Increased glycosylphosphatidylinositol-anchored protein-deficient granulocytes define a benign subset of bone marrow failures in patients with trisomy 8

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Original Article

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Running heads: Trisomy 8 with GPI-AP$^-$ cells

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Abstract

Trisomy 8 (+8), one of the most common chromosomal abnormalities found in patients with myelodysplastic syndromes (MDS), is occasionally seen in patients with otherwise typical aplastic anemia (AA). Although some studies have indicated that the presence of +8 is associated with the immune pathophysiology of bone marrow (BM) failure, its pathophysiology may be heterogeneous. We studied 53 patients (22 with AA and 31 with low-risk MDS) with +8 for the presence of increased glycosylphosphatidylinositol-anchored protein-deficient (GPI-AP$^-$) cells, their response to immunosuppressive therapy (IST), and their prognosis. A significant increase in the percentage of GPI-AP$^-$ cells was found in 14 (26%) of the 53 patients. Of the 26 patients who received IST, including nine with increased GPI-AP$^-$ cells and 17 without increased GPI-AP$^-$ cells, 14 (88% with increased GPI-AP$^-$ cells and 41% without increased GPI-AP$^-$ cells) improved. The overall and event-free survival rates of the +8 patients with and without increased GPI-AP$^-$ cells at five years were 100% and 100% and 59% and 57%, respectively. Examining the peripheral blood for the presence of increased GPI-AP$^-$ cells may thus be helpful for choosing the optimal

treatment for +8 patients with AA or low-risk MDS.

Key words: Trisomy 8, bone marrow failure, GPI-AP$^-$ cells, immunosuppressive therapy

Introduction

Karyotypic abnormalities in patients with bone marrow failure are generally regarded as a hallmark of clonal hematopoietic disorders with the propensity toward transformation into acute myeloid leukemia (AML). The incidence of cytogenetic abnormalities in aplastic anemia (AA) and myelodysplastic syndromes (MDS) is approximately 4% and 50 %, respectively (1, 2). Trisomy 8 (+8), one of the most frequent chromosomal abnormalities found in patients with MDS, is occasionally seen in patients with otherwise typical AA (3-8). A recent study based on 2072 MDS patients showed that 8% of these patients had +8 in isolation (9). For both AML and MDS, +8 is listed in the 'intermediate-risk cytogenetic group' (6, 9, 10). Several studies have shown that MDS patients with +8 are highly responsive to immunosuppressive therapy (IST) (3, 7, 11). However, +8 in AA patients is associated with an

increased risk of evolving into MDS/AML (5, 8). Thus, the prognostic significance of +8 in patients with AA or low-risk MDS remains unclear.

Small populations of glycosylphosphatidylinositol-anchored protein-deficient (GPI-AP$^-$) blood cells are often detected in the peripheral blood (PB) of patients with AA or low-risk MDS, such as refractory anemia (RA) and refractory cytopenia with multilineage dysplasia (RCMD) in the FAB classification (12-14). The GPI-AP$^-$ blood cells are detectable even in patients with BM failure that have chromosomal abnormalities, including +8 (15-17). Parlier and Longo reported the first patient with GPI-AP$^-$ blood cells and + 8 who had ringed sideroblasts (18, 19). Our recent study showed a close association of del(13q) with the presence of increased GPI-AP$^-$ cells as well as a favourable response to immunosuppressive therapy (IST) (16). In patients with AA or RA possessing +8, the presence of GPI-AP$^-$ cells may affect response to IST as well as prognosis. To test this hypothesis, we analyzed clinical data of 53 BM failure patients with +8 whose blood cells were examined for the presence of GPI-AP$^-$ cells.

Patients and Methods

Patients

This study included retrospective analysis of clinical records for 1228 BM failure patients: 733 with AA and 495 with low-risk MDS, including 286 with refractory cytopenia with unilineage dysplasia (RCUD), 149 with RCMD, and 60 with unclassified MDS (MDS-U). In all patients, blood samples were examined for the presence of GPI-AP$^-$ granulocytes and erythrocytes at our laboratory between May 1999 and July 2010. BM smear slides and trephine biopsy specimens were reviewed by two independent hematologists. BM cellularity was expressed as the percentage of BM volume occupied by hematopoietic cells in the trephine biopsy specimens. Hypocellular marrow was defined as <30% cellularity in patients <70 years, or <20% cellularity in patients ≥70 years (20). Chromosomal analysis was performed using the G-banding method and the presence of +8 clones were confirmed by fluorescent in-situ hybridization (FISH) when the number of +8 revealed by G-banding was less than or equal to two. The results of G-banding were described according to the International System for Human Cytogenetic Nomenclature (ISCN) (21). The ethics committee of Kanazawa University

Graduate School of Medical Science approved the study protocol, and all patients provided informed consent prior to sampling.

Therapy and response criteria

Horse anti-thymocyte globulin (ATG, Lymphoglobulin, Genzyme, Cambridge, MA, USA) in combination with cyclosporine (CsA) was given to patients with Severe aplastic anemia (SAA). Four to 6 mg/kg of CsA was administered to patients with moderate AA (MAA) or MDS. Trough levels of CsA were maintained between 150 and 250 ng/mL. Six patients (4 with AA and 2 with MDS) received 10 to 20 mg/day of metenolone acetate in addition to CsA. Responses to IST were defined according to the established criteria (22, 23).

Monoclonal antibodies

Monoclonal antibodies (mAbs) used for flow cytometry were FITC-conjugated anti-CD59 (P282E, IgG2a; Beckman Coulter, Brea, CA, USA), FITC-conjugated anti-CD55 (IA10, IgG2a; BD Pharmingen, San Diego, CA, USA), PE-conjugated anti-CD11b/Mac-1 (ICRF44, IgG1; BD Pharmingen)

and PE-conjugated anti-glycophorin A (JC159, IgG1; Dako, Glostrup, Denmark).

Detection of GPI-AP$^-$ cells by flow cytometry

All blood samples were analysed within 24 hours of collection to avoid false positive results due to cell damage. Staining with each mAb was performed according to the lyse-stain protocol as previously described (24, 25). The presence of CD55$^-$CD59$^-$glycophorin A$^+$ erythrocytes at the level of ≥0.005% and/or CD55$^-$CD59$^-$CD11b$^+$ granulocytes at the level of ≥0.003% was defined as an abnormal increase ("positive") based on results obtained from 183 healthy individuals (26). With careful handling of samples and elaborate gating strategies, cut-off values can be lowered to these levels without producing false positive results (24, 27, 28).

Statistical analysis

Prevalence of increased GPI-AP$^-$ cells among different patient populations was compared using the chi-square test. The Kaplan-Meier method and the Cox proportional hazards model were used to estimate time-to-event

analysis. Overall survival (OS) was calculated in months from date of diagnosis until date of death or last follow-up. Event-free survival (EFS) was defined as the time from diagnosis to AML evolution or death. Two-sided *P*-values were calculated, and $P<0.05$ was considered statistically significant. All statistical analyses were performed using the EZR software package (Saitama Medical Center, Jichi Medical University), a graphical user interface for R (The R Foundation for Statistical Computing, version 2.13.0) (29).

Results

Incidence of BM failure patients with +8

Of 754 patients with AA 22 (2.9%) possessed +8; instead, of 483 patients with low-risk MDS 31 (6.4%) possessed +8. Their clinical features are summarized in Table I. The median age of patients with +8 was 61, and BM was hypocellular in 32 patients, normocellular in 15, and hypercellular in six. Thirty-five patients had trisomy 8 alone (+8 alone), while 18 patients had additional chromosomal abnormalities (+8 others). The median percentage of +8 cells in karyotyped cells was 15%. Diagnoses of 31 MDS patients

according to the 2008 WHO classification included nine patients with RCUD, 16 with RCMD, and six with MDS-U. None of the patients with +8 had ringed sideroblasts. All MDS patients were classified as Int-1 according to the International Prognostic Scoring System (IPSS).

Prevalence of patients possessing increased GPI-AP$^-$ cells

As shown in Table 1, 14 (26.4%) of patients with +8 had GPI-AP$^-$ cells that accounted for 0.003% to 39.124% (median, 0.049%) of granulocytes. One patient who possessed 0.002% GPI-AP$^-$ granulocytes was judged positive because 0.026% of the patent's erythrocytes were GPI-AP$^-$ cells (Supplementary Fig. 1). None of the patients evolved into clinical PNH during the observation period of 2-10 years. The prevalence of increased GPI-AP$^-$ cells was lower than that (43%) in 937 BM failure patients (637 with AA and 300 with MDS) with normal karyotype (16). Of 22 AA patients with +8 nine (41%) had increased GPI-AP$^-$ cells; instead, of 31 low-risk MDS patients with +8 five (16%) had increased GPI-AP$^-$ cells. (P=0.04)

Response to IST in BM failure patients with +8

Twenty-six patients (49%) were treated with IST and 25 of these had evaluable responses. IST included CsA alone in 15 patients, CsA and ATG in five patients, and CsA and metenolone acetate in six patients. The overall response rate to IST in the +8 patients was 56% (14/25 patients). Of 16 AA patients with +8 treated with CsA and ATG (5) or CsA±metenolone acetate (11), nine (56%) responded. Nine MDS patients with +8 were treated with CsA±metenolone acetate and five (56%) improved (P=0.97). Of 8 patients positive for GPI-AP$^-$ cells treated with IST 7 (88%) responded; instead, of 17 patients negative for GPI-AP$^-$ cells 7 (41%) responded (P=0.03). Comparison of patients with +8 with 141 BM failure patients (120 with AA and 21 with MDS) with normal karyotypes that were included in our previous study (16) showed that +8 patients had lower response rates to IST than patients with normal karyotypes; 56% in +8 AA patients vs. 81% in normal karyotype AA patients (P=0.03) and 56% in +8 MDS patients vs. 62% in normal karyotype MDS patients (P=0.75) although the differences were not statistically significant in MDS patients (16).

Prognosis in BM failure patients with +8

None of the 14 +8 patients with increased GPI-AP$^-$ cells progressed to advanced MDS or AML during the follow-up period of 2 to 239 months (median, 67 months). On the other hand, five of the 39 +8 patients without GPI-AP$^-$ cells developed AML. The five-year OS and EFS rates of the 53 patients with +8 patients were 69.4% and 68.1%, respectively (Fig. 1A). The five-year OS/EFS rates of +8 patients with increased GPI-AP$^-$ cells were 100%/100%; instead, the five-year OS/EFS rates of +8 patients without increased GPI-AP$^-$ cells were 58.6%/56.9% (P=0.0347, P=0.0269, respectively; Fig. 1B). The five-year OS rates of +8 patients with +8 alone were 81.7%; instead, the five-year OS rates of +8 patients with +8 with other abnormalities were 45.5% (P= 0.0196; Fig. 1C). When age, gender, diagnosis, cellularity, clone size, karyotype complexity, and GPI-AP$^-$ cells were included in the multivariate analysis, higher age (60 years or older) and the absence of GPI-AP$^-$ cells represented independent negative predictors for OS (Table 2).

To further evaluate the significance of GPI-AP$^-$ cells in +8 patients, the five-year OS rates of BM failure patients with +8 were compared with those of 246 BM failure patients (179 with AA and 67 with MDS) with normal

karyotype that were included in our previous study (16). There was no significant difference in the survival rates between the two groups with increased GPI-AP$^-$ cells (100% vs 92.7% $P = 0.914$; Fig. 2A) while the survival rate of +8 patients without increased GPI-AP$^-$ cells (58.6%) was lower than that of patients with normal karyotype not possessing increased GPI-AP$^-$ cells (79.5%, $P = 0.0007$; Fig. 2B).

Discussion

The current retrospective study of a large number of BM failure patients revealed distinctive clinical features of BM failure patients with +8 abnormalities. Of the 483 patients with low-risk MDS, 31 (6.6%) possessed +8, which was comparable to the 8% reported in a recent study of 2072 MDS patients (2). That study did not provide any detailed diagnoses of the patients with +8. The present study detected GPI-AP$^-$ cells in 26.4% of patients with +8, and the prevalence of increased GPI-AP$^-$ cell percentages was higher in AA patients (41%) than in those with low-risk MDS (16%). This study is the first to reveal the prevalence of increased GPI-AP$^-$ cell

percentages based on a large number of AA and low-risk MDS patients with +8.

Approximately half of the patients with +8 were treated with IST, with an overall response rate of 56%. The relatively high response rate was probably achieved because IST was only administered to patients who had clinical features associated with a good response to IST, such as a short disease duration and the presence of thrombocytopenia with decreased megakaryocytes (30). The response rates were similar between AA (56%) and low-risk MDS patients (56%). However, there was a significant difference in the response rate between the patients with and those without increased GPI-AP$^-$ cells (88% vs. 41%).

Consistent with our current data, several studies demonstrated that AA and MDS patients with +8 are likely to respond to IST (3, 7, 11). There may thus be a common mechanism underlying the preferential commitment of hematopoietic progenitor clones with +8 in immune-mediated BM failures. One study revealed an increased expression of the WT1 gene by BM mononuclear cells from MDS patients with +8, which may elicit specific T cell responses to WT1 peptides and lead to the suppression of non +8

hematopoietic progenitor cells by bystander effects of activated T cells (11). The same group proposed that BM $CD34^+$ cells of + 8 patients exhibit resistance to apoptosis and increased myc expression as the mechanisms underlying the proliferative advantage of +8 clones (31). We were unable to examine WT1 gene expression and the number of WT1-specific T cells in our +8 patients who were responsive to IST. However, we believe that the specific immune responses to +8 clones may not be the main mechanism underlying the immune-mediated BM failure, for the following reasons: First, if the immune response is directed against +8 clones, successful T-cell suppression by IST should lead to the expansion of the abnormal clone. In reality, the changes in the percentage of +8 clones in patients responding to IST were highly variable and did not show a steady increase (Supplementary Fig 2). Second, the likelihood of responding to IST was determined by the presence of GPI-AP^- cells, not by the +8 clones; the +8 patients did not respond better to IST than patients with a normal karyotype (56% of AA patients with +8 vs. 81% of AA patients with a normal karyotype and 56% of MDS patients with +8 vs. 62% of MDS patients with a normal karyotype). Third, leukocytes with copy number- neutral loss of heterozygosity in the short arm of chromosome

6 (6pLOH) should be detected in patients with +8 if they are targets of cytotoxic T-cell attacks, based on our previous study showing that leukocytes with 6pLOH are detectable in 13% of AA patients (32). However, none of the six patients with +8 studied in the present population had leukocytes with 6pLOH (data not shown).

The IPSS classifies +8 as an intermediate risk factor for the progression of MDS (10, 33). The prognostic significance of +8 was confirmed by recent studies that involved MDS with at least 5% blasts (34). However, its significance in patients with AA and low risk MDS with less than 5% blasts has not been extensively studied. In contrast to previous reports (3, 11), this study revealed that AA and MDS with less than 5% blasts comprise a subset of patients with a propensity to evolve into AML. Recently, Schanz et al. studied 2902 MDS patients including 133 patients with +8 who had a median blast percentage of 4% in their BM and revealed that the median overall survival of the 133 patients was 23 months (6). However, this study included 1190 (42.7%) patients with blast percentages>5% in the BM. The median overall survivals in our 53 patients with +8 were 78 months in AA and 43 months in MDS patients. This study is the first to estimate the

overall survival in AA and low-risk MDS patients with +8 whose blast percentage in the BM is less than 5% based on a large number of patients.

On the other hand, the finding that the five-year EFS of +8 patients with an increased GPI-AP$^-$ cell percentage was 100% suggests that this subset of +8 BM failures is a benign type of BM failure similar to that of AA patients with normal karyotypes possessing increased GPI-AP$^-$ cells rather than a clonal disorder associated with a high risk of developing AML. The median age (66 years vs 59 years) and prevalence of hypercellular marrow (14% vs 10%) in patients with and without GPI-AP$^-$ cells were similar.

By comparing clinical courses between +8 patients and normal karyotype patients, both patient groups with increased GPI-AP$^-$ cells proved to have good prognosis regardless of the presence of +8, while in patients without increased GPI-AP$^-$ cells, the survival rate of +8 patients was significantly lower than that of patients with normal karyotype, strongly suggesting the importance of detecting GPI-AP$^-$ cells in predicting the prognosis of +8 patients. The WHO 2008 classification defined +8 as an intermediate-risk abnormality of MDS. The BM failure patients with +8 possessing an increased number of GPI-AP$^-$ cells may therefore be treated

in an inappropriate way such as with hypomethylating agents and allogeneic stem cell transplantation from unrelated donors. Therefore, our present findings suggest that it is important to determine if increased GPI-AP$^-$ cells are detectable when BM failure patients are found to have +8. The significance of detecting GPI-AP$^-$ cells in +8 patients needs to be confirmed by prospective studies involving a large number of BM failure patients.

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Authorship contributions

K.H. and N.S. contributed equally to this work and participated in designing and performing the research. K.H. conducted statistical analysis; N.S., T.K., Y.S., C.S, K.M., H.Y. and A.T. contributed patient samples and data; S.N. initiated and designed the study; K.H. wrote the manuscript with contributions from N.S. All authors critically reviewed the final manuscript.

Disclosure of conflicts of interest

The authors report no potential conflicts of interest.

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Table 1. Clinical features of bone marrow failure patients with trisomy 8

UPN	Age	Sex	Dx	Cellularity	IPSS	% of +8 cells	Other abnormalities	% GPI-AP$^-$ Granulocytes	% GPI-AP$^-$ Erythrocytes	GPI-AP$^-$ cells	Treatment	Response	Outcome	Cause of death	AML transformation
1	69	M	SAA	Hypo	NE	5	-	0.034	0.038	Positive	ATG+CsA	PR	Death	Infection	No
2	21	F	SAA	Hypo	NE	25	+	39.124	2.106	Positive	ATG+CsA	PR	Alive		No
3	50	F	SAA	Hypo	NE	15	-	0.002	0.026	Positive	ATG+CsA→BMT	NR	Alive		No
4	26	F	SAA	Hypo	NE	10	-	0	0	Negative	ATG+CsA	PR	Death	Infection	No
5	16	M	SAA	Hypo	NE	20	-	0	0	Negative	ATG+CsA→BMT	NR	Death	Infection	No
6	26	M	SAA	Hypo	NE	15	-	0	0	Negative	Allo-BMT	NA	Death	Infection	Yes
7	68	M	SAA	Hypo	NE	5	-	0.002	0.002	Negative	Allo-BMT	NA	Alive		Yes
8	46	F	SAA	Hypo	NE	55	+	0	0.004	Negative	CsA→Allo-BMT	NR	Death	GVHD	Yes
9	66	F	MAA	Hypo	NE	10	-	0.007	0	Positive	CsA	PR	Death	Infection	No
10	61	M	MAA	Hypo	NE	10	-	6.201	8.657	Positive	CsA	PR	Alive		No
11	50	M	MAA	Hypo	NE	10	-	0.033	0.039	Positive	CsA+AS	PR	Alive		No
12	71	M	MAA	Hypo	NE	10	-	0.049	0.092	Positive	CsA+AS	PR	Alive		No
13	68	M	MAA	Hypo	NE	5	-	0.363	0.045	Positive	No treatment	NA	Alive		No
14	65	F	MAA	Hypo	NE	80	-	0.64	0.327	Positive	No treatment	NA	Alive		No
15	69	M	MAA	Hypo	NE	15	-	0	0.001	Negative	CsA	NR	Death	Lung Cancer	No
16	35	M	MAA	Hypo	NE	5	-	0	0	Negative	CsA	NR	Alive		No
17	79	M	MAA	Hypo	NE	45	+	0	0	Negative	CsA	NR	Alive		No
18	71	F	MAA	Hypo	NE	30	-	0	0.002	Negative	CsA	PR	Alive		No
19	10	M	MAA	Hypo	NE	80	-	0	0.004	Negative	CsA+AS	NR	Death	Pneumonia	No
20	33	M	MAA	Hypo	NE	35	-	0	0	Negative	CsA+AS	PR	Alive		No
21	60	F	MAA	Hypo	NE	25	-	0	0.001	Negative	No treatment	NA	Alive		No

22	31	F	MAA	Hypo	NE	NE	-	0	0	Negative	No treatment	NA	Alive		No
23	42	F	RCUD(RA)	Normo	Int-1	50	+	0	0	Negative	CsA	NR	Death	Heart failure	No
24	87	F	RCUD(RA)	Hypo	Int-1	35	+	0	0.003	Negative	CsA+AS	NR	Death	Infection	No
25	70	M	RCUD(RA)	Hyper	Int-1	5	-	0	0	Negative	CsA+AS	NR	Death	Heart failure	No
26	81	M	RCUD(RA)	Normo	Int-1	5	+	0	0	Negative	AS,VitK	Progression	Death	Progression	No
27	51	M	RCUD(RA)	Hypo	Int-1	70	+	0	0	Negative	Allo-BMT	NA	Alive		No
28	56	F	RCUD(RA)	Normo	Int-1	85	-	0	0.001	Negative	PSL	SD	Alive		No
29	75	M	RCUD(RA)	Normo	Int-1	15	-	0	0.001	Negative	AS	Progression	Death	Progression	No
30	72	F	RCUD(RA)	Hypo	Int-1	75	+	0	0	Negative	No treatment	NA	Alive		No
31	66	F	RCUD(RA)	Normo	Int-1	10	+	0	0.01	Negative	No treatment	NA	Alive		No
32	81	M	RCMD	Hyper	Int-1	5	-	0.034	0	Positive	NA	NA	Alive		No
33	88	F	RCMD	Hyper	Int-1	50	-	0.142	0.23	Positive	AS	PR	Alive		No
34	18	F	RCMD	Hypo	Int-1	55	+	0.003	0.008	Positive	Allo-PBSCT	NA	Alive		No
35	59	F	RCMD	Hyper	Int-1	5	-	0.001	0.001	Negative	CsA	CR	Alive		No
36	65	M	RCMD	Hypo	Int-1	5	-	0	0.001	Negative	CsA	HI-1	Alive		No
37	28	F	RCMD	Normo	Int-1	20	-	0	0	Negative	CsA	HI-1	Alive		No
38	61	F	RCMD	Hyper	Int-1	100	-	0	0	Negative	CsA	HI-2	Death	Bleeding	No
39	59	M	RCMD	Hypo	Int-1	35	+	0.001	0.003	Negative	CsA	Progression	Death	Progression	Yes
40	51	F	RCMD	Hypo	Int-1	13	+	0	0.002	Negative	PSL	SD	Death	Infection	No
41	89	M	RCMD	Normo	Int-1	50	-	0	0	Negative	AraC	Progression	Death	Progression	No
42	72	M	RCMD	Normo	Int-1	10	+	0	0	Negative	VitK	NA	Death	Pneumonia	No
43	50	F	RCMD	Normo	Int-1	NE	-	0	0.001	Negative	AS,VitK	SD	Alive		No
44	77	F	RCMD	Normo	Int-1	10	+	0	0.003	Negative	AS	Progression	Death	Progression	Yes
45	7	M	RCMD	Normo	Int-1	10	-	0	0.002	Negative	Allo-BMT	NA	Death	TMA	No
46	63	F	RCMD	Normo	Int-1	NE	+	0	0	Negative	No treatment	NA	Death	Progression	No
47	56	F	RCMD	Normo	Int-1	40	-	0	0.002	Negative	No treatment	NA	Alive		No

48	70	F	MDS-U	Hypo	Int-1	5	+	0.005	0.023	Positive	CsA	CR	Alive		No
49	81	M	MDS-U	Hypo	Int-1	95	+	6.851	0.272	Positive	CsA	NA	Alive		No
50	75	M	MDS-U	Hyper	Int-1	5	-	0	0	Negative	AS	HI-3	Death	Infection	No
51	77	M	MDS-U	Normo	Int-1	35	+	0	0	Negative	AS,VitK	Progression	Death	Progression	No
52	34	F	MDS-U	Hypo	Int-1	NE	-	0	0	Negative	Allo-PBSCT	NA	Alive		No
53	55	F	MDS-U	Normo	Int-1	45	-	0	0	Negative	No treatment	NA	Alive		No
Median	61					15									

Table 2. Results of multivariate analysis of prognostic factors for overall survival of patients with BM failure with trisomy 8

Variable	Categories	BMF with trisomy 8	
		Hazard ratio (95% CI)	***P*-value**
Age	≥60 years vs. <60 years	3.9 (1.1–13.6)	<0.05
Sex	Male vs. female	1.5 (0.6–4.2)	0.42
Diagnosis	AA vs. MDS	1.3 (0.3–5.8)	0.74
Cellularity	Hypocellular vs. others	0.5 (0.1–1.9)	0.31
Karyotype complexity	8+ alone vs. 8+ others	0.4 (0.1–1.3)	0.12
Clone size (% of +8 cells)	≥15% vs. <15%	0.5 (0.2–1.3)	0.16
GPI-AP$^-$ cells	Positive vs. negative	0.1 (0.02–0.7)	<0.05

GPI-AP$^-$ cells: glycosylphosphatidylinositol-anchored protein-deficient blood cells; AA: aplastic anemia;

MDS: myelodysplastic syndrome; CI: confidence interval; BMF: bone marrow failure.

Figure Legends

Figure.1. Overall and event-free survival rates of BM failure patients with trisomy 8

(A) Five-year overall survival (OS) and event-free survival (EFS) rates of +8 patients. (B) Five-year OS and EFS rates of +8 patients with and without increased GPI-AP$^-$ cells. (C) Five-year OS and EFS rates of +8 patients with +8 alone and +8 with other abnormalities (8+ others). The EFS was defined as the time from diagnosis to AML evolution or death.

Figure.2. Overall survival rates of BM failure patients with trisomy 8 and normal karyotype

(A) Five-year overall survival (OS) rates of +8 patients and normal karyotype patients with increased GPI-AP$^-$ cells. (B) Five-year OS rates of +8 patients and normal karyotype patients without increased GPI-AP$^-$ cells.

Supplementary Figure 1.

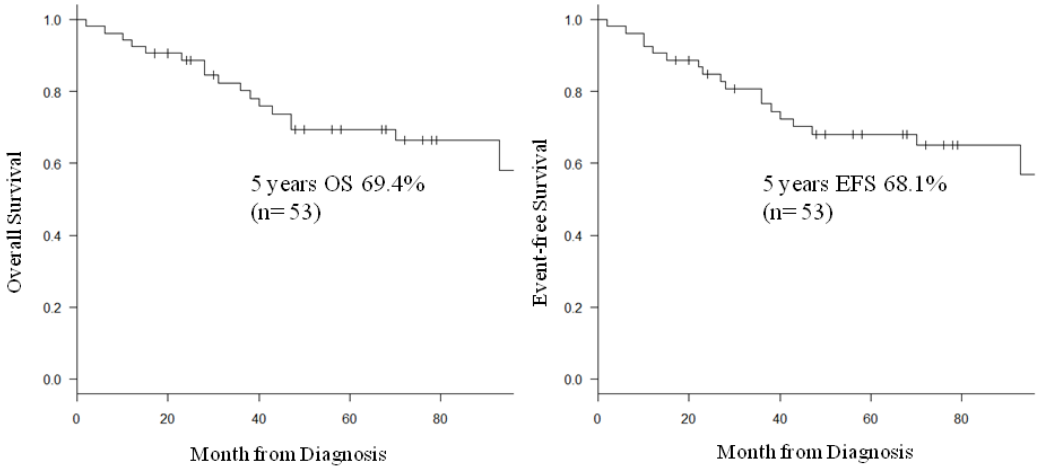
One patient who possessed 0.002% GPI-AP$^-$ granulocytes was judged positive because 0.026% of the patent's erythrocytes were GPI-AP$^-$ cells.

Supplementary Figure 2.

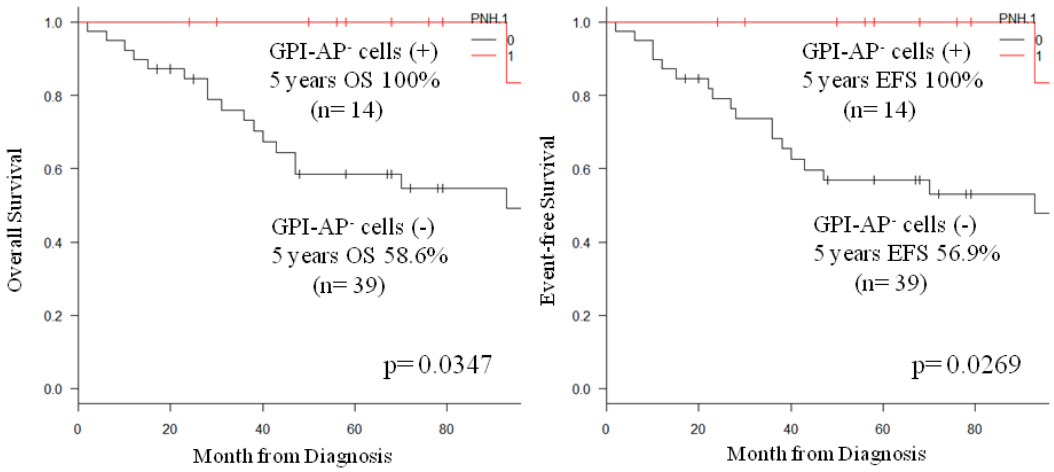
Changes in the proportion of +8 cells for six patients. The percentage of +8 clones revealed by G-banding increased in three patients (UPN3, 20, 33) and decreased in two patients (UPN2,9) after successful IST.

Figure 1

(A)


Overall Survival
1.0
0.8
0.6
0.4
0.2
0.0
0
20
40
60
80
Month from Diagnosis
5 years OS 69.4%
(n= 53)
Event-free Survival
Month from Diagnosis
5 years EFS 68.1%
(n= 53)

(B)


Overall Survival
PNH.1
0
1
GPI-AP- cells (+)
5 years OS 100%
(n= 14)
GPI-AP- cells (-)
5 years OS 58.6%
(n= 39)
p= 0.0347
Month from Diagnosis
Event-free Survival
GPI-AP- cells (+)
5 years EFS 100%
(n= 14)
GPI-AP- cells (-)
5 years EFS 56.9%
(n= 39)
p= 0.0269
Month from Diagnosis

(C)

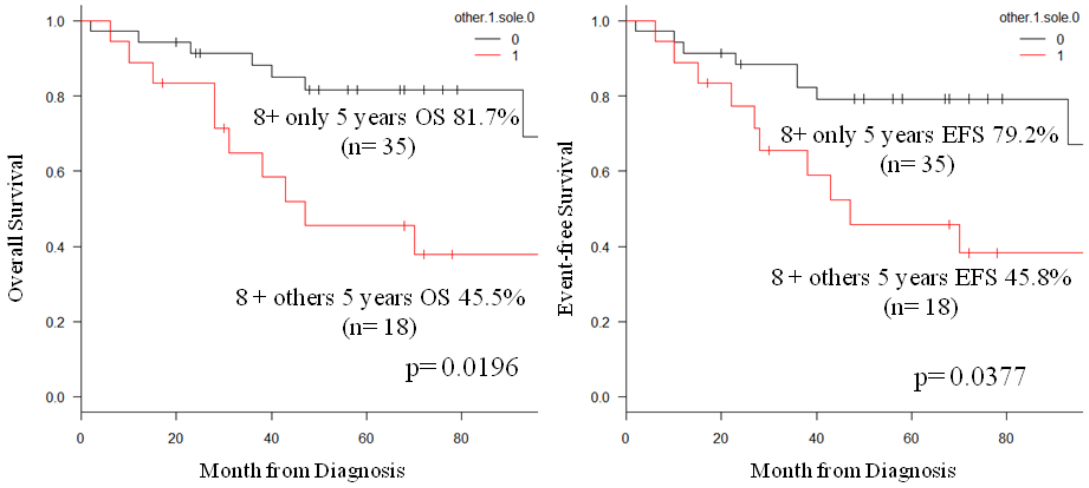
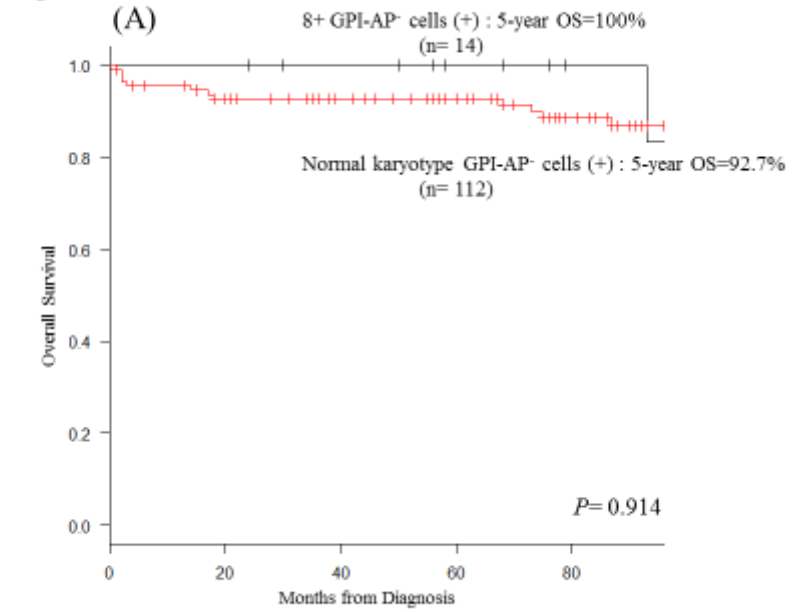
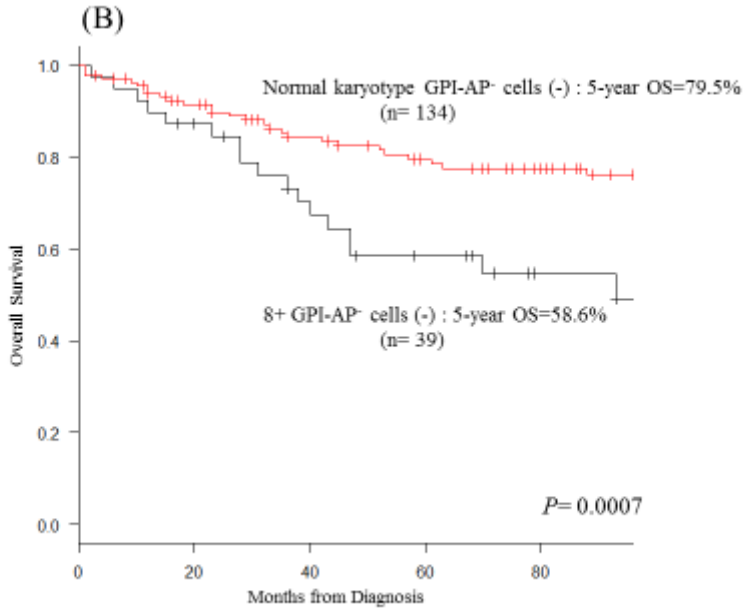
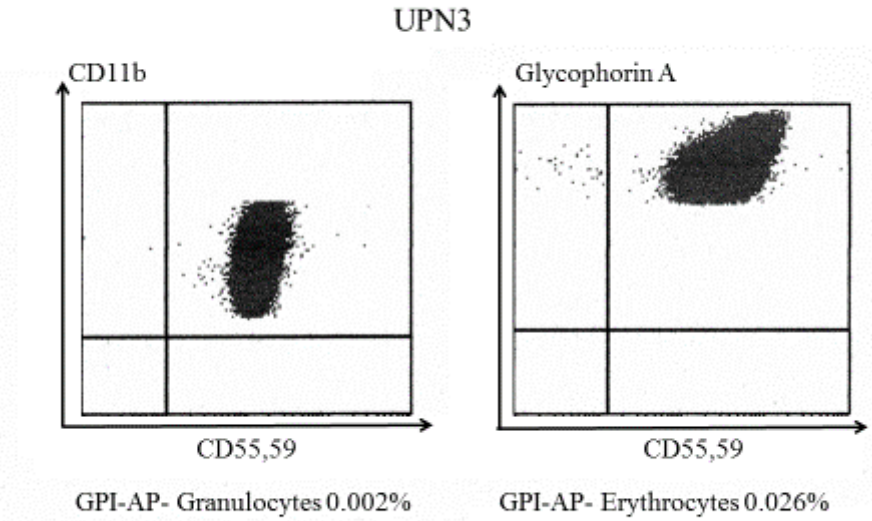

other.1.sole.0
0
1
8+ only 5 years OS 81.7%
(n= 35)
8 + others 5 years OS 45.5%
(n= 18)
p= 0.0196
Overall Survival
Month from Diagnosis
other.1.sole.0
0
1
8+ only 5 years EFS 79.2%
(n= 35)
8 + others 5 years EFS 45.8%
(n= 18)
p= 0.0377
Event-free Survival
Month from Diagnosis

Figure 2


(A)
8+ GPI-AP- cells (+) : 5-year OS=100%
(n= 14)
Normal karyotype GPI-AP- cells (+) : 5-year OS=92.7%
(n= 112)
P= 0.914
Overall Survival
Months from Diagnosis



Supplementary Figure 1



Supplementary Figure 2