Group/Spanish Registry of MDS (234 patients), German–Austrian MDS Study Group (198 patients), MD Anderson Cancer Center (85 patients), Tokyo Medical University (12 patients) and other centers participating in the International Working Group on MDS Cytogenetics (12 patients) databases were the subject of this analysis. Several patients in this study had been included in previously published reports,[5,9,11] but without focusing on deletion 5q. Cases belonging to the Spanish Haematological Cytogenetic Working Group, Spanish Registry of MDS and MD Anderson Cancer Center were scrutinized and double checked before inclusion for avoiding duplication.

The cases were collected between November 1972 and September 2008. The diagnosis of MDS was made according to the classification proposal of the French–American–British (FAB) study group.[1] Patients with a diagnosis of refractory anemia with excess blasts in transformation or chronic myelomonocytic leukemia by FAB criteria were excluded because they are no longer considered as MDS by the WHO classification system. Whenever possible, patients were reclassified by WHO 2001 criteria.[2] Patients with an ambiguous diagnosis of MDS and those who had previously received chemotherapy or radiotherapy (therapy-related MDS) were excluded. In all patients included in this study, deletion 5q had been detected by conventional cytogenetics. The cytogenetic analysis of BM specimens was performed at the individual centers following standard chromosome-banding procedures, being cross-validated among centers in previously published studies. Inclusion in the study required the analysis of at least 10 metaphases per case. The criteria defined by the International System for Human Cytogenetic Nomenclature in 2005 were used for identification of abnormal clones.[16] For example, a karyotype was considered complex when more than two independent cytogenetic abnormalities were found. When two or more clones with two aberrations were noted, the patient was categorized in the complex aberration group, whereas patients with two karyotypically independent clones with a single change in one clone and two anomalies in the second one were not considered as complex chromosomal abnormalities. Loss of Y chromosome was considered as one chromosomal abnormality. In this series, an unrelated clone was defined as a clone with cytogenetic aberrations that did not derive from the progenitor clone with the deletion 5q. The unrelated clones were considered as additional aberrations, accompanying the deletion 5q, for the definition of its cytogenetic complexity.[17] All the cytogenetic information corresponding to the German–Austrian MDS Study Group was initially reviewed by JS and DH; and the Spanish Haematological Cytogenetic Working Group/Spanish Registry of MDS cytogenetic information by MM, BE and FS. The final revision was carried out by FS, deleting those cases with incomplete cytogenetic information. The final diagnosis was provided by each institution, all of them with recognized experience in this pathology.

In keeping with the guidelines of the Declaration of Helsinki Principles, this retrospective noninterventional study was conducted with the approval of the internal review board from the participating institutions belonging to each registry/cooperative group/center or following individual institutional guidelines.

Prognostic factors

Different patient and disease characteristics, recorded at the time of diagnosis, were examined in the prognostic factor analysis to establish their possible relationship with OS and AML transformation. Basic demographic data included age and sex. Hematological parameters were hemoglobin level, absolute neutrophil count (ANC), platelet count, number of cytopenias and proportion of blast cells in BM, all of them taking cutoff points and groups defined by the 1997 IPSS into account.[10] For platelet count, an additional cutoff point of 150×10^9 per liter was analyzed. Initially, we chose to test this value based on the higher platelet count that characterizes the '5q−syndrome' and the low number of patients with severe thrombocytopenia in this subset. After showing its association with prognosis in those patients, we decided to examine its potential impact in the overall series as well.

Classification systems included FAB[1] and WHO 2001[2] classifications, and IPSS scoring system. The IPSS risk categories considered were those in the original report (low, intermediate-1, intermediate-2 and high).[10] Cytogenetic findings recorded and analyzed were the presence of additional chromosome abnormalities, including the number of additional abnormalities (karyotype complexity) and the most prevalent specific additional abnormalities found (chromosome 1, chromosome 3, −7, 7q−, +8, +11, +13, 12p−, chromosome 17, −18/18q−, 20q−, +21, −X/−Y and unrelated clones, taking into account if they were accompanying deletion 5q as a single additional chromosome abnormality or in the context of a complex karyotype), the proportion of metaphases carrying deletion 5q, and the most frequent break points of the 5q-deleted region (q13q31, q13q33, q22q33, q12q33, q14q34 and other break points). Initially, the number of additional chromosomal abnormalities was grouped into six categories: none (isolated deletion 5q), one, two, three, four and five or more additional abnormalities. After showing that the clinical outcome for patients with two or more additional abnormalities was almost identical, only three cytogenetic categories were considered for all subsequent analysis: isolated deletion 5q, deletion 5q plus one additional abnormality and deletion 5q plus two or more additional abnormalities.

Statistical analysis

Comparisons of proportions and ranks of variables between different groups were performed by χ^2-test, Fisher's exact test, Student's *t*-test, Mann–Whitney *U*-test or one-way ANOVA with *post hoc* Tukey's test, as appropriate.

The Kaplan–Meier product limit method was used to estimate the probability of OS and risk of AML transformation,[18–21] OS was measured from hematological diagnosis to death or last follow-up. All deaths, whether related or not to MDS, were considered as the end point of the follow-up interval. Patients treated with intensive AML-type chemotherapy (11 patients), hematopoietic stem cell transplantation (3 patients) or with lenalidomide (3 patients) were considered as censored data at the time of starting treatment, when the starting date of treatment was available. AML transformation was measured from diagnosis to AML development. Patients dying from any cause before developing AML were considered as censored data in the date of death for the calculation of AML transformation curves. To avoid any potential bias in the estimation of the risk of AML transformation, only patients from those registries/centers with information about AML evolution was available in most of instances were included in the calculation of AML transformation risk. Statistical comparisons between different actuarial curves were based on log-rank tests.[19–21]

Multivariate analysis using the Cox proportional hazards regression method for temporal events was used to identify the most significant independent prognostic variables for OS and AML transformation.[22] Characteristics selected for possible inclusion in the multivariate model were those for which there

was some indication of a significant association with OS or AML transformation in the univariate analysis (Table 4), $P<0.05$. Only cases with complete data for all variables were included in the regression procedure. The forward stepwise procedure was stopped when the *P*-value for entering an additional variable was above 0.05. All *P*-values reported are two sided. The selected *P*-value for considering differences statistically significant in all analyses was <0.05. All analyses were performed using the statistical package SPSS version 17.0 (SPSS Inc., Chicago, IL, USA).

Results

Characteristics of the patients

The overall series included 183 men (34%) and 358 women (66%) with a median age of 68 years (range, 33–92 years). The main characteristics of the patients at the time of diagnosis are summarized in Table 1. The median value for hemoglobin level, ANC and platelet count was 9.0 g per liter (range, 2.5–14.0), 1.8×10^9 per liter (range, 0.10–38.40) and 181×10^9 per liter (range, 4–1610), respectively, whereas median BM blast count was 4.0%. Most of the patients were classified as RA (49.2%) or RAEB (42.7%) according to the FAB classification; and '5q−syndrome' (39.7%), RAEB-2 (29.0%) or RAEB-1 (21.7%) by the WHO 2001 criteria.

In total, 299 patients (55.3%) had deletion 5q as the sole chromosomal abnormality, 93 (17.2%) had one additional abnormality and 149 (27.5%) had a complex karyotype with two or more associated abnormalities. The most frequent single additional anomalies to deletion 5q were del(12p) ($n=11$), trisomy 21 ($n=10$), trisomy 8 ($n=9$) and del(20q) ($n=8$). Of note, there were no patients with deletion 5q and loss of chromosome Y. However, as expected, majority of patients were women (ratio 1:2.1). In the context of complex karyotypes, aberrations most commonly found were those affecting chromosome 17 ($n=40$), −18/18q− ($n=36$), trisomy 8 ($n=35$), del(20q) ($n=30$), monosomy 7 ($n=28$) and involvement of chromosome 3 ($n=25$).

Ten of the cases included in the series (2.0%) had unrelated clones (without deletion 5q), with trisomy 8 (four cases) and del(12p) (two cases), being the most frequent cytogenetic aberrations.

The most common 5q-deleted regions in 383 cases, in which this information was available, were q13q33 (49.4%), q13q31 (15.9%), q22q33 (7.8%) and 20.9% other unspecific break points. There was a strong correlation between the number of chromosomal abnormalities found in addition to deletion 5q and different hematological parameters, other cytogenetic findings, FAB and WHO subtype, and IPSS classification (Table 2). Comparing patients with $\geqslant 2$ additional abnormalities with patients belonging to a group encompassing two cytogenetic categories (del(5q) and del(5q) + 1), we observed that there were differences in sex distribution ($P<0.001$), and hemoglobin level between both groups ($P=0.074$). Platelet count and ANC showed differences between both groups ($P<0.001$) and a higher incidence of cytopenias as well ($P<0.001$). The proportion of blasts in BM was higher ($P<0.001$) as well as the higher proportion of cases with metaphases carrying the deletion 5q ($P<0.001$).

FAB and WHO diagnoses, according to the number of chromosomal abnormalities found in addition to deletion 5q, are shown in Figure 1.

Apart from differences in characteristics inherent to the definition of '5q−syndrome' (for example, absence of additional chromosomal abnormalities and lower proportion of blasts in

Table 1 Patient characteristics

Characteristic	*Number of patients, n (%)*
Total number of patients	541
Age	532
<60 years	129 (24.2)
⩾60 years	403 (75.8)
Sex	541
Male	183 (33.8)
Female	358 (66.2)
Hemoglobin	438
<10 g per 100 ml	308 (70.3)
⩾10 g per 100 ml	130 (29.7)
Absolute neutrophil count	320
$<1.8 \times 10^9$ per liter	156 (48.8)
$\geqslant 1.8 \times 10^9$ per liter	164 (51.2)
Platelet count	439
$<100 \times 10^9$ per liter	129 (29.4)
$\geqslant 100 \times 10^9$ per liter	310 (70.6)
Cytopenias	325
None	48 (14.8)
One	115 (35.4)
Two	105 (32.3)
Three	57 (17.5)
BM blast count	497
<5%	293 (58.8)
5–10%	90 (18.1)
11–20%	115 (23.1)
FAB subtype	508
RA	250 (49.2)
RARS	41 (8.1)
RAEB	217 (42.7)
WHO subtype	373
RA	4 (1.1)
RARS	2 (0.5)
RCMD	18 (4.8)
RCMD-RS	11 (2.9)
'5q−syndrome'	148 (39.7)
RAEB-1	81 (21.7)
RAEB-2	108 (29.0)
MDS-U	1 (0.3)
Karyotype complexity	541
Isolated 5q−	299 (55.3)
5q− + 1 abnormality	93 (17.2)
5q− + 2 abnormalities	26 (4.8)
5q− + 3 abnormalities	21 (3.9)
5q− + 4 abnormalities	19 (3.5)
5q− + ⩾5 abnormalities	83 (15.3)
Deletion 5q break points	383
q13q31	61 (15.9)
q13q33	189 (49.4)
q22q33	30 (7.8)
q12q33	13 (3.4)
q14q34	10 (2.6)
Others	80 (20.9)
Percentage of del(5q) metaphases	365
<100%	233 (63.8)
100%	132 (36.8)
IPSS risk group	329
Low	89 (27.1)
Intermediate-1	110 (33.4)
Intermediate-2	83 (25.2)
High	47 (14.3)

Abbreviations: BM, bone marrow; FAB, French–American–British; IPSS, International Prognostic Scoring System; MDS-U, MDS unclassifiable; RA, refractory anemia; RAEB, RA with excess of blasts; RARS, RA with ringed sideroblasts; RCMD, refractory cytopenia with multilineage dysplasia; RCMD-RS, RCMD with ringed sideroblasts; WHO, World Health Organization.

Table 2 Patient characteristics according to the karyotype complexity

	Isolated del(5q) (1)		*del(5q)+1 abnormality (2)*		*del(5q)+⩾2 abnormalities (3)*		*P-value*		
	Median (Q1–Q3)	n *(%)*	*Median (Q1–Q3)*	n *(%)*	*Median (Q1–Q3)*	n *(%)*	*(1) vs (2)*	*(1) vs (3)*	*(2) vs (3)*
Age	68 (59–76)	292	67 (59–76)	93	68 (59–76)	147	0.772[a]	0.357[a]	0.294[a]
<60 years		77 (26.4)		24 (25.8)		28 (19.0)			
⩾60 years		215 (73.6)		69 (74.2)		119 (81.0)			
Sex		299		93		149	0.440[b]	<0.001[b]	0.031[b]
Male		84 (28.1)		30 (32.3)		69 (46.3)			
Female		215 (71.9)		63 (67.7)		80 (53.7)			
Hemoglobin	8.9 (2.0)[c]	255	9.3 (1.9)[c]	77	8.7 (1.6)[c]	106	0.327[d]	0.455[d]	0.078[d]
<10 g per 100 ml		176 (69)		47 (61.0)		85 (80.2)			
⩾10 g per 100 ml		79 (31)		30 (39.0)		21 (19.8)			
Absolute neutrophil count	2.2 (1.4–3.0)	167	1.6 (1.1–2.8)	49	1.1 (0.5–2.2)	104	0.111[a]	<0.001[a]	0.003[a]
$<1.8 \times 10^9$ per liter		58 (34.7)		25 (51.0)		73 (70.2)			
$\geqslant 1.8 \times 10^9$ per liter		109 (65.3)		24 (49.0)		31 (29.8)			
Platelet count	243 (145–377)	253	196 (106–295)	79	59 (33–113)	107	0.006[a]	<0.001[a]	<0.001[a]
$<100 \times 10^9$ per liter		35 (13.8)		18 (22.8)		76 (71.0)			
$\geqslant 100 \times 10^9$ per liter		218 (86.2)		61 (77.2)		31 (29.0)			
BM blast count	3.0 (1.0–5.0)	275	4.0 (2.0–10.0)	81	9.0 (4.0–13.0)	142	0.009[a]	<0.001[a]	<0.001[a]
<5%		203 (73.8)		51 (63.0)		39 (27.5)			
5–10%		43 (15.6)		9 (11.1)		38 (26.7)			
11–20%		29 (10.5)		21 (25.9)		65 (45.8)			
Percentage of del(5q) metaphases	75 (52.2–100.0)	179	88.7 (64.4–100.0)	76	98.1 (69.8–100.0)	110	0.227[b]	<0.001[b]	0.051[b]
<100%		129 (72.1)		49 (64.5)		55 (50.0)			
100%		50 (27.9)		27 (35.5)		55 (50.0)			
Cytopenias		170		50		105	0.074[e]	<0.001[b]	<0.001[e]
None		38 (22.4)		7 (14.0)		3 (2.8)			
One		79 (46.5)		21 (42.0)		15 (14.3)			
Two		46 (27.0)		15 (30.0)		44 (41.9)			
Three		7 (4.1)		3 (14.0)		43 (41.0)			
IPSS risk group		173		51		105	<0.001[e]	<0.001[b]	<0.001[b]
Low		89 (51.4)		0 (0.0)		0 (0.0)			
Intermediate-1		65 (37.6)		34 (66.7)		11 (10.5)			
Intermediate-2		18 (10.4)		15 (29.4)		50 (47.6)			
High		1 (0.6)		2 (3.9)		44 (41.9)			
FAB subtype		277		85		146	0.023[b]	<0.001[b]	<0.001[b]
RA		182 (65.7)		42 (49.4)		26 (17.8)			
RARS		21 (7.6)		11 (12.9)		9 (6.2)			
RAEB		74 (26.7)		32 (37.6)		111 (76.0)			
WHO subtype		217		42		114	<0.001[e]	<0.001[e]	0.016[e]
'5q–syndrome'		148 (68.2)		0 (0.0)		0 (0.0)			
RA		1 (0.5)		2 (4.8)		1 (0.9)			
RARS		0 (0.0)		1 (2.4)		1 (0.9)			
RCMD		3 (1.4)		8 (19.0)		7 (6.1)			
RCMD-RS		1 (0.5)		5 (11.9)		5 (4.4)			
RAEB-1		35 (16.0)		9 (21.4)		37 (32.4)			
RAEB-2		29 (13.4)		17 (40.5)		62 (54.4)			
MDS-U		0 (0.0)		0 (0.0)		1 (0.9)			

Abbreviations: BM, bone marrow; FAB, French–American–British; IPSS, International Prognostic Scoring System; MDS-U, MDS unclassifiable; RA, refractory anemia; RAEB, RA with excess of blasts; RARS, RA with ringed sideroblasts; RCMD, refractory cytopenia with multilineage dysplasia; RCMD-RS, RCMD with ringed sideroblasts; WHO, World Health Organization.
Q1, percentile 25; Q3, percentile 75.
[a]Mann–Whitney *U*-test.
[b]χ^2-test.
[c]This value corresponds to the mean and s.d., in brackets.
[d]One-way ANOVA with *post-hoc* Tukey's test;
[e]Fisher's exact test.

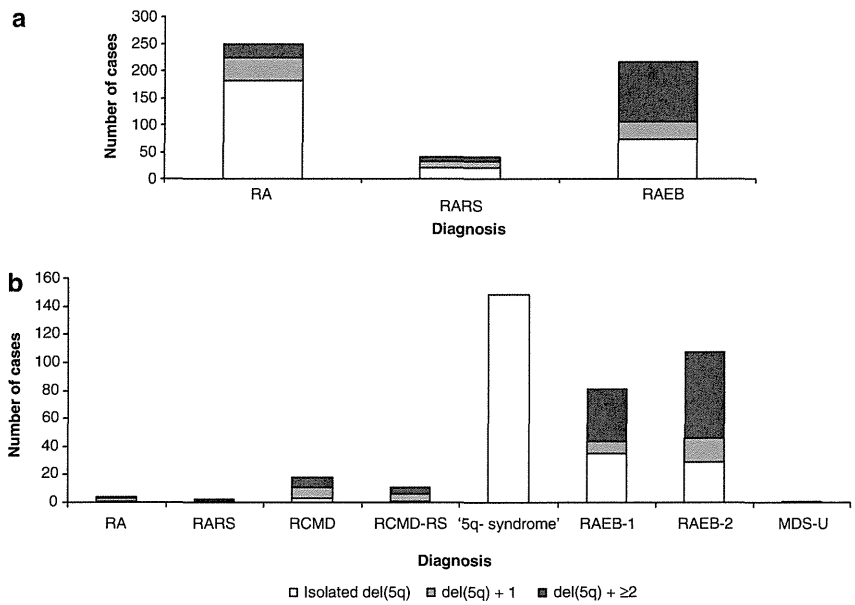

Figure 1 Incidence of the three defined cytogenetic categories (isolated del(5q), del(5q) + 1, del(5q) + ⩾2) among the different morphological subtypes. (**a**) According to the FAB classification. (**b**) According to the WHO classification. Abbreviations: RA, refractory anemia; RARS, RA with ringed sideroblasts; RAEB, RA with excess of blasts; RCMD, refractory cytopenia with multilineage dysplasia; RCMD-RS, RCMD with ringed sideroblasts; MDS-U, MDS unclassifiable.

BM), this subset of patients ($n=148$) had a higher median ANC ($P=0.001$) and median platelet count value ($P<0.001$) and, consequently, a lower number of cytopenias ($P<0.001$) than the remaining patients. Further, patients with '5q−syndrome' showed a lower median percentage of metaphases carrying deletion 5q than the rest of the patients (median 70 vs 90%; $P<0.001$) (Table 3). No significant differences in break points were observed between patients with '5q−syndrome' and the remaining patients (data not shown).

Outcome and prognostic factors in the overall series

Overall survival and AML transformation data were available in 512 (94.6%) and 299 (55.3%) patients, respectively. With a median follow-up of 17.2 months (range, 1–326) for surviving patients, 258 patients remained alive and the median OS for the whole series was 36.8 months. In total, 66 patients evolved to AML during follow-up, with the actuarial risk of AML evolution at 5 years of 38.8%. As depicted in Table 4, univariate analysis showed that both OS and risk of AML transformation were significantly influenced by age ($P<0.001$ and $P=0.042$, respectively), sex ($P<0.001$ and $P=0.029$, respectively), ANC ($P<0.001$ and $P=0.004$, respectively) and platelet count, number of cytopenias, proportion of BM blasts, FAB and WHO subtype, IPSS risk group and number of chromosomal abnormalities found in addition to deletion 5q ($P<0.001$ for all variables, both OS and AML evolution), as well as the percentage of metaphases carrying deletion 5q ($P<0.001$ and $P=0.003$, respectively). In addition, OS was shorter in those with lower hemoglobin levels ($P=0.030$). Different deletion break points showed an impact on outcome in terms of OS ($P=0.008$). Although there was one break point (q22q33) that showed less median survival time, this did not differ statistically from the rest of the break points ($P=0.228$). Figure 2 shows the actuarial curves of OS (Figure 2a) and AML transformation (Figure 2b) in the three cytogenetic groups defined according to the number of chromosomal abnormalities found in addition to deletion 5q: isolated deletion 5q, deletion 5q plus one additional abnormality and deletion 5q plus two or more additional abnormalities. As can be appreciated, all the three aforementioned cytogenetic groups were found to have a significantly different risks of AML transformation ($P<0.001$ for all comparisons), but regarding OS only two risk groups could be clearly identified, patients with deletion 5q alone or with one additional chromosomal abnormality and patients with two or more additional abnormalities. Although patients with deletion 5q plus one additional abnormality had a somewhat shorter OS than patients with isolated deletion 5q (median OS, 63.4 and 46.0 months, respectively) differences in OS between these two groups were not statistically significant ($P=0.131$). We were not able to determine the potential impact in the outcome of any of the additional aberrations because of the low number of cases as a single anomaly accompanying to the deletion 5q. In contrast, patients with two or more additional abnormalities showed a significantly shorter OS than the other two groups of patients (median OS, 6.8 months; $P<0.001$).

The same prognostic impact of the three cytogenetic groups, defined by the number of chromosomal abnormalities found in addition to deletion 5q, on OS and risk of AML transformation was evident when the analysis was restricted to patients with $<5\%$ and $<10\%$ blasts in BM (Figure 3).

As shown in Table 5, in multivariate analysis the characteristics showing an independent prognostic impact concerning OS and AML transformation risk were the number of chromosomal abnormalities found in addition to deletion 5q ($P<0.001$ for both outcomes); the platelet count ($P<0.001$ and $P=0.001$, respectively) and the proportion of blasts in BM ($P<0.001$ and $P=0.016$, respectively). Age and sex also added significant prognostic information for OS ($P=0.001$ and $P=0.020$, respectively). The independent prognostic impact of platelet count in multivariate analysis was observed studying this variable both as a dichotomous and continuous one. When this

Table 3 Comparative of clinical characteristics of patients with '5q−syndrome'

	'5q−syndrome'		*'non-5q−syndrome'*		P-*value*
	Median (Q1–Q3)	n *(%)*	*Median (Q1–Q3)*	n *(%)*	
Age	70 (59–79)	147	67 (60–75)	385	0.070[a]
<60 years		39 (26.5)		90 (23.4)	
⩾60 years		108 (73.5)		295 (76.6)	
Sex		148		393	0.035[b]
Male		43 (29.1)		140 (35.6)	
Female		105 (70.9)		253 (64.4)	
Hemoglobin	9.0 (1.9)[c]	133	8.9 (1.9)[c]	305	0.420[d]
<10 g per 100 ml		92 (69.2)		216 (70.8)	
⩾10 g per 100 ml		41 (30.8)		89 (29.2)	
Absolute neutrophil count	2.2 (1.5–3.3)	86	1.6 (0.9–2.7)	234	<0.001[a]
$<1.8 \times 10^9$ per liter		26 (30.2)		130 (55.6)	
$\geqslant 1.8 \times 10^9$ per liter		60 (69.8)		104 (44.4)	
Platelet count	295 (174–412)	130	138 (60–262)	309	<0.001[a]
$<100 \times 10^9$ per liter		13 (10.0)		116 (37.5)	
$\geqslant 100 \times 10^9$ per liter		117 (90.0)		193 (62.5)	
BM blast count	2.0 (1.0–3.0)	141	6.0 (3.0–11.0)	357	<0.001[a]
<5%		141 (100.0)		152 (42.6)	
5–10%		0 (0.0)		90 (25.2)	
11–20%		0 (0.0)		115 (32.2)	
IPSS score		89		240	<0.001[b]
Low		70 (78.7)		19 (7.9)	
Intermediate-1		19 (21.3)		91 (37.9)	
Intermediate-2		0 (0.0)		83 (34.6)	
High		0 (0.0)		47 (19.6)	
Percentage of del(5q) metaphases	70.0 (40.0–93.1)	73	90.0 (61.1–100.0)	292	<0.001[b]
<100%		60 (82.2)		173 (59.2)	
100%		13 (17.8)		119 (40.8)	

Abbreviations: BM, bone marrow; IPSS, International Prognostic Scoring System.
Q1, percentile 25; Q3, percentile 75.
[a]Mann–Whitney *U*-test.
[b]χ^2-test.
[c]This value corresponds to the mean and s.d., in brackets.
[d]Student's *t* test.

variable was introduced simultaneously in the regression procedure in both ways, the dichotomized manner was selected for entering the model. For this reason and for practical purposes, all results offered are those obtained with platelet count as a dichotomized variable.

Outcome and prognostic factors in patients with '5q−syndrome'

When the analysis was restricted to 144 patients with the '5q−syndrome' diagnosis and available follow-up data, median OS was 68.8 months and actuarial risk of AML transformation at 5 years was 17.1%. In univariate analysis, male patients (median OS, 40.9 vs 80.0 months for women; $P=0.020$), patients older than 60 years of age (median OS, 45.0 vs 134.5 months for patients ⩽60 years of age; $P=0.005$) and those with a platelet count lower than 150×10^9 per liter (median OS, 32.2 vs 80.0 months for patients with a platelet count greater than 150×10^9 per liter; $P<0.001$) had a significantly shorter OS.

Multivariate analysis showed that the main factors influencing OS were platelet count (hazards ratio (HR), 3.2; $P=0.001$) and age (HR, 2.2; $P=0.034$). None of the parameters evaluated demonstrated a significant association with AML transformation risk neither on univariate nor on multivariate analysis.

Outcome and prognostic factors in patients of low and intermediate-1 risk

Patients belonging to the low and intermediate-1 IPSS category are considered as good prognosis, as well as those MDS with deletion 5q. Comparing the outcomes of both groups of patients in our series, as expected, low IPSS patients has a median survival time higher than the intermediate-1 patients, though these differences were not statistically significant (58.9 vs 45.0 months; $P=0.182$). The actuarial AML risk at 5 years was also similar (21.2 vs 25.6%; $P=0.437$). Focusing on low-risk patients, all presented isolated 5q deletion and <5% of BM blasts. The univariate analysis did not detect any prognostic factor regarding OS and AML, for those variables that there were enough patients per group. The intermediate-1 group had patients belonging to the three cytogenetic and BM blast count predefined categories. The OS univariate analysis showed the prognosis impact of cytogenetic categories ($P=0.020$), age ($P=0.003$) and platelet count ($P=0.002$). Regarding AML,

Table 4 Results of univariate analyses of prognostic factors for OS and AML transformation in the overall series

	Overall survival				*AML transformation*			
	n *(%)*	*Median survival (mo)*	*Patients alive at 5 years (%)*	P-*value*	n *(%)*	*Time to 25% probability (mo)*	*Cumulative probability of AML evolution at 5 years (%)*	P-*value*
Age	506 (93.5)			<0.001	297 (54.9)			0.042
<60 years	121 (23.9)	80.0	52.4		66 (22.2)	13.5	47.7	
⩾60 years	385 (76.1)	33.0	28.1		231 (77.8)	41.8	36.0	
Sex	512 (94.6)			<0.001	299 (55.3)			0.029
Male	174 (34.0)	25.0	21.2		108 (36.1)	14.9	52.1	
Female	338 (66.0)	44.9	41.9		191 (63.9)	42.1	32.4	
Hemoglobin	429 (79.3)			0.030	290 (53.6)			0.252
<10 g per 100 ml	302 (70.4)	35.0	33.7		200 (69.0)	22.9	41.9	
⩾10 g per 100 ml	127 (29.6)	54.5	42.7		90 (31.6)	44.2	32.8	
Absolute neutrophil count	318 (58.8)			<0.001	285 (52.7)			0.004
$<1.8 \times 10^9$ per liter	155 (48.7)	15.0	17.1		136 (47.7)	13.2	47.3	
$\geqslant 1.8 \times 10^9$ per liter	163 (51.3)	38.7	45.0		149 (52.3)	51.6	28.7	
Platelet count	428 (79.1)			<0.001	290 (53.6)			<0.001
$<100 \times 10^9$ per liter	127 (29.7)	8.2	8.3		100 (34.5)	6.7	67.6	
$\geqslant 100 \times 10^9$ per liter	301 (70.3)	47.0	57.1		190 (65.5)	48.6	30.4	
Cytopenias	323 (59.7)			<0.001	286 (52.9)			<0.001
None	47 (14.6)	65.9	53.4		44 (15.4)	NR	15.9	
One	115 (35.6)	50.9	36.1		100 (35.0)	34.5	44.8	
Two	104 (32.2)	19.7	20.5		92 (32.1)	15.0	32.2	
Three	57 (17.6)	7.9	5.2		50 (17.5)	67	76.6	
BM blast count	479 (88.5)			<0.001	296 (54.7)			<0.001
<5%	277 (57.8)	50.9	44.3		151 (51.0)	51.1	31.3	
5–10%	88 (18.4)	19.7	26.2		63 (21.3)	13.5	42.1	
11–20%	114 (23.8)	11.0	12.5		82 (27.7)	8.4	55.9	
IPSS risk group	327 (60.4)			<0.001	289 (53.4)			<0.001
Low	88 (26.9)	58.9	49.1		78 (27.0)	65.0	21.2	
Intermediate-1	109 (33.3)	45.0	34.3		94 (32.5)	52.4	25.6	
Intermediate-2	83 (25.4)	13.4	15.2		74 (25.6)	9.1	65.0	
High	47 (14.4)	6.5	0.0		43 (14.9)	5.2	100.0	
FAB subtype	488 (90.2)			<0.001	2889 (53.4)			<0.001
RA	232 (47.5)	57.0	47.3		117 (40.5)	51.4	29.8	
RARS	41 (8.4)	38.9	36.1		20 (6.9)	10.8	—	
RAEB	215 (44.1)	14.9	17.3		152 (52.6)	9.7	48.1	
WHO subtype	362 (66.9)			<0.001	255 (47.1)			<0.001
'5q−syndrome'	140 (38.7)	65.9	51.3		86 (33.7)	65.0	18.2	
RA	4 (1.1)	31.6	33.3		3 (1.2)	—	100.0	
RARS	2 (0.6)	2.7	0.0		2 (0.8)	—	100.0	
RCMD	17 (4.7)	31.0	15.9		10 (3.9)	—	100.0	
RCMD-RS	11 (3.0)	20.8	16.4		6 (2.4)	4.7	100.0	
RAEB-1	79 (21.8)	18.0	20.0		62 (24.3)	15.4	30.6	
RAEB-2	108 (29.8)	10.4	13.0		85 (33.3)	8.7	63.4	
MDS-U	1 (0.3)	9.7	0.0		1 (0.4)	—	—	
Percentage of del(5q) metaphases	353 (65.2)			<0.001	250 (46.2)			0.003
<100%	225 (63.7)	39.6	35.2		170 (68.0)	51.1	34.4	
100%	128 (36.3)	16.2	20.1		80 (42.0)	8.4	53.4	
Deletion 5q break points	370 (68.4)			0.008				0.386
q13q31	60 (16.2)	57.1	47.4		33 (15.9)	52.4	47.4	
q13q33	181 (48.9)	39.6	38.7		110 (53.2)	26.0	38.7	
q22q33	30 (8.1)	24.0	28.2		13 (6.3)	13.2	28.2	
q12q33	13 (3.5)	57.4	46.7		10 (4.8)	—	46.7	
q14q34	10 (2.7)	73.0	33.8		1 (0.5)	—	33.8	
Others	76 (20.6)	19.7	26.9		40 (19.3)	15.4	26.9	
Karyotype complexity	512 (94.6)			<0.001	299 (55.3)			<0.001
Del(5q)	275 (53.7)	63.4	50.6		160 (53.5)	65.0	21.1	
Del(5q) + 1	89 (17.4)	46.0	40.4		43 (14.4)	14.9	57.6	
Del(5q) + 2	26 (5.1)	13.9	0.0		16 (5.4)	4.7	100.0	
Del(5q) + 3	21 (4.1)	8.1	0.0		15 (5.0)	2.6	100.0	
Del(5q) + 4	19 (3.7)	7.6	0.0		13 (4.3)	3.9	100.0	
Del(5q) + ⩾5	82 (16.0)	5.7	2.3		52 (17.4)	4.2	100.0	

Abbreviations: AML, acute myeloid leukemia; BM, bone marrow; FAB, French–American–British; IPSS, International Prognostic Scoring System; MDS-U, MDS unclassifiable; NR: not reached; OS, overall survival; RA, refractory anemia; RAEB, RA with excess of blasts; RARS, RA with ringed sideroblasts; RCMD, refractory cytopenia with multilineage dysplasia; RCMD-RS, RCMD with ringed sideroblasts; WHO, World Health Organization.

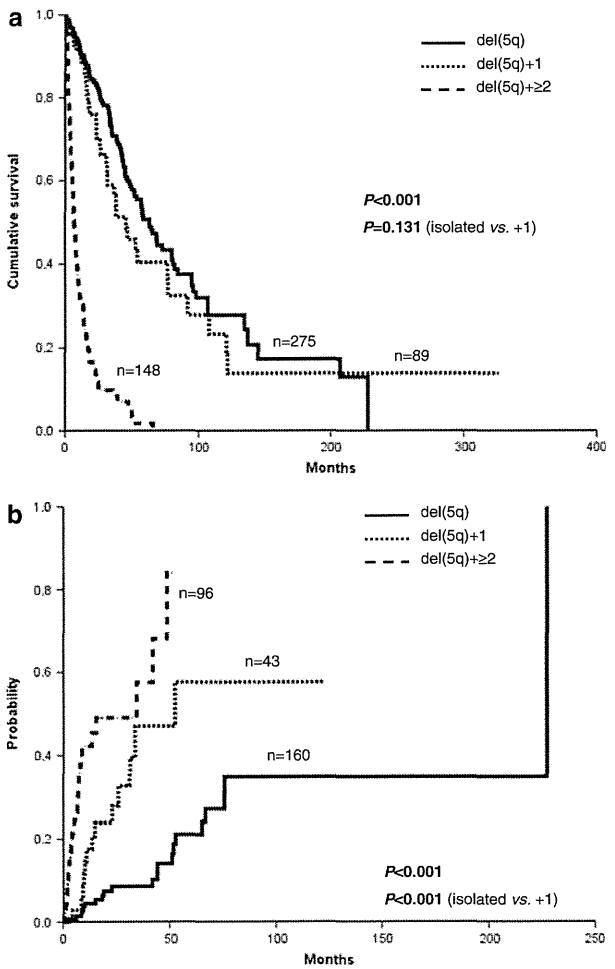

Figure 2 Kaplan–Meier curves according to the three defined cytogenetic categories (isolated del(5q), del(5q)+1, del(5q)+≥2). (**a**) Actuarial probability of overall survival. (**b**) Cumulative probability of AML transformation.

cytogenetic categories ($P=0.008$) and sex ($P=0.027$) revealed their prognostic impact in the intermediate-1 subset of patients.

Discussion

In this paper we present the results of a larger multicenter cooperative study that recruited the largest to-date known series of *de novo* MDS patients with deletion 5q in the pre-lenalidomide era. This has allowed us to assess the clinical characteristics, natural history and prognostic factors, with special emphasis on cytogenetic findings; being the risk of transformation to AML one of the highlights of this study. This was one of the controversial points for the approval of lenalidomide by the European Medicines Agency. Although a phase III clinical trial comparing lenalidomide vs placebo has shown some preliminary data about the risk of AML transformation in patients treated and not treated with lenalidomide,[12] herein, we have studied extensively this parameter in nontreated patients, taking different prognostic factors into account.

We confirmed the strong relationship between the number of additional chromosomal abnormalities (apart from deletion 5q) and outcomes, and we are able to show that the patterns of these additional karyotype abnormalities define two distinct risk groups concerning the probability of OS and three concerning the risk of AML transformation. Platelet count and sex were the only variables independently associated with OS in a specific subanalysis of patients with WHO-defined '5q−syndrome'.

As to cytogenetic abnormalities, we found that the most frequent single additional abnormalities to deletion 5q were: del(12p), trisomy 21, trisomy 8 and del(20q), the incidences of which were within the ranges reported in the literature.[23] It should be noted, however, that the number of aberrations of chromosome 7 (−7/7q−) occurring as the sole additional abnormality in this series ($n=5$) was not large enough to help us to clarify its prognostic value, though a slightly nonstatistically significant decrease in OS was observed in this subset of patients (data not shown).

Regarding break points observed in our series, our results agree with previous studies.[9,24–26] However, some of the variability in the reported deletion break points may result from the difficulties of interpretation in suboptimal chromosomal preparations and the interpersonal variability as well. For the whole series, we observed an association between the deleted regions and its outcomes, in terms of OS. Nevertheless, we did not find association of the length of the deleted segment with respect to OS. Of note, no significant differences in break points were observed between patients with the '5q−syndrome' and the rest of the series, in contrast with which was previously reported.[27]

Karyotype complexity is a well-known prognostic factor in MDS.[5,8,10,11,28,29] However, in MDS patients with deletion 5q prognostic value of the number of chromosomal abnormalities in addition to deletion 5q (for example, complexity of the karyotype) is still a matter of debate, with previous reports showing conflicting results. In 2003, Stewart *et al.*[30] analyzed outcomes of hematopoietic stem cell transplants in patients with MDS or AML and deletion 5q as the sole karyotypic abnormality ($n=20$) vs deletion 5q in combination with other chromosomal abnormalities ($n=37$). Overall, patients with deletion 5q as the sole karyotypic abnormality had lower rate of relapse and increased relapse-free survival. In addition to that, the blast count (<5%) was the only factor significantly associated with relapse-free survival. In 2004, Giagounidis *et al.*[9] reported a series of 76 MDS patients with deletion 5q in which those with one additional abnormality to deletion 5q had a significant worse prognosis. However, the analysis was restricted to a subset of just 10 patients with a single additional abnormality. Recently, Holtan *et al.*[31] studying 130 deletion 5q MDS patients (including 39 with isolated deletion 5q and 16 plus one additional aberration) found similar survival for these two groups. Finally, in the largest series reported before the present one, Haase *et al.*[5] did not find statistical differences in OS between both groups of patients (82 patients with one additional abnormality out of 168 deletion 5q MDS patients). In this enlarged series, we also failed to find a significantly different OS between patients with a sole deletion 5q ($n=275$) and those with a single additional abnormality ($n=89$), despite this latter group showed a somewhat shorter survival (46.0 vs 63.4 months; $P=0.131$). Nevertheless, this similarity was not kept for the risk of AML evolution, an outcome not extensively evaluated in previous studies, as patients with a single additional abnormality showed a higher risk of evolution to AML (57.6 vs 21.1% at 5 years; $P<0.001$). Patients with two or more additional abnormalities had a dismal prognosis in terms of OS and risk to AML transformation. The data regarding transformation to AML will be of importance, specially, in the

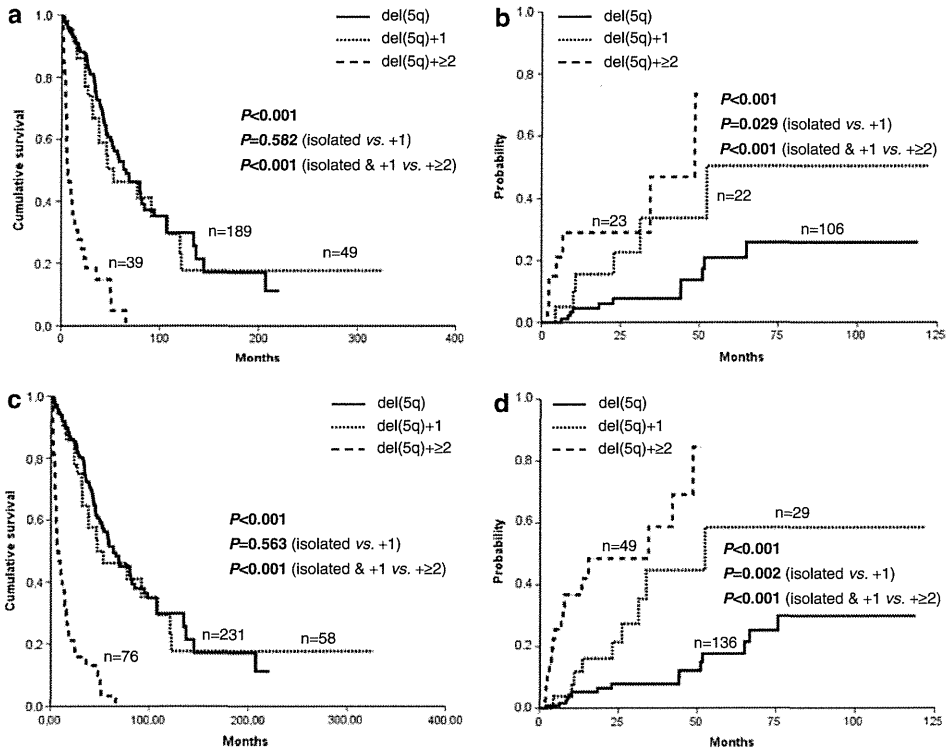

Figure 3 Kaplan–Meier curves according to the three defined cytogenetic categories (isolated del(5q), del(5q) + 1, del(5q) + ⩾2) in patients with <5 and <10% blasts in bone marrow (BM). (**a**) Actuarial probability of overall survival for patients with a BM blast count <5%. (**b**) Cumulative probability of AML transformation for patients with a BM blast count <5%. (**c**) Actuarial probability of overall survival for patients with a BM blast count <10%. (**d**) Cumulative probability of AML transformation for patients with a BM blast count <10%.

Table 5 Results of multivariate analysis of prognostic factors for OS and AML transformation in the overall series

Variable	*Overall survival*			*AML transformation*		
	Categories	*Hazards ratio (95% CI)*	P-*value*	*Categories*	*Hazards ratio (95% CI)*	P-*value*
Karyotype complexity	del(5q) and del(5q)+1 vs del(5q)+⩾2	4.1 (2.9–5.7)	<0.001	del(5q) vs del(5q)+1 vs del(5q)+⩾2	2.9 (2.0–4.1)	<0.001
Platelet count	$\leqslant 150 \times 10^9$/l vs $>150 \times 10^9$/l	2.0 (1.5–2.8)	<0.001	$\leqslant 150 \times 10^9$/l vs $>150 \times 10^9$/l	2.2 (1.2–3.9)	0.001
BM blast count	<5% vs 5–10% vs 11–20% vs >20%	1.4 (1.2–1.7)	<0.001	<5 vs >5%	1.4 (1.1–1.9)	0.016
Age	<60 vs ⩾60 years	1.6 (1.2–2.3)	0.001	—	—	—
Sex	Female vs male	0.7 (0.5–0.9)	0.020	—	—	—

Abbreviations: AML, acute myeloid leukemia; BM, bone marrow; CI, confidence interval; OS, overall survival.

assessment of clinical trials, a controversial point for the approval of drugs in hematological malignancies.

Multivariate analysis confirmed the independently adverse impact of the complexity of the karyotype (for instance, plus ⩾2 additional aberrations) in both OS and risk of AML transformation. In contrast, differences in outcome between patients with isolated deletion 5q and those with a single additional abnormality do not seem to be fully attributable to the extra aberration *per se*. In fact, these two groups showed significant differences in variables such as BM blasts and platelet count (Table 2), which could account, at least in part, for the different outcomes.

Nowadays, the IPSS score[10] still being the 'gold standard' for MDS stratifications and prognostication. In 2007, Malcovati *et al.*[32] published a new scoring system based on the WHO classification, called WHO classification-based prognostic scoring system, which includes the IPSS cytogenetic risk categories, the WHO classification and transfusion requirements.Unfortunately, this latter variable was not available in most of our patients and, thus we were not able to evaluate the potential prognostic importance of transfusion requirements and WHO classification-based prognostic scoring system in MDS patients with 5q deletion.

Finally, we analyzed the characteristics and outcome of 148 patients fulfilling the '5q−syndrome' WHO (2001) definition (144 with available follow-up data). WHO (2008) classification[3] restricts this diagnosis to MDS patients with an isolated deletion 5q without any additional chromosomal abnormality (with the

exception of a loss of the Y chromosome) and a BM blast count below 5%. Ironically, none of the patients with deletion 5q MDS in this series showed a concurrent loss of Y chromosome, although it was observed in the context of complex karyotypes. The multivariate analysis of prognostic factors in patients with WHO 2001-defined '5q−syndrome' showed that a platelet count lower or equal to 150×10^9 per liter and advanced age were adversely related to OS. In contrast, none of the parameters evaluated showed a significant association with AML transformation risk. This is the first series that includes a large number of cases with '5q−syndrome' defined according to the WHO classification; our findings could help to a better prognostic characterization of this entity. Although, Patnaik *et al.*[33] in 2010, published a large series fulfilling the current WHO-2008 definition, they were as not large as our subset of patients. However, the multivariate analysis give additional data, showing that the tranfusion need at diagnosis and dysgranulopoiesis are important prognostic factors, as well as age. In addition, they contribute with data from molecular studies that is very useful in this subset of patients. They performed mutational analysis from *JAK2*, *MPL* and *IDH1* genes, which revealed mutations except for the *IDH1* gene, they are more associated with high-risk MDS or AML.[33,34]

In summary, the results of this retrospective collaborative study, which is the largest available series of patients with primary MDS and deletion 5q, most of them receiving supportive care, show the independent prognostic impact of the number of additional chromosomal abnormalities to deletion 5q, to question the currently accepted WHO definition of the '5q−syndrome'. In addition, it is the first to show the prognostic importance of platelet count and age in patients with '5q−syndrome'. Further, this series could be very useful for the design of clinical trials in MDS patients with deletion 5q. This may be of special relevance in view of the controversies arisen by the results observed in patients treated with lenalidomide.

Conflict of interest

The authors declare no conflict of interest.

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Phase I and II study of azacitidine in Japanese patients with myelodysplastic syndromes

Toshiki Uchida,[1,17,18] Yoshiaki Ogawa,[2,18] Yukio Kobayashi,[3] Takayuki Ishikawa,[4,19] Haruhiko Ohashi,[5] Tomoko Hata,[6] Noriko Usui,[7] Masafumi Taniwaki,[8] Kazunori Ohnishi,[9] Hideki Akiyama,[10] Keiya Ozawa,[11] Kazuma Ohyashiki,[12] Shinichiro Okamoto,[13] Akihiro Tomita,[14] Shinji Nakao,[15] Kensei Tobinai,[3] Michinori Ogura,[1] Kiyoshi Ando[2] and Tomomitsu Hotta[16]

[1]Department of Hematology and Oncology, Nagoya Daini Red Cross Hospital, Nagoya; [2]Division of Hematology and Oncology, Department of Internal Medicine, Tokai University School of Medicine, Kanagawa; [3]Hematology and Stem Cell Transplantation Division, National Cancer Center Hospital, Tokyo; [4]Department of Hematology and Oncology, Graduate School of Medicine, Kyoto University, Kyoto; [5]Clinical Research Center, National Hospital Organization Nagoya Medical Center, Nagoya; [6]Department of Hematology and Molecular Medicine Unit, Nagasaki University, Nagasaki; [7]Division of Oncology and Hematology, Department of Internal Medicine, The Jikei University School of Medicine, Tokyo; [8]Division of Hematology and Oncology, Kyoto Prefectural University of Medicine, Kyoto; [9]Cancer Center, Hamamatsu University School of Medicine, Shizuoka; [10]Hematology Division, Tokyo Metropolitan Komagome Hospital, Tokyo; [11]Division of Hematology, Department of Medicine, Jichi Medical University, Tochigi; [12]First Department of Internal Medicine, Tokyo Medical University, Tokyo; [13]Division of Hematology, Keio University School of Medicine, Tokyo; [14]Department of Hematology and Oncology, Nagoya University Graduate School of Medicine, Nagoya; [15]Department of Cellular Transplantation Biology, Kanazawa University Graduate School of Medicine, Ishikawa; [16]National Hospital Organization Nagoya Medical Center, Nagoya, Japan

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Azacitidine, an inhibitor of DNA methyltransferase, is reported to have antileukemic efficacy and is approved for the treatment of myelodysplastic syndromes in Western countries. We have conducted a Phase I/II study of azacitidine in Japanese patients with myelodysplastic syndromes to evaluate its pharmacokinetics, efficacy, and safety. In all, 53 patients received 75 mg/m^2 azacitidine subcutaneously or intravenously once daily for seven consecutive days on a 28-day cycle. The C_{max} following intravenous administration was approximately 3.7-fold higher than that following subcutaneous administration, whereas the area under the plasma concentration–time curve from time zero to infinity was comparable for subcutaneous and intravenous administration. The bioavailability of azacitidine following subcutaneous administration was 91.1%, indicating that azacitidine is nearly completely absorbed after subcutaneous administration. The hematologic improvement and hematologic response rates were 54.9% (28/51) and 28.3% (15/53), respectively, and there were no differences between the two routes of administration. Azacitidine was generally well tolerated and clinically manageable in Japanese patients with myelodysplastic syndromes. Adverse events occurred in ≥20% of patients included hematologic toxicity, gastrointestinal events, and general disorders, such as malaise. Grade 3/4 adverse events that occurred in ≥50% of patients were all due to hematologic toxicity. The safety profile of azacitidine was generally similar for both routes of administration, with the exception of injection site reactions observed following subcutaneous administration. These results indicate that azacitidine can be expected to be a useful therapeutic agent in Japanese patients with myelodysplastic syndromes. (*Cancer Sci* 2011; 102: 1680–1686)

Myelodysplastic syndromes are hematopoietic stem cell disorders characterized by ineffective hematopoiesis leading to peripheral blood cytopenias and, in many patients, there is a risk of progression to AML.[1,2] Peripheral blood cytopenias, anemia, neutropenia, and thrombocytopenia are the hallmark symptoms of MDS,[3] often resulting in death due to complications such as infection and hemorrhage.

Although no clear association has been identified between genetic aberrations and the pathogenesis of MDS, the presence and expansion of malignant clones of pluripotent hematopoietic stem cells have been detected. Epigenetic alterations, such as hypermethylation of DNA, have been associated with the pathogenesis of MDS.[4,5] Marked methylation of the promoter domain of a tumor-suppressor gene encoding a cell cycle-regulating factor decreases the expression of the gene and induces abnormal cell proliferation.

Azacitidine is a cytidine nucleoside analog, the activity of which against abnormal hematopoietic cells may be mediated by demethylation of DNA and cytotoxic effects. In a Phase III study conducted by the CALGB, azacitidine yielded significantly higher response rates in all types of MDS classified according to the FAB classification[6] compared with best supportive care.[7,8] In addition, in a recently reported international multicenter Phase III study (the AZA-001 study), azacitidine significantly prolonged survival in patients with higher-risk MDS compared with conventional care regimens.[9] Azacitidine is the first drug that has been demonstrated to alter the natural history of MDS.[7,9]

Pharmacokinetic evaluation of azacitidine is limited[10,11] and has not been performed in Japanese patients. Although azacitidine is currently approved for s.c. and i.v. administration in the US, no clinical trial has compared the PK, safety, and efficacy of azacitidine administered s.c. and i.v. Thus, we undertook the present study, an open-label, multicenter study, to evaluate the PK, efficacy, and safety of azacitidine following s.c. and i.v. administration in Japanese patients with MDS.

Materials and Methods

Patient selection. Patients aged between 20 and 79 years who had been diagnosed with an MDS subtype (e.g. RA, RARS, RAEB, or RAEB-T), according to FAB classification,[6] were enrolled in the present study. Patients with secondary (treatment-related) MDS with RAEB-T were excluded from the study. Other enrollment criteria included an Eastern Cooperative Oncology Group performance status of 0 or 1,[12] an estimated life expectancy ≥12 weeks, adequate hepatic function (total bilirubin ≤1.5-fold the ULN, aspartate aminotransferase or alanine aminotransferase levels ≤2-fold the ULN), adequate renal function (serum creatinine ≤1.5-fold the ULN, serum carbonates

[17]To whom correspondence should be addressed.
E-mail: tuchida@nagoya2.jrc.or.jp
[18]These authors contributed equally to this work.
[19]Present address: Department of Hematology and Clinical Immunology, Kobe City Medical Center General Hospital, Kobe, Japan.

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Table 1. Patient characteristics

Patient characteristics	Phase I (n = 10)	No. patients in the Phase I and II studies (%)		
		All (n = 53)	Subcutaneous administration (n = 26)	Intravenous administration (n = 27)
Sex				
Male	8	36 (67.9)	17 (65.4)	19 (70.4)
Female	2	17 (32.1)	9 (34.6)	8 (29.6)
Age (years)				
Median	64	65	64	66
Range	58–77	35–77	37–77	35–77
ECOG performance status				
0	6	33 (62.3)	16 (61.5)	17 (63.0)
1	4	20 (37.7)	10 (38.5)	10 (37.0)
FAB classification				
RA	4	16 (30.2)	7 (26.9)	9 (33.3)
RARS	1	3 (5.7)	1 (3.8)	2 (7.4)
RAEB	4	20 (37.7)	11 (42.3)	9 (33.3)
RAEB-T	1	14 (26.4)	7 (26.9)	7 (25.9)
WHO classification				
RA	3	9 (17.0)	6 (23.1)	3 (11.1)
RCMD	1	5 (9.4)	1 (3.8)	4 (14.8)
RARS	0	0	0	0
RCMD-RS	1	3 (5.7)	1 (3.8)	2 (7.4)
RAEB-1	2	11 (20.8)	6 (23.1)	5 (18.5)
RAEB-2	2	14 (26.4)	8 (30.8)	6 (22.2)
MDS-U	0	0	0	0
del (5q)	0	2 (3.8)	0	2 (7.4)
AML	1	9 (17.0)	4 (15.4)	5 (18.5)
IPSS				
Low	0	0	0	0
Intermediate-1	5	23 (43.4)	10 (38.5)	13 (48.1)
Intermediate-2	3	15 (28.3)	10 (38.5)	5 (18.5)
High	2	15 (28.3)	6 (23.1)	9 (33.3)
Bone marrow blasts (%)				
<5	5	20 (37.7)	9 (34.6)	11 (40.7)
5–10	2	13 (24.5)	8 (30.8)	5 (18.5)
10–20	2	12 (22.6)	6 (23.1)	6 (22.2)
20–30	1	8 (15.1)	3 (11.5)	5 (18.5)
Median	4.9	8.5	8.45	8.8
Range	0.0–23.0	0.0–29.9	0.0–29.9	0.0–28.6
Cytogenetic abnormalities				
–	6	19 (35.8)	10 (38.5)	9 (33.3)
+	4	34 (64.2)	16 (61.5)	18 (66.7)
Karyotype risk				
Good	6	24 (45.3)	12 (46.2)	12 (44.4)
Intermediate	3	13 (24.5)	6 (23.1)	7 (25.9)
Poor	1	16 (30.2)	8 (30.8)	8 (29.6)
Cytopenia				
0/1	1	8 (15.1)	3 (11.5)	5 (18.5)
2/3	9	45 (84.9)	23 (88.5)	22 (81.5)
Time since original diagnosis (years)				
<1	4	22 (41.5)	10 (38.5)	12 (44.4)
1–2	1	9 (17.0)	5 (19.2)	4 (14.8)
2–3	1	6 (11.3)	3 (11.5)	3 (11.1)
≥3	2	9 (17.0)	6 (23.1)	3 (11.1)
Unknown	2	7 (13.2)	2 (7.7)	5 (18.5)

AML, acute myelogenous leukemia; ECOG, Eastern Cooperative Oncology Group; FAB, French–American–British; IPSS, International Prognostic Scoring System; MDS-U, myelodysplastic syndromes, unclassifiable; RA, refractory anemia; RAEB, refractory anemia with excess blasts; RAEB-1, refractory anemia with excess blasts-1; RAEB-2, refractory anemia with excess blasts-2; RAEB-T, refractory anemia with excess blasts in transformation; RARS, refractory anemia with ringed sideroblasts; RCMD-RS, refractory cytopenia with multilineage dysplasia and ringed sideroblasts; WHO, World Health Organization.

≥19 mEq/L), and ≥4 weeks since the most recent treatment for MDS, such as immunosuppressive drugs or androgens (patient who had received chemotherapy or radiotherapy were excluded from the study). Patients with RA or RARS needed to meet at least one of the following criteria: (i) hemoglobin <10 g/dL requiring red blood cell transfusion for at least 3 months before study entry; (ii) platelet count $<5 \times 10^4/mm^3$ or significant clinical hemorrhage; and/or (iii) absolute neutrophil count

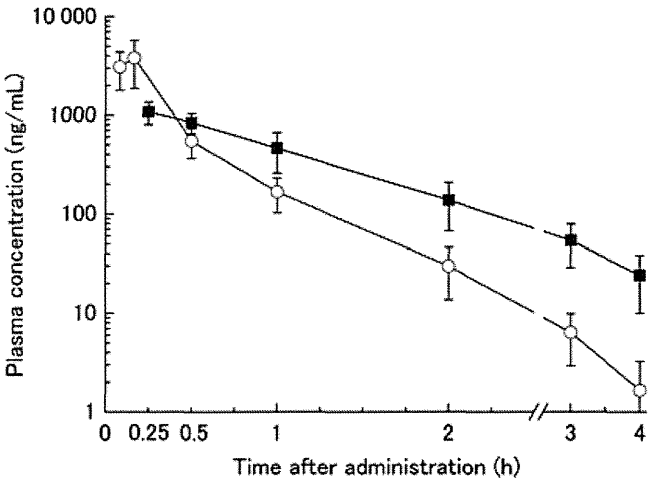

Fig. 1. Time-course of mean plasma concentrations of azacitidine (n = 9) following s.c. (■) or i.v. (○) administration. The 8-h plasma concentrations were not plotted because they were measurable in three patients only (s.c. administration) or were below the limit of detection in all patients (i.v. administration). Data show the mean ± SD.

<1000/mm^3, with susceptibility to infection requiring treatment with antibiotics. The Institutional Review Board of each participating hospital approved the study protocol and the study was conducted in accordance with Good Clinical Practice for Trials of Drugs and the Declaration of Helsinki. All patients provided written informed consent.

Study design. Azacitidine was administered at 75 mg/m^2 once daily for 7 days on a 28-day cycle either s.c. or i.v. (10-min infusion). For s.c. administration, azacitidine suspension was prepared at a final concentration of 25 mg/mL. For doses >100 mg (4 mL), the dose was divided equally between two syringes and injected into two separate sites. A serotonin 5-HT$_3$ receptor antagonist was administered approximately 30 min prior to azacitidine to prevent nausea and vomiting.

The study was divided into three parts: a Phase I part, a Phase II part, and an extended administration part. Patients were alternately assigned to receive s.c. or i.v. administration in order of their enrollment in the study. In the Phase I part of the study, PK and safety were examined after the administration of two cycles of azacitidine with a cross-over design for route of administration (s.c. to i.v. or i.v. to s.c.) to evaluate PK. In the Phase II part of the study, azacitidine was administered for a minimum of four cycles and its safety and efficacy were evaluated. Patients who received azacitidine for two cycles in the Phase I part of the study continued with azacitidine for an additional two cycles in the Phase II part of the study. In the extended administration part of the study, treatment was continued for patients who met the criteria of PR or HI in the Phase II part of the study as long as they continued to benefit, or until disease progression, for a maximum of 18 cycles.

During the study, the dose of azacitidine could be adjusted (either delayed or decreased) at the beginning of any cycle on the basis of hematologic laboratory results (nadir counts), renal function, serum electrolytes (serum bicarbonate, blood urea nitrogen, and serum creatinine), and the occurrence of Grade 3/4 non-hematologic toxicity. If no HI was evident by Cycle 2 and if no Grade 3/4 non-hematologic toxicity occurred (other than nausea or vomiting), the dose of azacitidine could be increased by 100 mg/m^2 for the next cycle.

A change in the route of administration was allowed only at the discretion of the investigator depending on the patient's condition (but not during the administration period). Patients who met the criteria of CR received azacitidine for an additional three cycles and were then followed-up off treatment.

Pharmacokinetics. The PK of azacitidine was evaluated on Day 1 in each cycle of the Phase I part of the study. Blood samples were taken before administration and then 15 and 30 min and 1, 2, 3, 4, and 8 h after s.c. administration. For i.v. administration, blood samples were taken before administration and then at 5, 10, and 30 min and 1, 2, 3, 4, and 8 h after administration. Plasma concentrations of azacitidine were measured by LC/MS/MS. The lower limit of quantification was 1.0 ng azacitidine/mL plasma.

The following PK parameters were calculated for each patient for both routes of administration, when applicable. Both C_{max} and t_{max} were determined from observed values. The elimination rate constant (β) was estimated by linear regression of the logarithm of the terminal plasma concentrations as a function of time from at least three data points showing the highest correlation coefficient and $t_{½,β}$ was calculated as 0.693/β. A linear trapezoidal rule area was used to calculate the AUC_{0-t}. Extrapolation to infinity was obtained as $Ct/β$ and this area was added to the AUC_{0-t} to provide an estimate of $AUC_{0-\infty}$. The systemic bioavailability (%) after s.c. administration was calculated from the ratio of the s.c. geometric mean $AUC_{0-\infty}$ to the i.v. geometric mean $AUC_{0-\infty}$. The PK parameters were calculated using WinNonlin Professional v. 5.2.1 (Pharsight, Mountain View, CA, USA).

Efficacy evaluation. The primary and secondary efficacy endpoints in the present study were the HI and HR rates, respectively, determined according to IWG 2006 criteria.[13] The primary endpoint of HI included evaluation of HI-E, HI-P, and HI-N. Furthermore, HI was defined as the presence of HI in at least one cell line. The secondary endpoint of HR included evaluation of CR, PR, and mCR, defined as marrow improvement evidenced by ≤5% myeloblasts and a ≥50% decrease in myeloblasts over the pretreatment period without an improvement in cytopenias. Efficacy was evaluated after completion of Cycle 4 and after completion of the last cycle, and the best response was defined as the best HI or HR achieved during the study period. The HI was evaluated in patients with abnormal pretreatment values defined according to IWG 2006 response criteria.[13] Transfusion independence was also evaluated in patients who were blood transfusion dependent at baseline. A patient was considered to be transfusion independent at baseline if the patient had had no transfusions during the 56 days prior to and

Table 2. Pharmacokinetic parameters for azacitidine after subcutaneous and intravenous administration

Route of administration	n	Dose (mg/m^2)	C_{max} (ng/mL)	t_{max} (h)	$AUC_{0-\infty}$ (ng × h/mL)	$t_{½,β}$ (h)	BA† (%)
Subcutaneous	9	75	1120 ± 210	0.361 ± 0.253	1180 ± 250	1.05 ± 0.61	91.1
Intravenous	9	75	4170 ± 1850	0.158 ± 0.028	1440 ± 520	0.441 ± 0.041	–
Intravenous	1	25	953	0.0833	455	0.389	–

Unless noted otherwise, data are given as the mean ± SD. †n = 8. –, not calculated; C_{max}, maximum plasma concentration; t_{max}, time to maximum concentration; AUC_{0-t}, area under the plasma concentration–time curve from time zero to the last measurable time point; $AUC_{0-\infty}$, area under the plasma concentration–time curve from time zero to infinity; $t_{½,β}$, half-life in the beta-phase; BA, bioavailability.

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Table 3. Best response

Response	*N*	*n*	%	95% CI
Any HI†	51	28	54.9	40.3–68.9
HI-E	46	21	45.7	
HI-P	33	22	66.7	
HI-N	29	14	48.3	
Progression/relapse after HI	53	3	5.7	
Any HR	53	15	28.3	16.8–42.3
CR	53	8	15.1	
PR	53	0	0	
mCR	53	7	13.2	
Stable disease	53	26	49.1	
Failure	53	0	0	
Relapse after CR or PR	53	0	0	
Disease progression	53	8	15.1	
Not evaluable‡	53	4	7.5	

†International Working Group 2006 response criteria[13] define the cases subject to evaluation of hematologic improvement (HI) as follows: baseline hemoglobin <11 g/dL for erythroid improvement (HI-E), baseline platelet count $<10 \times 10^4/mm^3$ for platelet improvement (HI-P), and baseline absolute neutrophil count $<1000/mm^3$ for neutrophil improvement (HI-N). ‡Hematologic responses were not evaluated in the four patients who dropped out of the study during the first cycle of azacitidine treatment. HR, hematologic response; CR, complete remission; PR, partial remission; mCR, marrow complete remission.

including the first day of treatment. Otherwise, the patient was considered to be transfusion dependent. A patient was considered to be transfusion independent during the study period if the patient had no transfusions over the course of ≥56 consecutive days during the study period.

Safety evaluation. The occurrence of AE was monitored from the first administration of azacitidine to Day 29 of the last cycle. Furthermore, the occurrence of AE was monitored until just before any change in treatment in cases in which treatment was discontinued or changed to other treatment due to progression of MDS or for other reasons. The AE were classified according to the Medical Dictionary for Regulatory Activities, Japanese edition (MedDRA/J v. 12.0, http://www.pmrj.jp/jmo/php/indexj.php, accessed 22 Jun, 2011), whereas the severity of the AE was evaluated according to the National Cancer Institute's Common Terminology Criteria for Adverse Events, v. 3.0 (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/ctcaev3.pdf, accessed 22 Jun, 2011).

Results

Patient characteristics. In all, 54 patients were enrolled in the present study and 53 received azacitidine (10 in the Phase I part of the study and 43 in the Phase II part). One patient dropped out of the study before initiation of treatment because of the discovery of a serious complication (emphysema) after enrollment. Thirty-four patients (64.2%) continued on treatment in the extended administration part of the study. The baseline characteristics of the 53 patients are given in Table 1 and were generally similar for patients in the s.c. and i.v. groups. Nine patients (17.0%) had AML based on the WHO classification.[14] All patients had de novo MDS.

The median number of cycles of azacitidine administered was 7 (range 1–18). Forty-two of 53 patients (79.2%) received four or more cycles, 32 (60.4%) received six or more cycles, 15 (28.3%) received 12 or more cycles, and eight (15.1%) completed 18 cycles of treatment. Of the 32 patients who received six or more cycles, 20 (62.5%) remained on 75 mg/m^2 azacitidine throughout the study period with no dose adjustments.

On the basis of hematology laboratory values (nadir counts), the next cycle was delayed for 30 patients (56.6%) and, of these, 18 (60.0%) had their dose reduced. The dose of azacitidine was not increased above 75 mg/m^2 for any of the patients.

The route of administration was changed for seven patients during the study period. Four patients were changed from s.c. to i.v. due to injection site reactions ($n = 3$) and hemorrhagic event (purpura; $n = 1$), whereas three patients were changed from i.v. to s.c. administration owing to difficulties in securing a route of access because of small blood vessels ($n = 2$) and extravasation ($n = 1$).

Table 4. Subgroup analysis of best response

Response	% (*n*/*N*)									
	All subjects	FAB classification				IPSS			Route of administration†	
		RA	RARS	RAEB	RAEB-T	Int-1	Int-2	High	Subcutaneous	Intravenous
Any HI	54.9 (28/51)	50.0 (8/16)	100 (3/3)	57.9 (11/19)	46.2 (6/13)	60.9 (14/23)	46.2 (6/13)	53.3 (8/15)	53.8 (14/26)	56.0 (14/25)
HI-E	45.7 (21/46)	40.0 (6/15)	100 (3/3)	50.0 (9/18)	30.0 (3/10)	47.6 (10/21)	41.7 (5/12)	46.2 (6/13)	54.5 (12/22)	37.5 (9/24)
HI-P	66.7 (22/33)	46.2 (6/13)	50.0 (1/2)	100 (9/9)	66.7 (6/9)	62.5 (10/16)	71.4 (5/7)	70.0 (7/10)	68.8 (11/16)	64.7 (11/17)
HI-N	48.3 (14/29)	60.0 (3/5)	0 (0/1)	50.0 (8/16)	42.9 (3/7)	30.0 (3/10)	55.6 (5/9)	60.0 (6/10)	40.0 (6/15)	57.1 (8/14)
Any HR	28.3 (15/53)	18.8 (3/16)	33.3 (1/3)	35.0 (7/20)	28.6 (4/14)	21.7 (5/23)	33.3 (5/15)	33.3 (5/15)	26.9 (7/26)	29.6 (8/27)
CR	15.1 (8/53)	18.8 (3/16)	33.3 (1/3)	15.0 (3/20)	7.1 (1/14)	17.4 (4/23)	13.3 (2/15)	13.3 (2/15)	11.5 (3/26)	18.5 (5/27)
PR	0	0	0	0	0	0	0	0	0	0
mCR	13.2 (7/53)	0	0	20.0 (4/20)	21.4 (3/14)	4.3 (1/23)	20.0 (3/15)	20.0 (3/15)	15.4 (4/26)	11.1 (3/27)
RBC transfusion independent‡	55.6 (15/27)	55.6 (5/9)	100 (3/3)	46.2 (6/13)	50.0 (1/2)	66.7 (10/15)	33.3 (2/6)	50.0 (3/6)	60.0 (9/15)	50.0 (6/12)

†The route of administration for each patient was defined as the route via which the patient received most cycles of azacitidine treatment (there were several patients in whom the route of administration changed during the treatment period). ‡A patient was considered red blood cell (RBC) transfusion independent at baseline if he or she had undergone no RBC transfusions during the 56 days prior to and including the first day of treatment. The patient was otherwise considered RBC transfusion dependent. A patient was considered RBC transfusion independent during the treatment period if he or she did not undergo any RBC transfusions over a period of ≥56 consecutive days during the treatment period (e.g. Day 1–Day 56, Day 2–Day 57 etc.). HI, hematologic improvement; E, erythroid; P, platelet; N, neutrophil; HR, hematologic response; CR, complete remission; PR, partial remission; mCR, marrow complete remission; RA, refractory anemia; RAEB, refractory anemia with excess blasts; RAEB-T, refractory anemia with excess blasts in transformation; RARS, refractory anemia with ringed sideroblasts; FAB, French–American–British; IPSS, International Prognostic Scoring System; Int-1, intermediate-1; Int-2, intermediate-2.

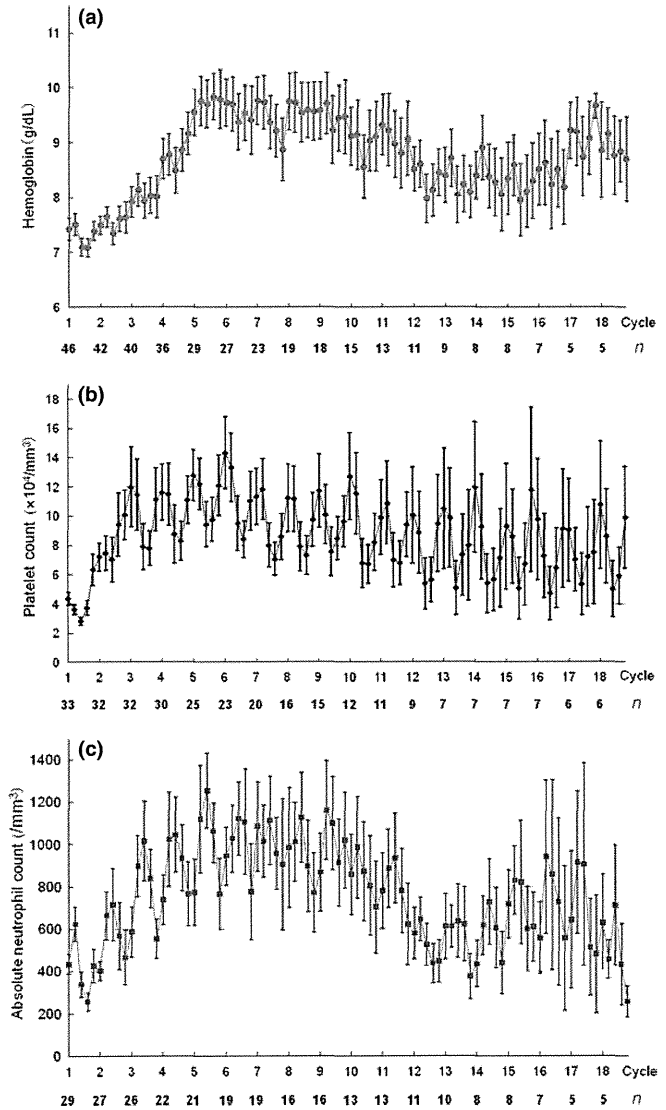

Fig. 2. (a) Hemoglobin concentrations, (b) platelet count, and (c) absolute neutrophil count following azacitidine treatment over 18 cycles. Data are the mean ± SEM. *n*, number of patients evaluable for hematologic improvement at each cycle.

Pharmacokinetics. The time course of plasma concentrations of azacitidine after s.c. or i.v. administration are shown in Figure 1 and the PK parameters are give in Table 2. Of 10 patients, one discontinued s.c. administration in the second cycle and another had the i.v. dose reduced to 25 mg/m^2 azacitidine on the basis of hematology laboratory values (nadir counts) in the second cycle. The bioavailability of azacitidine in the remaining eight patients receiving at 75 mg/m^2, s.c., azacitidine was 91.1%. The C_{max} for i.v. administration was approximately 3.7–fold higher than for s.c. administration, whereas the $AUC_{0-\infty}$ was comparable between the two routes of administration.

Efficacy. The best responses obtained in the present study are listed in Table 3. The HI rate was 54.9% (28/51) and the median time to reach the criterion for any improvement was 53.5 days (range 20–217). The HR was 28.3% (15/53) and the median time to reach the criterion for any remission was 113 days (range 49–247). All CR patients had two or three cell line cytopenias at baseline, as defined by the IPSS classification.[15] In addition, of the seven mCR patients, six had two or three cell line cytopenias at baseline, four achieved trilineage HI and one achieved bilineage HI. Subgroup analyses of best response among FAB, IPSS classifications, or route of administration are given in Table 4. There were no marked differences within each subgroup.

Of the 27 patients who were RBC transfusion dependent at baseline, 55.6% became transfusion independent during the study period and the proportion of such patients did not differ markedly with FAB or IPSS classifications (Table 4). Transfusion independence on platelet transfusion was not evaluable, because only two patients were platelet transfusion dependent at baseline.

The HI rates following s.c. and i.v. administration were 53.8% (14/26) and 56.0% (14/25), respectively, whereas the corresponding HR rates were 26.9% (7/26) and 29.6% (8/27), respectively (Table 4). There was no difference in efficacy between s.c. and i.v. administration of azacitidine.

The time course of mean values in three hematopoietic lineages in the patients evaluable for HI is shown in Figure 2. Hemoglobin, platelet, and absolute neutrophil counts all increased progressively until Cycle 5 or 6 and were maintained until Cycle 10.

Safety. The AE that occurred in ≥20% of patients included hematologic toxicity, gastrointestinal events, and general disorders, such as malaise (Table 5). Grade 3/4 AE, which occurred in ≥50% of patients, were all due to hematologic toxicity. Most Grade 3/4 hematologic toxicities were manageable by dose delay or dose reduction. The median time to onset of nadir hematology values across all cycles was 17 days for hemoglobin and platelets, and 25 days for absolute neutrophil counts.

There were no Grade 4 non-hematologic AE and The Grade 3 non-hematologic AE occurring in ≥10% of patients were limited to pneumonia (11.3%; 6/53) and a decrease in blood phosphorus (18.9%; 10/53). Most AE tended to be more pronounced during the first or second cycles of treatment than during later cycles. There were no AE that appeared to increase in frequency over time, suggesting that there were no delayed or cumulative toxicities. There were no relevant differences in the AE profile when analyzed according to FAB or IPSS classifications.

The incidence of AE was comparable between the s.c. and i.v. administration groups, with the exception of injection site reactions, which occurred more frequently following s.c. administration (Table 5). The injection site-related AE observed in the present study were all Grade 2 or lower, and no patients discontinued or interrupted their participation in the study owing to injection site reactions.

A total of 40 SAE was observed in 18 patients (34.0%). The SAE observed in two or more patients included febrile neutropenia (n = 7), pneumonia (n = 4), sepsis (n = 2), neutropenic infection (n = 2), thrombocytopenia (n = 2), and pericarditis (n = 2). With the exception of gastric cancer observed in a 74-year-old patient with RA, the seriousness criterion for SAE was "requiring or prolonging hospitalization." None of the patients died during the study period; however, one patient died 59 days after the last dose of azacitidine during the follow-up period for AE due to progression of MDS.

Discussion

This is the first report evaluating the PK, efficacy, and safety of azacitidine administered s.c. or i.v. in Japanese patients with MDS. In the Phase I portion of the study, the bioavailability of azacitidine following s.c. administration was 91.1%, indicating that azacitidine is nearly completely absorbed after s.c. administration, with negligible degradation or metabolism prior to entry into the circulation. The PK profile for azacitidine in Japanese patients was similar that seen in Caucasian patients with MDS,[10] suggesting that there are negligible ethnic differences in the PK of azacitidine.

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Table 5. All adverse events occurring in at least 20% of the 53 patients administered azacitidine

Preferred term†	*n* (%)						
	NCI-CTC grade			Route of administration			
				Subcutaneous		Intravenous	
	1–4	3	4	Cycle 1 (*n* = 27)	Cycle 4 (*n* = 21)	Cycle 1 (*n* = 26)	Cycle 4 (*n* = 22)
Hematologic							
Thrombocytopenia	46 (86.8)	9 (17.0)	25 (47.2)	20 (74.1)	11 (52.4)	21 (80.8)	7 (31.8)
Leukopenia	45 (84.9)	19 (35.8)	22 (41.5)	16 (59.3)	12 (57.1)	18 (69.2)	12 (54.5)
Neutropenia	44 (83.0)	3 (5.7)	40 (75.5)	20 (74.1)	14 (66.7)	19 (73.1)	12 (54.5)
Hemoglobin decreased	39 (73.6)	7 (13.2)	31 (58.5)	17 (63.0)	8 (38.1)	14 (53.8)	9 (40.9)
Erythropenia	36 (67.9)	7 (13.2)	18 (34.0)	16 (59.3)	8 (38.1)	12 (46.2)	8 (36.4)
Hematocrit decreased	32 (60.4)	5 (9.4)	15 (28.3)	14 (51.9)	6 (28.6)	9 (34.6)	8 (36.4)
Lymphopenia	29 (54.7)	12 (22.6)	5 (9.4)	10 (37.0)	9 (42.9)	8 (30.8)	6 (27.3)
Febrile neutropenia	16 (30.2)	16 (30.2)	0	5 (18.5)	0	4 (15.4)	0
Non-hematologic							
Constipation	39 (73.6)	1 (1.9)	0	11 (40.7)	6 (28.6)	17 (65.4)	11 (50.0)
Malaise	28 (52.8)	0	0	10 (37.0)	3 (14.3)	7 (26.9)	1 (4.5)
Pyrexia	24 (45.3)	4 (7.5)	0	9 (33.3)	1 (4.8)	4 (15.4)	4 (18.2)
ALT increased	23 (43.4)	2 (3.8)	0	11 (40.7)	6 (28.6)	7 (26.9)	2 (9.1)
Diarrhea	23 (43.4)	0	0	7 (25.9)	1 (4.8)	6 (23.1)	1 (4.5)
Blood albumin decreased	21 (39.6)	3 (5.7)	0	8 (29.6)	2 (9.5)	7 (26.9)	2 (9.1)
AST increased	21 (39.6)	2 (3.8)	0	9 (33.3)	4 (19.0)	6 (23.1)	3 (13.6)
Injection site reaction	21 (39.6)	0	0	18 (66.7)	7 (33.3)	1 (3.8)	0
Anorexia	20 (37.7)	3 (5.7)	0	9 (33.3)	1 (4.8)	5 (19.2)	1 (4.5)
Rash	20 (37.7)	0	0	6 (22.2)	2 (9.5)	5 (19.2)	2 (9.1)
Blood ALP increased	19 (35.8)	0	0	4 (14.8)	6 (28.6)	3 (11.5)	5 (22.7)
Nausea	18 (34.0)	1 (1.9)	0	6 (22.2)	0	7 (26.9)	3 (13.6)
Protein urine present	17 (32.1)	0	0	4 (14.8)	2 (9.5)	3 (11.5)	1 (4.5)
Blood glucose increased	16 (30.2)	3 (5.7)	0	0	6 (28.6)	3 (11.5)	3 (12.6)
Blood bilirubin increased	16 (30.2)	1 (1.9)	0	3 (11.1)	4 (19.0)	5 (19.2)	1 (4.5)
Blood LDH increased	15 (28.3)	1 (1.9)	0	5 (18.5)	3 (14.3)	2 (7.7)	1 (4.5)
Protein total decreased	15 (28.3)	1 (1.9)	0	8 (29.6)	1 (4.8)	2 (7.7)	1 (4.5)
Blood urine present	15 (28.3)	0	0	1 (3.7)	2 (9.5)	3 (11.5)	2 (9.1)
Blood phosphorus decreased	13 (24.5)	10 (18.9)	0	3 (11.1)	0	3 (11.5)	1 (4.5)
Nasopharyngitis	13 (24.5)	0	0	1 (3.7)	1 (4.8)	1 (3.8)	0
Injection site erythema	13 (24.5)	0	0	6 (22.2)	3 (14.3)	0	1 (4.5)
Stomatitis	11 (20.8)	0	0	2 (7.4)	2 (9.5)	2 (7.7)	1 (4.5)
Pruritus	11 (20.8)	0	0	5 (18.5)	2 (9.5)	3 (11.5)	3 (13.6)
Back pain	11 (20.8)	0	0	2 (7.4)	2 (9.5)	0	0

†Adverse events occurring at a frequency of 20% or more are listed. Adverse events are tabulated according to route of administration in each cycle because there were several cases in which the route of administration changed between cycles. NCI-CTC, National Cancer Institute Common Terminology Criteria; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; LDH, lactate dehydrogenase.

The HI and HR rates were 54.9% and 28.3%, respectively. In the present study, better efficacy was observed than in the CALGB9221 study conducted in a cohort of MDS patients similar to those in the present study,[8] although it is difficult to compare the results between the two studies owing to differences in the response criteria. In the present study, the HR rates in higher-risk MDS patients were 32.4% (11/34) in RAEB and RAEB-T and 33.3% (10/30) in Intermediate-2 and High. Although overall survival was not evaluated in the present study, the favorable efficacy results in higher-risk MDS patients suggests that a prolongation of survival in Japanese patients, similar to the results reported in the AZA-001 study,[9] could be expected. The HI rates in lower-risk MDS patients were 57.9% (11/19) in RA and RARS and 60.9% (14/23) in Intermediate-1. Furthermore, two-thirds of the lower-risk MDS patients who were blood transfusion dependent at baseline became transfusion independent during the study period. These findings indicate that azacitidine treatment results not only in a reduction in the risk of infection and hemorrhage due to cytopenias (leukocytopenia and thrombocytopenia), but also an improvement in the quality of life by eliminating the need for blood transfusions in lower-risk MDS patients.

In Western countries, azacitidine is administered as long as the patient continues to benefit. In the present study, patients who achieved HI required additional treatment cycles to achieve HR. In addition, the HI and HR rates were higher at completion of the last cycle of azacitidine than at completion of Cycle 4 (data not shown). Therefore continued treatment with azacitidine appears to be appropriate not just for Western patients, but also for all patients with MDS, including Japanese patients, as long as the patients continue to benefit from treatment. Hematology laboratory values in three hematopoietic lineages tended to decrease with time in and after Cycle 11 (Fig. 2). In the present study, patients who met the criteria for CR received azacitidine for an additional three cycles and were then followed-up off treatment. Because the median number of cycles in eight CR patients was 7.5 (range 5–11), the removal of these patients was considered to be one of the reasons for the observed decreases in laboratory values.

There was no difference in the efficacy of azacitidine between s.c. and i.v. administration. Because the dosing volume was

larger (4 mL maximum) for s.c. administration, there was a high incidence of injection site reactions in patients who received azacitidine s.c. and some of these patients had difficulty continuing with s.c. administration. Furthermore, s.c. administration was difficult in patients who had a low platelet count due to MDS and who were at risk of s.c. hemorrhage, whereas i.v. administration was problematic in patients in whom it was difficult to secure an i.v. route of administration due to small blood vessels or in those who had vascular disorders. In the present study, the route of administration of azacitidine was changed in seven patients. Selection of an alternative route of administration depending on patient condition is clinically significant not only in terms of enabling treatment with azacitidine, but also in terms of continuing it.

Azacitidine was well tolerated and clinically manageable in Japanese patients with MDS. The AE that occurred were generally associated with the known effects of azacitidine and were manageable with symptomatic therapy and/or dose delays, dose reductions, or dose discontinuation. With the exception of the injection site reactions observed following s.c. administration, the AE profile was generally similar between the s.c. and i.v administration groups. The safety profile of azacitidine in the Japanese patients in the present study was comparable to that reported previously in Caucasian patients.[7–9]

In conclusion, our findings demonstrate that the PK, efficacy, and safety of azacitidine are comparable in Japanese and non-Japanese patients, with no marked differences in the safety or efficacy profiles of azacitidine between s.c. and i.v. routes of administration. The risk–benefit ratio of azacitidine in Japanese patients with MDS was comparable to that in non-Japanese patients, indicating that this drug is a promising therapeutic agent for Japanese MDS patients.

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Disclosure Statement

Azacitidine was provided by Nippon Shinyaku Co., Ltd. The authors have no potential conflicts of interest to report.

Abbreviations

AE	adverse events
AML	acute myelogenous leukemia
$AUC_{0-\infty}$	area under the plasma concentration–time curve from time zero to infinity
AUC_{0-t}	area under the plasma concentration–time curve from time zero to the last measurable time point
CALGB	Cancer and Leukemia Group B
C_{max}	maximum plasma concentration
CR	complete remission
Ct	final concentration observed
FAB	French–American–British
HI	hematologic improvement
HI-E	erythroid response
HI-N	neutrophil response
HI-P	platelet response
HR	hematologic response
HSCT	hematopoietic stem cell transplantation
IPSS	International Prognostic Scoring System
i.v.	intravenous
IWG	International Working Group
LC/MS/MS	high-performance liquid chromatography with tandem mass spectrometry
mCR	marrow complete remission
MDS	myelodysplastic syndromes
PK	pharmacokinetics
PR	partial remission
RA	refractory anemia
RAEB	refractory anemia with excess blasts
RAEB-T	refractory anemia with excess blasts in transformation
RARS	refractory anemia with ringed sideroblasts
RBC	red blood cell
SAE	serious adverse events
s.c.	subcutaneous
$t_{½,\beta}$	half-life in the β phase
t_{max}	time to maximum concentration
ULN	upper limit of the institutional normal range
WHO	World Health Organization

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Microarray CGH analyses of chromosomal 20q deletions in patients with hematopoietic malignancies

Michiko Okada [a,*], Yumiko Suto [b], Momoki Hirai [c], Masayuki Shiseki [d], Akemi Usami [a], Kaori Okajima [a], Masanao Teramura [d], Naoki Mori [d], Toshiko Motoji [d]

[a] *Chromosome Laboratory, Shiseikai Dai-Ni Hospital, Tokyo, Japan;* [b] *Central Blood Institute, Japanese Red Cross Society, Tokyo, Japan;* [c] *International Research and Educational Institute for Integrated Medical Science, Tokyo Women's Medical University, Tokyo, Japan;* [d] *Division of Hematology, School of Medicine, Tokyo Women's Medical University, Tokyo, Japan*

The chromosomal abnormality del(20q) is mostly found in various myeloid disorders, including myelodysplastic syndromes, myeloproliferative neoplasms, and acute myeloid leukemia. Here, microarray comparative genomic hybridization (aCGH) analyses of 14 patients cytogenetically confirmed to carry the del(20q) aberration in their bone marrow demonstrated that all deletions were interstitial and both the proximal and distal breakpoints varied among individuals. The centromeric breakpoints were located in the 20q11.21-12 region, and the telomeric breakpoints, in the 20q13.13-13.33 region. The extent of the deletion ranged from 11.2 to 27.3 Mb, and the commonly deleted region (CDR) was estimated to be 7.2 Mb in size. Two commonly retained regions were present, the proximal region adjacent to the centromere (20q11.1-11.21) and a subtelomeric one (20q13.33). The CDR of our study was more distal than reported previously. Furthermore, in three patients fluorescence in situ hybridization (FISH) demonstrated that del(20q) cells were detected at a higher frequency in the karyotype analyses than by interphase FISH and aCGH analyses. As the size and breakpoints of del(20q) have been reported to vary among patients, the presence of one or more tumor suppressor genes in the CDR has been suggested. Our study will contribute to the identification of candidate tumor suppressor genes on 20q.

Keywords Hematopoietic malignancies, del(20q) chromosome, microarray comparative genomic hybridization, commonly deleted region, interphase fluorescence in situ hybridization

The chromosomal abnormality del(20q) is found in various myeloid disorders, including myelodysplastic syndromes (MDSs), myeloproliferative neoplasms (MPNs), and acute myeloid leukemia (AML). MDS patients with a del(20q) have a relatively good prognosis (1). The deletion has been reported to be rare in patients with lymphoid malignancies (2), although it can arise in multipotent precursors of myeloid cells and B cells (3) and in pluripotent stem cells (4). However, during the last 20 years, we observed lymphoid malignancies in 8 of 73 patients who had del(20q) in their bone marrow cells. Therefore, we included two lymphoid patients in this study.

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* Corresponding author.
E-mail address: okadam@dh.twmu.ac.jp

Delineation of the bands deleted on 20q is difficult using conventional cytogenetic techniques because of the small size and indistinct banding, especially in bone marrow samples. The deletions were suggested to be interstitial and grouped into two categories, large deletions involving the loss of both G(+) bands from 20q and small deletions involving the loss of one G(+) band (20q12) (5). Molecular studies of del(20q) have defined a commonly deleted region (CDR) of 1.7–6.62 Mb using bacterial artificial chromosome (BAC)/P1-derived artificial chromosome (PAC) fluorescence in situ hybridization (FISH) or microsatellite polymerase chain reaction (PCR) (6,7). Recently, Huh et al. reported two CDRs, of 2.5 Mb and 1.8 Mb (8).

We preliminarily estimated the CDR of del(20q) to be 8.2 Mb in length from a study of eight patients at the Third Asian Chromosome Colloquium (ACC3) (9) using microarray

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comparative genome hybridization (aCGH) analyses. In the present report, we have added four more patients and reduced the estimated length of the CDR to 7.2 Mb. The purpose of this study was to accurately characterize del(20q) chromosomes from 12 patients with hematopoietic malignancies. Using 60-mer oligonucleotide arrays, we characterized their proximal and distal breakpoints in chromosome 20 to be between 30,100,973–39,933,662 bp, and 47,180,979–58,444,848 bp, (build 36.1, hg18) respectively.

Materials and methods

The experimental protocol involved the following steps: (A) Chromosomes were analyzed in bone marrow aspirates from 14 patients (Table 1). A portion of the aspirates and the rest of the fixed cells after the chromosome analysis were stored at −20°C. (B) We conducted aCGH analyses on the fixed cells or the bone marrow aspirates after storage for 0.5–8 years (Table 2). (C) The preserved cells of three patients, who showed differences in clone size by karyotype and array analyses, were used for FISH and additional karyotype analyses about 2 years after the array experiments (Table 3).

Patients

We selected 14 patients with a deletion of the long arm of chromosome 20, detected by Q-banding, for aCGH analyses. Six of the patients had MDS, one had MPN, five had AML, and two had lymphoid malignancies, Waldenström macroglobulinemia (WM) and non-Hodgkin lymphoma (NHL) in a leukemic phase (Table 1). The patients were diagnosed using the World Health Organization (WHO) classifications for myeloid neoplasms and acute leukemia (10), and hematopoietic and lymphoid tissues (11).

Chromosome analyses

Chromosome examinations were performed with a Q-banding technique using bone marrow cells cultured overnight without any mitogens, except in patient 12. A 7-day culture in the presence of phorbol 12-myristate 13-acetate (TPA) was also used for karyotype analyses of patient 12 to stimulate B-cell–lineage growth (Table 1). Karyotypes were described according to the International System for Human Cytogenetic Nomenclature 2009 (12).

Microarray CGH analyses

DNA was extracted from uncultured bone marrow aspirates from six patients. In the other eight patients, bone marrow cells fixed in Carnoy solution were used after an unstimulated overnight culture. All of these specimens had been preserved at −20°C for 0.5–8 years. The fixed cells were washed three times with 99% ethanol before use. DNA was isolated with a genomic DNA preparation kit (Mammalian Genome Mini Prep, Sigma-Aldrich, St. Louis, MO). Pooled human DNA (Promega, Fitchburg, WI) was used as a reference. The microarrays used in this study were 60-mer in situ synthesized oligonucleotide arrays produced by Agilent Technologies (Santa Clara, CA) based on the Human March 2006 Assembly (National Center for Biotechnology Information [NCBI], build 36.1, hg18). We used a genomewide catalogue CGH array consisting of approximately 244,000 probes with a mean interprobe interval of 8.9 b. All hybridizations were

Table 1 Clinical and cytogenetic characteristics of patients

Patient	Diagnosis	Sex/Age, y	Karyotype
1	MDS(RA)	M/73	46,XY,del(20)(q13.1 or q13.1q13.3)[26]/46,XY[3]
2	MDS(RA)	F/51	46,XX,del(20)(q11.2)[22]
3	MDS(RCMD)	M/69	46,XY,del(20)(q11.2)[30]
4	MDS(RCMD)	F/84	46,XX,del(20)(q11.1q13.1∼13.2) or del(20)(q13.1q13.3)[26]/46,XX[2]
5	MDS(RCMD)	M/76	46,XY,del(20)(q11q13.3)[11]/46,XY[16]
6	MPN(PV)	M/85	46,XY,del(20)(q11.?2q13.?1)[33]
7	MDS(RCMD)→AML	M/72	46,XY,del(20)(q11.2q13.3)[25]
8	MDS(RCMD)→AML	M/69	47,XY,+8,del(20)(q12 or q11q13.1)[30]
9	MPN(ET)→AML	M/65	46,XY,+1,der(1;7)(q10;p10),del(20)(q11.2 or q11.1q13.3)[30]
10	AML	F/74	47,XX,+8,del(20)(q11.2q13.1∼13.2)[32]
11	AML	M/76	46,XY,inv(9)(p12q13),der(16)t(1;16)(q12∼21;q11∼12)[1]/46,idem, del(20)(q11)[29]
12	WM	M/75	46,XY,inv(9)(p12q13),del(20)(q11.2)[25]/46,XY,inv(9)(p12q13)[3] (overnight culture without any stimulation) 46,XY,inv(9)(p12q13),del(20)(q11.2)[15]/ 46,XY,del(7)(q21q36),inv(9)(p12q13)[1]/46,XY,inv(9)(p12q13)[8] (7-day culture with TPA stimulation)
13	NHL in a leukemic phase	F/83	46,XX,del(20)(q11.2q13.3)[14]/46,XX[15]
14	MDS(RCMD)	M/63	46,XY,del(20)(q12q13.3)[9]/46,XY[17]
15[a]	AML with inv(16) (p13.1q22) in CR	M/36	46,XY[29]

Abbreviations: RA, refractory anemia; RCMD, refractory cytopenia with multilineage dysplasia; PV, polycythemia vera; ET, essential thrombocythemia.

[a] This is a control patient in the FISH experiment with no history of carrying a del(20q), and not included in aCGH studies.

Table 2 Results of aCGH analyses

Patient	Start (NT)	End (NT)	Deletion size (Mb)	Deleted bands detected by array analysis
1	37,488,367	50,316,897	12.83	20q12-13.2
2	30,411,323	49,098,594	18.69	20q11.21-13.13
3	30,319,299	51,455,290	21.14	20q11.21-13.2
4	36,957,293	54,407,570	17.45	20q11.23-13.31
5	31,134,883	58,444,848	27.31	20q11.21-13.33
6	32,233,259	47,180,979	14.95	20q11.22-13.13
7	31,049,105	49,076,424	18.03	20q11.21-13.13
8	34,408,519	48,145,526	13.74	20q11.23-13.13
9	34,049,128	50,034,067	15.98	20q11.23-13.2
10	39,933,662	51,171,130	11.24	20q12-13.2
11	34,351,399	57,476,399	23.13	20q11.23-13.32
12	30,100,973	56,986,098	26.89	20q11.21-13.32
13	Deletion was not detected in these two patients			
14				

Abbreviation: NT, nucleotide number.

performed according to the manufacturer's recommended protocols.

The restriction enzymes *AluI* and *RsaI* were used to digest 500 ng of genomic DNA, which we then fluorescently labeled using an Agilent Genomic Labeling Kit. The test samples were labeled with cyanine 5-deoxyuridine triphosphate, and the reference samples were labeled with cyanine 3-deoxyuridine triphosphate. Labeled DNA was denatured and pre-annealed with human Cot-1 DNA (Invitrogen, Carlsbad, CA) and Agilent blocking reagent prior to hybridization for 40 hours at 20 rpm in a 65°C hybridization oven. Standard washing procedures were followed. Array slides were scanned (GenePix 4000B; Axon Instruments, Foster City, CA) at a resolution of 5 mm, and image analysis was performed using the default CGH settings of Feature Extraction Software 9.1.3.1 (Agilent Technologies). Agilent Genome Workbench 5.0 was used to visualize, detect, and analyze genomic gain/loss patterns in the aCGH profiles using the ADM-2 algorithm and the optimum analytical conditions (threshold 6.0, default value).

FISH analyses

FISH experiments were performed on 3 patients (patients 12, 13, and 14) and a control (patient 15). Slides were prepared from the stored cells of overnight cultures described in the chromosome analyses. Karyotypes were additionally analyzed on those slides in each patient. A dual-color FISH probe was used to estimate the clone size of del(20q) cells. The probe was a mixture of the approximately 450-kb Texas Red–labeled *PTPRT* gene probe (composed of 3 BAC clones, GSP1579H07, GSP1187C03, and GSP1586H05) and 680-kb fluorescein isothiocyanate (FITC)–labeled DNA BAC clones (GSP1226G02, GSP1558G11, GSP1546F12, and GSP1152H07) designed by Genomic Signal Processing Laboratory (Kawasaki City, Japan). The *PTPRT* gene is located at 20q12~q13, the most proximal region within our CDR. The FITC-labeled probe is at 20q11.22, 5 Mb from the *PTPRT* gene. Del(20q) cells with large deletions (patients 12 and 13) were expected to have one green and one red signal.

Table 3 Results of FISH analysis

	Del(20q) chromosome[a]			FISH pattern of dual-color 20q probe			
				No. of cells with the pattern (%)			
Patient	Banding	No. of cells with del(20q) (%)	Total observed cells	G2R1[b]	G1R1[c]	G2R2[d]	Total
12	del(20)(q11.2)	24 (80.0)	30	I 7(1.9)	83(22.4)	280(75.7)	370
				M 0(0.0)	4(66.7)	2(33.3)	6
13	del(20)(q11.2q13.3)	3 (30.0)	10	I 10(2.2)	59(12.9)	388(84.9)	457
				M	ND		
14	del(20)(q12q13.3)	4 (23.5)	17	I 24(11.7)	5(2.4)	176(85.9)	205
				M	ND		
15 (control)	Normal karyotype	0 (0.0)	29	I 4(1.9)	1(0.5)	200(97.6)	205
				M	ND		

Abbreviations: I, interphase; M, metaphase; ND, no determination.

[a] Chromosomes were analyzed additionally on slides prepared for FISH experiments.

[b] G2R1 is a FISH pattern with 2 green and 1 red signal. Del(20q) cells of patient 14 demonstrate this pattern in the interphase nuclei.

[c] G1R1 is a FISH pattern with 1 green and 1 red signal. Del(20q) cells of patient 12 and patient 13 demonstrate this pattern.

[d] G2R2 is a FISH pattern with 2 green and 2 red signals, the pattern of cells having 2 normal chromosome 20.