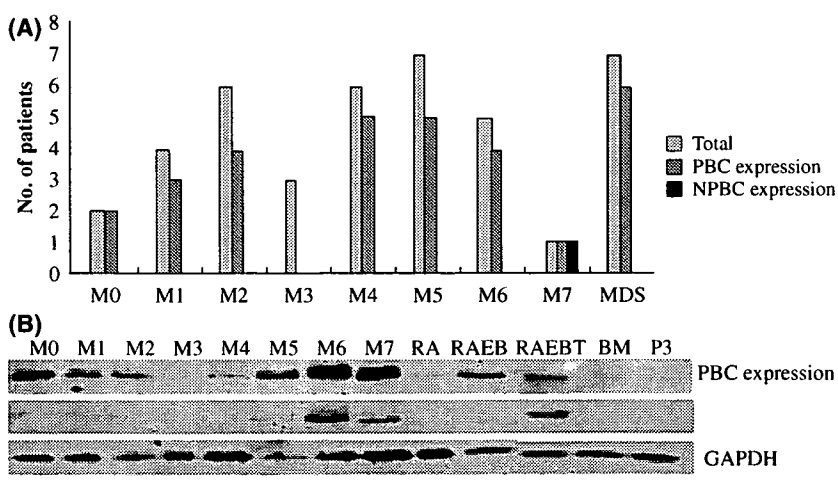

Fig 3. Beta-catenin expression of AML cells assessed by immunoblotting. (A) The graph shows data obtained for the expression of NPBC in mononuclear cells from 41 patients with AML, and the data indicate that expression of NPBC is specific to some FAB subtypes, especially to M6 and M7. (B) Representative immunoblots for PBC and NPBC in AML samples. RA, refractory anaemia; RARS, RA with ringed sideroblasts; RAEB, RA with excess blasts; RAEBT, refractory anaemia with excess blasts in transformation.

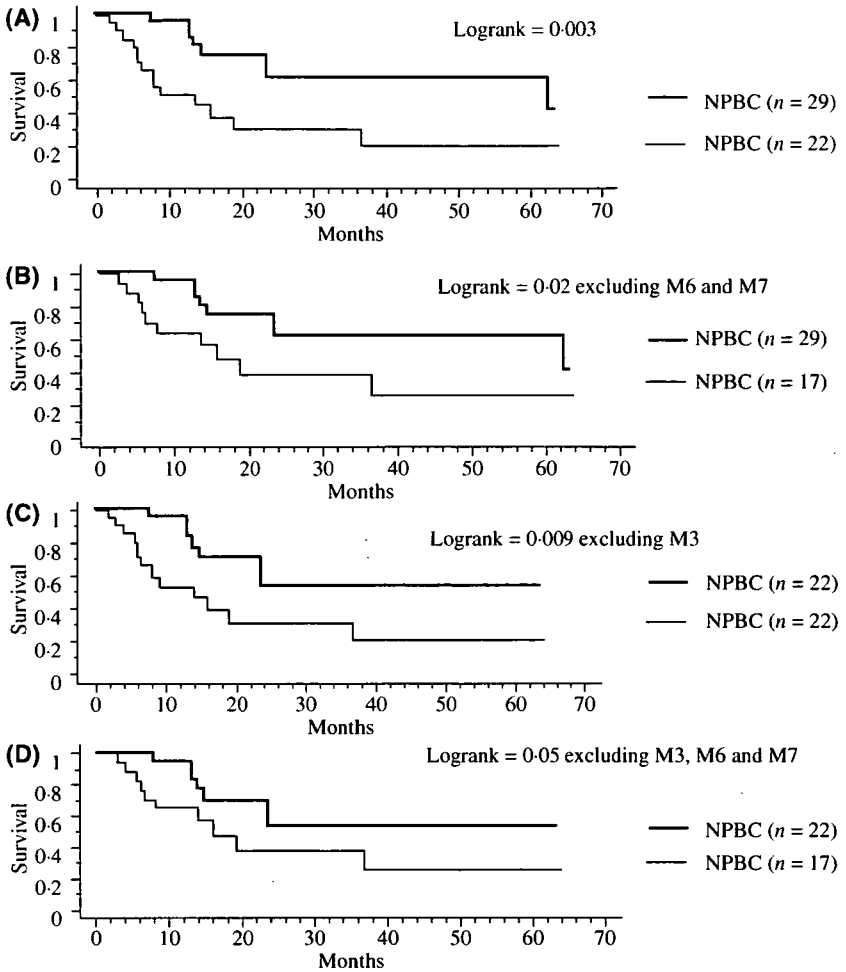

Fig 4. Kaplan–Meier cumulative survival curves were calculated for 51 AML patients (A) and 46 patients excluding subtype M6/M7 (B), 44 patients excluding subtype M3 (C), 39 patients excluding subtype M3/M6/M7 (D), respectively, according to the presence of nuclear NPBC. Comparison of the survival curves using the log-rank test identified nuclear NPBC$^+$ as a prognostic factor.

(Fig 1A). These findings suggest that increased nuclear NPBC levels were the result of aberrant signal transduction in the Wnt pathway or an abnormality of beta-catenin itself.

In this study, the expression levels of total and non-phosphorylated beta-catenin were correlated but varied significantly among leukemia samples. In M6 and M7 samples, the expression of beta-catenin was significantly augmented, whereas it was hardly detected in M3 and normal BM samples. These variations suggest the possibility that Wnt/beta-catenin signaling is mediated by multiple factors, such as immaturity, lineage, and oncogenic signals.

We established a correlation between nuclear NPBC$^+$ and poor survival in AML patients. The prognostic value of total beta-catenin expression has been previously studied in AML patients (Ysebaert *et al*, 2006), but the present study clearly showed for the first time that nuclear NPBC is associated with prognosis. The association was still observed even if M6/M7 and/or M3 patients were excluded from the analysis. Nuclear NPBC might be a new prognostic marker for AML and MDS that can be evaluated by histopathological examination.

Wnts are a family of paracrine and autocrine factors that regulate cell growth and cell fate (Reya *et al*, 2003). The Wnt autocrine signaling mechanism was initially discovered in human breast and ovarian tumor cell lines as well as in MM primary samples (Bafico *et al*, 2004; Derksen *et al*, 2004). Since several Wnt family members have been reported in BM stromal cells, it is possible that leukaemia cells respond to different proteins of the Wnt/beta-catenin pathway secreted by stromal cells in a paracrine fashion (Austin *et al*, 1997; Van Den Berg *et al*, 1998; Etheridge *et al*, 2004). Nuclear NPBC was detected in some cell lines only when they were transplanted in non-obese diabetic/severe combined immunodeficient/gammacnull (NOG) mice (data not shown). Thus, the leukaemia niche may have an important role in nuclear NPBC expression during AML.

MDS is a clonal hematopoietic stem cell disorder characterized by multi-lineage dysplasia and pancytopenia in which further genetic events may be required for the rapid expansion of leukaemic blasts (Heaney & Golde, 1999; Hirai, 2003). Although nuclear NPBC has been studied in many cancers as

well as haematological malignancies, it has not been studied in MDS (Morin *et al*, 1997; Barker & Clevers, 2000; Giles *et al*, 2003). This is the first study to report the expression of beta-catenin in MDS patients. Here we showed that nuclear NPBC was related to the IPSS score and that secondary AML from MDS showed the highest percentage of nuclear NPBC expression. The Wnt signaling pathway may play an important role in the pathogenesis of the transformation of MDS into AML. Regarding chromosomal abnormalities, −7/−7q and/or complex karyotypes were significantly associated with the presence of nuclear NPBC. According to recent data (Liu *et al*, 2006), the gene encoding alpha-catenin (*CTNNA1*) is suppressed by deletion and/or methylation. Both alpha-catenin and beta-catenin bind to the inner membrane of hematopoietic cells, and cadherin binds to actin filaments via the catenin complex. If the expression of alpha-catenin is suppressed in the 5q- genotype or for other reasons, beta-catenin may be abnormally located or activated. In this study, abnormalities of chromosome 5 were associated with the presence of NPBC but this was not statistically significant. The reason why NPBC is associated with chromosome 7 abnormalities remains unclear. A gene encoded by chromosome 7 may be associated with the regulation of the Wnt/beta-catenin pathway. Several possible candidate genes including *SFRP4*, *WNT2*, and *FZD1* and *FZD9* are located at human chromosome 7. There are reports that sFRP4 plays a role in tumor suppression via the Wnt pathway (Hrzenjak *et al*, 2004; Horvath *et al*, 2007), although the specific relationship remains unknown. Since many molecules are directly or indirectly associated with the phosphorylation, stabilization and nuclear translocation of beta-catenin, the presence of nuclear NPBC might provide a clue to find a new leukemia-associated signaling mechanism.

In conclusion, *in situ* detection of nuclear NPBC by immunohistochemistry of paraffin sections from BM specimens could be used to predict the prognosis of AML and MDS. Understanding the mechanisms leading to leukemogenesis in nuclear NPBC$^+$ AML and MDS may lead to new anti-leukemia therapies.

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M Bocchia[1], M Ippoliti[1], A Gozzetti[1], E Abruzzese[2], S Calabrese[1], M Amabile[3], MT Pirrotta[1], R Crupi[1], D Tozzuoli[1], MM Trawinska[2], M Defina[1], G Martinelli[3] and F Lauria[1]
[1]*Department of Hematology, Siena University, Siena, Italy;* [2]*Department of Hematology, Roma Tor Vergata University, Roma, Italy and* [3]*Hematology and Clinic Oncology 'Seragnoli' Institute, Bologna University, Bologna, Italy*
E-mail: bocchia@unisi.it

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Clinical characteristics and outcomes in patients with t(8;21) acute myeloid leukemia in Japan

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In acute myeloid leukemia (AML), t(8;21)(q22;q22) translocation is one of the most common karyotype abnormalities, occurring in 7–8% of adult patients.[1] This change is closely associated with AML-M2 subtype in the French-American-British (FAB) classification, a type of AML with high complete remission (CR) rate (85–90%) and favorable survival rate.[1–3] Studies conducted in western countries have demonstrated that survival is prolonged when consolidation chemotherapies comprising high-dose cytarabine are administered.[2]

A research group from the Cancer and Leukemia Group B reported that clinical characteristics of t(8;21) AML could alter depending on ethnicity, suggesting that direct adoption of treatment strategies based on clinical studies conducted in western countries to Japanese patients may not be advisable.[4] A recent study from Japan focused mainly on the clinical impact of cytogenetics.[5] Information on clinical characteristics and optimal treatments, such as the clinical impact of high-dose cytarabine, thus remains unavailable for Japanese patients.

To establish optimal therapeutic strategies applicable to Japanese patients, clarification of clinical characteristics and outcomes in Japanese patients with t(8;21) AML is crucial. The present retrospective multicenter study was conducted to investigate the clinical characteristics of Japanese patients with t(8;21) AML.

From January 2000 to December 2005, a total of 147 Japanese adult patients (≥15-years-old), who were newly diagnosed with *de novo* AML (FAB: M2) according to FAB classifications, were consecutively enrolled in nine collaborating hospitals. We retrospectively reviewed the medical records of these patients. These 147 patients included 46 patients with t(8;21) AML and 101 AML(M2) patients without t(8;21).

Diagnosis of t(8;21) AML was established based on chromosomal analysis (G-banding, $n=45$) and/or detection of RUNX1(AML1)/MTG8(ETO) fusion gene by real-time reverse transcription-polymerase chain reaction ($n=16$). Overall survival was calculated from diagnosis to death from any causes and event-free survival was defined as the time from diagnosis to the following events: first relapse of AML; treatment-failure; or death from any cause except leukemia. High-, standard- and low-dose cytarabine were defined as $\geqslant 2\,g/m^2$/day, 100–200 mg/m^2/day and $\leqslant 40\,mg/m^2$/day, respectively. No patients received any other doses of cytarabine. Of the 46 patients with t(8;21), 4 were enrolled in the AML 202 study of the Japan Adult Leukemia Study Group..

Overall survival rate was calculated using the Kaplan-Meier product limit method. A log-rank test was applied to assess impact by the factor of interest when appropriate. Estimated survival was calculated as of January 31, 2007. Uni- and multivariate Cox proportional hazard models were applied to estimate the impact of potential prognostic factors. Factors associated with at least borderline significance ($P<0.10$) in univariate analyses were subjected to multivariate analysis using backward stepwise proportional-hazard modeling. Values of $P<0.05$ were considered statistically significant. Multivariate Cox proportional hazard models were used to determine the influence of age, sex and karyotype (with or without t(8;21)) on survival of all 147 patients. All analyses were conducted using STATA version 9.2 software (STATA, College Station, TX, USA).

Characteristics of AML patients with t(8;21) are shown in Table 1. Patients with t(8;21) (median age, 49.5 years; range, 18–86 years) were significantly younger than AML(M2) patients without t(8;21) (median age, 60 years; range 17–90 years; $P<0.001$). AML(M2) patients without t(8;21) included 57 men and 44 women. The median follow-up of surviving patients was 27.0 months (range, 0.2–82.6 months) after diagnosis.

Twelve patients with t(8;21) AML died during follow-up at a median of 10.6 months (range, 3.1–80.1 months) after diagnosis due to primary disease ($n=10$), pneumonia ($n=1$) or sudden cardiac death ($n=1$). Overall survival rates at 3 years after diagnosis in patients with t(8;21) was 70% (95% confidence

Table 1 Characteristics and treatment of patients with t(8;21) AML

Variables		*Number*
Age (years)	Median, range	49.5 (18–86)
Sex	Male/female	32/14
Karyotypic abnormality[a]		
(A) t(8;21)(q22; q22) without additional karyotypic abnormality		12
(B) t(8;21)(q22; q22) with loss of sex (Y) chromosome		13
(C) t(8;21)(q22; q22) with abnormal chromosome 9		4
(D) t(8;21)(q22; q22) with $\geqslant 3$ additional abnormalities		9
(E) t(8;21)(q22; q22) with loss of X chromosome		3
(F) Other karyotypic abnormalities[b]		3
White blood cell count (/μl)	Median, range	9350 (900–54 970)
Red blood cell count (10E6/μl)	Median, range	2.4 (0.6–4.0)
Hemoglobin at diagnosis (g/dl)	Median, range	8.5 (2.3–12.8)
Platelet count (10E3/μl)	Median, range	34 (6–99)
Lactate dehydrogenase level (IU/l)	Median, range	453 (162–3831)
Extramedullary involvement	Present[c]/absent	8/37
Surface antigens on leukemia cells		
CD 7	Present/absent	2/42
CD 13	Present/absent	41/3
CD 19	Present/absent	29/17
CD 33	Present/absent	35/11
CD 34	Present/absent	42/2
CD 56[d]	Present/absent	32/7
HLA-DR	Present/absent	44/2
First induction therapy[e]		
Idarubicine 12 mg/m^2 d1–3+cytarabine 100 mg/m^2 d1–7		37
Daunorubicine 50 mg/m^2 d1–5+cytarabine 100 mg/m^2 d1–7		3
Low-dose cytarabine-based chemotherapy		4
Other regimen		1
Outcomes of first induction therapy		
Complete remission		36
Complete remission not achieved		9[f]
Consolidation therapy[g]		
High-dose cytarabine-based chemotherapy		21
Courses of high-dose cytarabine (1/2/3/4)		1[h]/4/12/4
Standard cytarabine-based chemotherapy		16
Low-dose cytarabine-based chemotherapy		2
Hematopoietic stem cell transplantation		
In first complete remission (autologous/allogeneic)		3/4[i]
In other stage (autologous/allogeneic)		0/8

Abbreviation: AML, acute myeloid leukemia.
[a]Two patients were diagnosed by detection of RUNX1/MTG8 fusion gene using reverse transcription-polymerase chain reaction.
[b]Those included 46,XY, t(8;21)(q22;q22), del(11)(p11p13) ($n=1$); 46,XY, t(7;21;8)(q22;q22;q22) ($n=1$); 45,XY, t(8;12;21)(q22;p11;q22), del(9)(q?) ($n=1$) and 46,XX,t(2;19)(q37;p13),t(8;21)(q22;q22) ($n=1$).
[c]Including skin ($n=2$), submandibular lymph nodes ($n=1$), mediastinum ($n=2$), cervical lymph nodes ($n=2$), spleen ($n=2$), submandibular lymph nodes ($n=1$), liver ($n=1$), lung ($n=1$) and subcutaneous ($n=1$).
[d]Mean fluorescence intensity of CD56 among karyotype group A-F was 68, 42, 48,83, 85 and 18%, respectively.
[e]One patient rejected chemotherapy.
[f]Five patients achieved complete remission after second course of induction therapy. Three of the remaining 4 patients died with disease progression at a median of 195 days (range, 92–243 days) after diagnosis. The final remaining patient underwent allogeneic peripheral blood stem cell transplantation from an HLA-matched sibling at day 114 after diagnosis.
[g]One patient did not receive consolidation therapy and data were unavailable for one patient.
[h]This patient received autologous hematopoietic stem cell transplantation in first complete remission.
[i]Those patients had received standard-dose cytarabine ($n=2$) or high-dose cytarabine ($n=2$) containing consolidation therapies before transplantation.

interval (CI), 51–83%). This rate was significantly better than that in AML (M2) patients without t(8;21) (overall survival at 3 years, 0.43 (95%CI, 0.32–0.54); log-rank test, $P=0.005$; Figure 1a). Among patients <60-years-old, overall survival rates of patients with t(8;21) AML and patients with non-t(8;21) AML(M2) were 71% (95%CI, 47–86%) and 58% (95%CI, 41–72%), respectively (log-rank test, $P=0.28$; Figure 1b). Event-free survival rate at 3 years in patients with t(8;21) was 54% (95%CI, 37–69%). Overall survival rates in patients with t(8;21) according to karyotype are shown in Figure 1c. No significant difference in overall survival (Figure 1c) or event-free survivals were noted between karyotypic groups (log-rank test, $P=0.27$ and $P=0.51$, respectively). There was not any significant association between presence of extramedullary involvement and additional karyotype abnormality ($P=0.49$).

Of the 45 patients who received induction therapy, 36 and 5 patients achieved CR after first and second courses of chemotherapy, respectively (Table 1). CR rate was 91%.

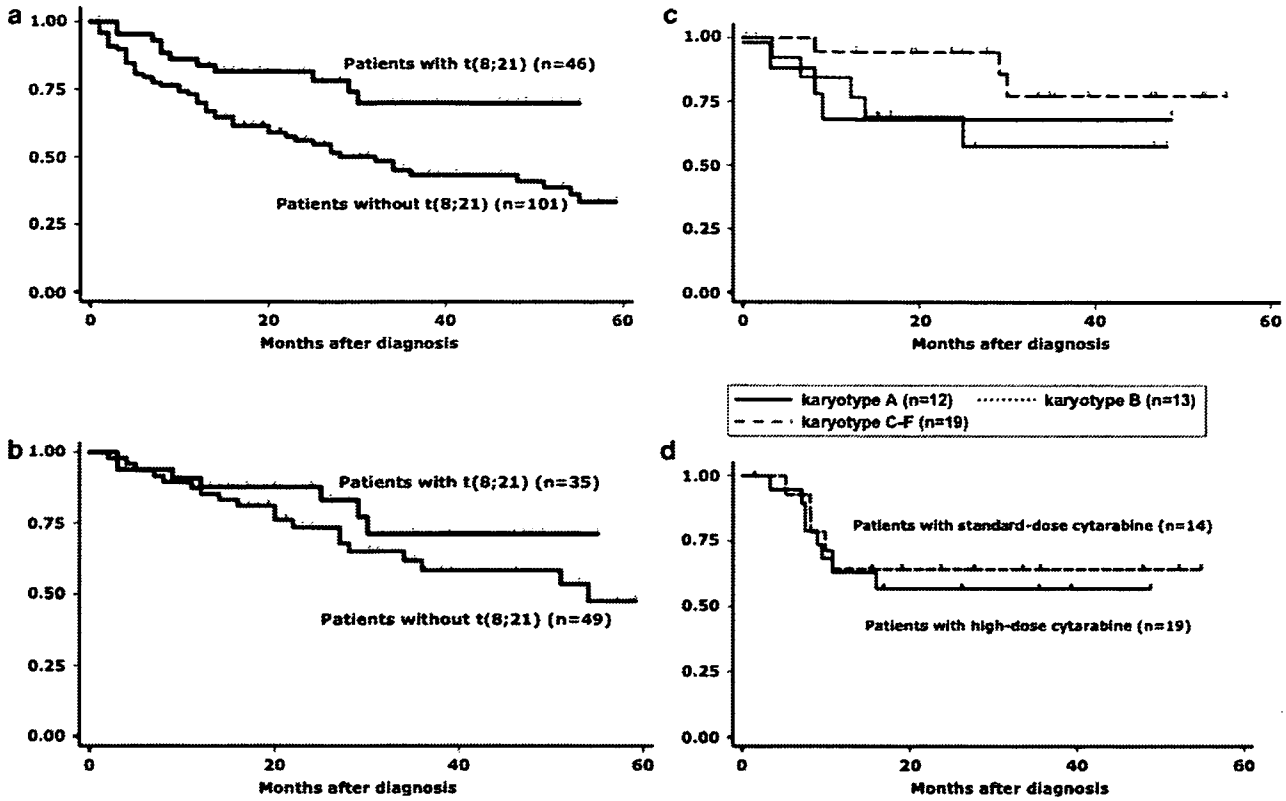

Figure 1 Overall survival rates. Overall survival rates of the AML(M2) patient with or without t(8;21) were shown in (**a**). Overall survival rate at 3 years after diagnosis in patients with t(8;21) was 0.70 (95%CI, 0.51–0.83). Overall survival rate at 3 years after diagnosis in patients with non-t(8;21) AML(M2) was 0.43 (95%CI, 0.32–0.54) (**a**). A significant difference was identified between groups (log-rank test, $P=0.005$). Overall survival rates for AML(M2) patients <60-years-old were shown in (**b**). Overall survival rate at 3 years after diagnosis in patients with or without t(8;21) was 0.71 (95%CI, 0.47–0.86) and 0.58 (95%CI, 0.41–0.72), respectively. No significant difference was seen between groups (log-rank test, $P=0.28$) (**b**). Overall survival rates according to karyotype at diagnosis were shown in Panel c. Survival rates of the following karyotype groups are shown: (A) t(8;21)(q22;q22) without other karyotype abnormality; (B) t(8;21)(q22;q22) with loss of sex (Y) chromosome; (C) t(8;21)(q22;q22) with abnormal chromosome 9; D) t(8;21)(q22;q22) with ⩾3 additional abnormalities; E) t(8;21)(q22;q22) with loss of X chromosome and F) other karyotype abnormalities. No significant difference was noted between groups (log-rank test, $P=0.27$) (**c**). Event-free survival rates with high- and standard-dose cytarabine were shown in (**d**). Event-free survival rates at 3 years after diagnosis in patients with consolidation therapy containing high- and standard-dose cytarabine were 57% (95%CI, 32–76%) and 64% (95%CI, 34–83%), respectively (log-rank test, $P=0.69$) The four patients who received the allogeneic stem cell transplantation in the first complete remission were excluded from the analysis (**d**). AML, acute myeloid leukemia; CI, confidence interval.

Of the 40 patients who received induction therapy containing standard-dose cytarabine, 38 achieved CR. Among those, 21 patients received high-dose cytarabine-containing consolidation therapy. One of the 21 patients died in CR during consolidation therapy, due to infection. Event-free survival rates in patients with and without high-dose cytarabine were shown in Figure 1d.

In multivariate analysis, age and white blood cell count at diagnosis represented significant unfavorable predictors of overall survival. White blood cell count and lactate dehydrogenase level at diagnosis represented significant unfavorable predictors of event-free survival. (Table 2) Among 147 patients with AML (M2), presence of t(8;21) was not a significant predictor of survival (hazard ratio, 0.65; 95%CI 0.34–1.24; $P=0.19$) in multivariate analysis.

The present study demonstrated a more favorable survival rate for patients with t(8;21) AML in Japan than seen in recent studies conducted in western countries,[4,6–8] even though median age in the present study (49.5 years) was higher than those in recent studies (28–43 years).[4,6–8] Median white blood cell count and platelet count in the present study, which have been reported as predictors of survival in previous studies,[4,6–8] were consistent with those in previous studies. Differences in patient backgrounds between recent studies and ours are thus unlikely to have affected survival rates. These results indicate that t(8;21) AML in Japanese patients is associated with more favorable outcomes than seen in patients from western countries. Prognosis of t(8;21) AML may differ according to ethnicity, although statically analysis was not conducted. Further large-scale studies to investigate differences in clinical outcome among patients of various ethnicities thus appear warranted.

Interestingly, differences in overall survival between t(8;21) AML patients and AML(M2) without t(8;21) patients were unclear after adjusting for age (Figure 1b). One possible explanation is that AML (M2) in Japanese patients is associated with favorable outcomes. Another explanation is that favorable outcomes for t(8;21) AML are greatly related to low patient age. To date, information on clinical differences after adjusting for age between t(8;21) AML and AML(M2) without t(8;21) limited, and is worth investigating in future studies.

Additional karyotype abnormalities have been reported as an unfavorable prognostic factor for t(8;21) AML.[1] The significance of these abnormalities may vary with ethnicity.[4] In the present study, the prognostic impact of additional karyotype abnormalities (including loss of the sex chromosome and abnormal chromosome 9) was uncertain, consistent with a previous study from Japan.[5] Additional karyotype abnormalities may not

Table 2 Risk factors for overall and event-free survival in patients with t(8;21) AML

Univariate factors	*Overall survival*			*Event-free survival*		
	HR	*95% CI*	*P*	*HR*	*95% CI*	*P*
Age (years)	1.04	1.00–1.08	0.08	1.01	0.98–1.05	0.37
Sex (male vs female)	2.25	0.48–10.4	0.30	1.75	0.58–5.32	0.32
Induction therapies (low-dose vs standard-dose cytarabine-containing regimens)	2.57	0.55–12.0	0.23	2.65	0.76–9.23	0.13
White blood cell count at diagnosis ($\geqslant$10E4/μl vs $<$10E4/μl)	6.78	1.48–31.0	0.01	4.41	1.57–12.41	0.005
Hemoglobin at diagnosis (linear by 1 g/dl increase)	1.01	0.81–1.25	0.94	1.08	0.90–1.30	0.40
Platelet count at diagnosis (linear by 10E4/μl increase)	1.06	0.86–1.32	0.58	1.01	0.84–1.22	0.91
Lactate dehydrogenase level at diagnosis (linear by 1 IU increase)	1.00	1.000–1.001	0.06	1.001	1.0002–1.0011	0.004
CD56 expression of leukemia cell (positive vs negative)[a]	0.59	0.15–2.39	0.46	0.78	0.22–2.78	0.71
Karyotype (t(8;21) with other additional abnormality vs t(8;21) without additional abnormality or t(8;21) with loss of sex (Y) chromosome)[a]	1.01	0.31–3.31	0.99	1.05	0.42–2.66	0.91
Extramedullary involvement (present vs absent)	0.92	0.20–4.25	0.91	0.87	0.25–3.02	0.83
Stepwise multivariate factors						
Age (years)	1.04	1.00–1.09	0.04	NA	NA	NA
White blood cell count at diagnosis ($\geqslant$10E4/μl vs $<$10E4/μl)	7.70	1.66–35.7	0.009	3.68	1.29–10.50	0.02
Lactate dehydrogenase level at diagnosis (linear by 1 IU increase)	NA	NA	NA	1.001	1.000–1.001	0.02

Abbreviations: AML, acute myeloid leukemia; 95% CI, 95% confidence interval; HR, hazard ratio; NA, not applicable.
[a]Patients with an unknown variable were included in the analysis using a dummy variable indicating missing data.

represent an important prognostic factor in Japanese patients. However, trisomy 4 still requires special consideration. All 3 Japanese patients with trisomy 4 in an earlier study died within 3 years.[5] This additional karyotype warrants further investigation, since the present study did not include these patients.

No clinical impact of high-dose cytarabine consolidation therapy in Japanese t(8;21) AML patients was demonstrated in the present study, inconsistent with previous studies from western countries.[1,2] Efficacy of high-dose cytarabine may differ between patients from Japan and western countries. Since intensive chemotherapy such as high-dose cytarabine carries a risk of treatment-related morbidity and mortality, clinicians must carefully select eligible patients who would benefit from this regimen. Overall survival rate in patients with high-dose cytarabine was not inferior to that in patients who received standard or low-dose regimens (data not shown). Our results indicate that high-dose cytarabine consolidation chemotherapy is feasible in Japanese patients with t(8;21) AML and that investigation of efficacy by conducting a randomized trial in Japan is warranted.

Despite providing novel and useful information on t(8;21) AML in Japan, some issues remain to be discussed. First, the patients known to have a less good prognosis, such as those with additional trisomy 4 was not included in the present study. Second, the information on tyrosine kinase mutations in the patients with t(8;21) was not presented in the present study. The tyrosine kinase mutations among various ethnicities are require to investigate in future studies, since those could influence the prognosis. Furthermore, specific mutations often associated with t(8;21), such as N-Ras and Flt3 besides c-kit are also worth investigating. The last detailed information of AML(M2) patients without t(8;21), including white blood cell count and karyotype at diagnosis, and induction and consolidation treatment were not available in the present study. Those require to be investigated in future studies.

In summary, the clinical characteristics of t(8;21) AML might differ between patients from Japan and western countries. Clinicians should be alert to potential clinical differences among ethnicities. Further large-scale studies on differences in clinical characteristics among various ethnicities including Japanese patients are required.

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[1]*Department of Hematology and Oncology, Toyohashi Municipal Hospital, Toyohashi, Japan;*
[2]*Department of Hematology and Oncology, Nagoya University Graduate School of Medicine, Nagoya, Japan;*
[3]*Department of Hematology/Oncology, Clinical Research Center, National Hospital Organization Nagoya Medical Center, Nagoya, Japan;*
[4]*Department of Hematology, Meitetsu Hospital, Nagoya, Japan;*
[5]*Department of Medicine, Fujita Health University School of Medicine, Toyoake, Japan;*
[6]*Department of Hematology, Komaki City Hospital, Komaki, Japan;*
[7]*Department of Hematology, Yokkaichi Municipal Hospital, Yokkaichi, Japan;*
[8]*Department of Hematology, Yamanashi Prefectual Central Hospital, Kofu, Japan;*
[9]*Department of Hematology, Okazaki City Hospital, Okazaki, Japan;*
[10]*Department of Infectious Diseases, Nagoya University School of Medicine, Nagoya, Japan and*
[11]*Department of HSCT Data Management, Nagoya University School of Medicine, Nagoya, Japan*
E-mail: narimt54@med.nagoya-u.ac.jp

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Appendix

This study was conducted at the following institutions: National Hospital Organization Nagoya Medical Center, Nagoya; Toyohashi Municipal Hospital, Toyohashi; Meitetsu Hospital, Nagoya; Fujita Health University Hospital, Toyoake; Komaki City Hospital, Komaki; Yokkaichi Municipal Hospital, Yokkaichi; Yamanashi Prefectural Central Hospital, Yamanashi; Okazaki City Hospital, Okazaki, Japan.

Congenital transfusion-dependent anemia and thrombocytopenia with myelodysplasia due to a recurrent *GATA1*^{G208R} germline mutation

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The X-linked gene *GATA1* encodes a 414-amino-acid hematopoietic transcription factor that controls erythroid and megakaryocytic differentiation. Virtually all cases of transient myeloproliferative disease and acute megakaryoblastic leukemia in children with Down syndrome harbor somatic *GATA1* mutations typically affecting exon 2 and leading to expression of the short isoform, GATA-1s, which lacks the transcriptional activation domain. Moreover, germline missense mutations in exon 4 of *GATA1* that predict alterations of amino acids Val205, Gly208, Arg216 or Asp218 of the N-terminal zinc-finger domain (residues 204–228) have been reported in nine families.[1–9] One additional family has been found to harbor a germline mutation, *GATA1* c.322G>C, which leads to expression of GATA-1s.[10] Consistent with X-linked inheritance and full penetrance, germline *GATA1* mutations disrupt hematopoiesis in males who harbor a hemizygous mutant *GATA1* allele. In contrast, female heterozygous carriers have no or minor hematopoietic defects such as mild chronic thrombocytopenia.[1–10] The spectrum of abnormalities caused by different *GATA1* mutations probably depends on the function of the predicted mutant protein such as the ability to associate with cofactor FOG-1.[9] Hematologic abnormalities include dyserythropoietic anemia and thrombocythemia (V205M, G208R, D218Y and GATA-1s),[1,3,9,10] thrombocytopenia with mild dyserythropoiesis (D218G, G208S),[2,8] thrombocytopenia with thalassemia (R216Q),[4,5] congenital erythropoietic porphyria (R216W),[7] and gray platelet syndrome (R216Q).[6] Splenomegaly is noted in some cases[5,7] and is likely to be due to ineffective and consecutive extramedullary hematopoiesis. To date, only the *GATA1*^{R216Q} mutation has been identified in more than one family,[4–6] hampering phenotype–genotype correlation.

We identified a second family with a *GATA1*^{G208R} mutation. The index patient was a male neonate born at term to healthy European non-consanguineous parents. Family history was unremarkable and the mother previously gave birth to a healthy boy. At birth, petechiae and ecchymoses on skin and mucosa as well as enlargement of liver and spleen were noted. Hemoglobin measured 8.9 g/dl, leukocytes 54 900/μl and thrombocytes 54 000/μl. Repeated platelet and packed red blood cell transfusions were administered. A bone marrow smear revealed dyserythropoiesis (Figure 1) and dysmegakaryopoiesis but no increase in blasts. A liver biopsy taken at the age of 16 days revealed siderosis, cholestasis and extramedullary hematopoiesis. Mutation analysis with published methods,[1,10] uncovered a hemizygous G to A transition at nucleotide position c.622 in exon 4 of *GATA1* predicting a p.G208R change in the highly conserved N-terminal zinc-finger domain of GATA-1. The patient inherited this allele from his heterozygous mother (Figure 1), who had a hemoglobin level of 11.3 g/dl, mean erythrocyte volume 81 fl, leukocytes 12 700/μl and thrombocytes 172 000/μl. At the time of this report, the patient was 6 months of age and was in stable condition requiring platelet transfusions every week and red packed cell transfusions every second to third week. To obtain the option of hematopoietic stem cells transplantation (HSCT) a donor search has been initiated. The same mutation, *GATA1*^{G208R} has been described in another individual with dyserythropoietic anemia and thrombocytopenia who was found to have anemia and thrombocytopenia at birth requiring transfusions.[3] This patient received his last red packed cell transfusion at 5 years of age and the frequency of mucosal and severe bleeding decreased in adulthood. At the age of 17 years he had a hemoglobin level of 9.6 g/dl, mean erythrocyte volume 103 fl and thrombocytes 12 000/μl.[3] Notably, this patient's mother was mildly thrombocytopenic with platelets measuring 140 000/μl.[3] The similar clinical presentation with transfusion-dependent cytopenia at birth underscores the notion