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JOURNAL OF CLINICAL ONCOLOGY ORIGINAL REPORT

Clinical Outcome of Children With Newly Diagnosed Philadelphia Chromosome–Positive Acute Lymphoblastic Leukemia Treated Between 1995 and 2005

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ABSTRACT

Purpose
In a previous analysis of 326 children with Philadelphia chromosome (Ph) –positive acute lymphoblastic leukemia (ALL) treated between 1986 and 1996, hematopoietic stem-cell transplantation from HLA-matched related donors, but not from unrelated donors, offered a superior outcome than chemotherapy alone. To evaluate the impact of recent improvements in chemotherapy and transplantation, we performed a similar analysis on patients treated in the following decade.

Patients and Methods
We analyzed 610 patients with Ph-positive ALL treated between 1995 and 2005 without tyrosine kinase inhibitor therapy. The median follow-up duration was 6.3 years.

Results
Complete remission was achieved in 89% of patients. The 7-year event-free survival and overall survival rates were superior in the present cohort compared with the previous cohort (32.0% ± 2.0% *v* 25.0% ± 3.0, respectively, $P = .007$; and 44.9% ± 2.2% *v* 36.0% ± 3.0%, respectively, $P = .017$). Compared with chemotherapy alone, transplantation with matched related donors or unrelated donors in first remission (325 patients) showed an advantage with increasing follow-up, suggesting greater protection against late relapses (hazard ratio at 5 years, 0.37; $P < .001$). In the multivariate Cox regression analysis accounting for treatment (transplantation *v* no transplantation), age, leukocyte count, and early response had independent impact on treatment outcome.

Conclusion
Clinical outcome of children and adolescents with Ph-positive ALL has improved with advances in transplantation and chemotherapy. Transplantations with matched related donors and unrelated donors were equivalent and offered better disease control compared with chemotherapy alone. Age, leukocyte count, and early treatment response were independent prognostic indicators. The results of this study will serve as a historical reference to evaluate the therapeutic impact of tyrosine kinase inhibitors on the outcome of Ph-positive ALL.

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INTRODUCTION

With current cure rates of 85% or greater in childhood acute lymphoblastic leukemia (ALL),[1] precise risk assessment is important to direct treatment. Patients with low-risk leukemia can be assigned to receive less intensive treatment to minimize late sequelae. Conversely, the subset of patients with high risk of relapse should be allocated to receive intensive treatment or novel therapies. With continuing improvement in therapy, the impact of many prognostic factors has been diminished or abolished altogether. Until recently, the Philadelphia chromosome (Ph) resulting from chromosomal translocation t(9;22), which occurs in 3% to 5% of children and 25% of adults with ALL, has consistently been associated with dismal treatment outcome. The translocation results in a fusion protein of 210 kDa (p210) when the *ABL1* proto-oncogene moves from chromosome 9 to the major breakpoint cluster region on chromosome 22, as usually observed in chronic myelogenous leukemia. The *ABL1* gene can also translocate to the minor breakpoint cluster region on chromosome 22, resulting in a 190-kDa fusion protein (p190) that occurs exclusively in ALL. More than 90% of children

with Ph-positive ALL have this subtype of t(9;22). Both the p210 and p190 proteins can be readily detected with techniques based on the polymerase chain reaction. [2-5] In a recent genome-wide analysis of diagnostic leukemia samples from 304 individuals with ALL, *IKZF1* (encoding the transcription factor Ikaros) was deleted in 83.7% of *BCR-ABL1* ALL.[6]

With conventional treatment including hematopoietic stem-cell transplantation (HSCT), only one third of children and adolescents with Ph-positive ALL have been long-term survivors.[7-19] A recent study showed that intensive chemotherapy in combination with continuous exposure to a tyrosine kinase inhibitor (imatinib) markedly improved early treatment outcome in a small group of children with Ph-positive ALL,[20] raising the question of whether HSCT remains the treatment of choice for children or young adults with Ph-positive ALL. In our previous study of 326 children and adolescents treated by 10 cooperative study groups or single institutions between 1986 and 1996, we demonstrated that HSCT with matched related donors, but not unrelated donors, was superior to chemotherapy alone.[21] With recent improvement in both chemotherapy and HSCT, we performed a similar analysis of patients treated between 1995 and 2005 without tyrosine kinase inhibitors, so that the results can serve as baseline data to guide future development of treatment for patients with Ph-positive ALL.

PATIENTS AND METHODS

Review of Data

Each study group reviewed its records to identify patients age less than 18 years with Ph-positive ALL registered in clinical trials between 1995 and 2005. Patients who were treated with any tyrosine kinase inhibitor during front-line chemotherapy were excluded from the analysis. We accepted either cytogenetic or molecular tests to identify the Ph status; patients who were negative at diagnosis but positive at relapse were not included. A predefined set of data, collected for each patient, was then sent to a coordinating center, where the findings were reviewed for consistency and completeness. Follow-up observations extended through 2008, with a median follow-up time of 6.3 years (range, 0.1 to 11.5 years). By consensus, none of the participating groups will be identified with their data sets in this report.

Patients and Treatment

Of the 762 patients with Ph-positive ALL identified, 610 were eligible and evaluable. At most of the participating centers, these children were identified early in the clinical course and were assigned to therapy for high-risk ALL. Indications for HSCT for patients in first complete remission varied among the different study groups. Nonetheless, HSCT from an HLA-matched related donor was generally accorded the highest priority among alternatives to chemotherapy alone. The lack of information on the availability of donors prevented us from determining whether all patients with a suitable donor underwent HSCT. Definition of early response to chemotherapy was given by each group according to protocol criteria; good early response was defined by either peripheral-blood count on day 8 (prednisone good response: $< 1{,}000$ blasts/μL in peripheral blood after 7 days of glucocorticoid therapy and one injection of intrathecal methotrexate)[22] or bone marrow evaluation on day 8 or day 15 (< 25% blasts) or day 21 (< 5% blasts) of remission induction.[23]

Statistical Analysis

The principal end points in the analysis of treatment results were event-free survival (EFS), disease-free survival (DFS), and overall survival (OS). EFS was defined as the time from diagnosis to first failure, which was defined as death during induction therapy, lack of achievement of remission during protocol-specified induction period, relapse at any site, death during remission, or development of second malignant neoplasm. DFS was defined as the time from complete remission until relapse at any site, death during complete remission, or development of a second malignant neoplasm. OS was defined as the time from diagnosis (or time from complete remission, when stated) to

Table 1. Pattern of Treatment Failure in Children With Ph-Positive ALL Who Achieved Complete Remission After Induction Therapy by Treatment (N = 542)

			Hematopoietic Stem-Cell Transplantation (n = 325)											
	Chemotherapy Only (n = 217)		Matched Related Donor (n = 115)		Mismatched Related Donor (n = 15)		Unrelated Donor (n = 166)		Autologous (n = 10)		Not Known (n = 19)		All Patients (N = 542)	
Event	No. of Patients	%	No. of Patients	%	No. of Patients	%	No. of Patients	%	No. of Patients	%	No. of Patients	%	No. of Patients	%
Time from CR1 to HSCT, months														
Median			4.0		5.0		6.0		5.7		3.6			
First to third quartile			3.0-5.7		3.9-6.8		4.3-7.9		5.2-6.4		3.2-6.5			
Relapse	146	67	49	43	2	13	45	27	7	70	3	16	252	46
Bone marrow	110		37		2		32		5		3		189	
CNS	13		1		0		1		1		0		16	
Testis	2		1		0		4		0		0		7	
Bone marrow + other	16		3		0		4		1		0		24	
Other	5		6		0		3		0		0		14	
Unknown	0		1		0		1		0		0		2	
Death in CCR	16	7	18	16	8	53	31	19	0	0	6	32	79	15
Therapy related	15		0		0		0				0		15	
HSCT	0		17		6		26				5		54	
Other	0		1		0		4				1		6	
Unknown	1		0		2		1				1		4	
Second neoplasm	0		1		0		3		0		0		4	1
CCR	55	25	47	41	5	33	87	52	3	30	10	53	207	38

Abbreviations: Ph, Philadelphia chromosome; ALL, acute lymphoblastic leukemia; CR1, first complete remission; CCR, continuous complete remission; HSCT, hematopoietic stem-cell transplantation.

death from any cause. Observations of patients were censored at the date of last contact when no events were observed.

The Kaplan-Meier method was used to estimate the probabilities of EFS, DFS, and OS, with SEs calculated according to Greenwood. Curves were compared using the log-rank test. Statistical methods were used to minimize potential sources of bias in comparing DFS and OS (from date of first complete remission) after HSCT or intensive chemotherapy alone. Kaplan-Meier plots that compared HSCT with chemotherapy alone were adjusted to account for the waiting time to transplantation. The curves originate at a landmark (median time to transplantation) and thus do not include patients who had events or whose data were censored before that time; the curves account for patients who underwent transplantation after the landmark by delayed entry. To deal with lack of proportional hazards between the two treatment groups, univariate comparison between these curves was performed at a predefined time point of 5 years from remission based on log-log transformation.[24]

Differences in time to transplantation and in the prognostic factors used to assign patients to HSCT were accounted for in Cox regression analyses. Treatment was considered to be a time-dependent factor. Thus, each patient was included in the chemotherapy-only group until transplantation, at which point he or she was shifted to the transplantation group. The model also included the covariates of age (0 to 3, 3 to 6, 6 to 10, 10 to 15, $v > 15$ years), leukocyte count (0 to 10, 10 to 25, 25 to 50, 50 to 100, $v > 100 \times 10^3/\mu L$), sex, and early response (poor responders according to bone marrow result or peripheral-blood result, response not known, v good responders). The time dependence of the treatment effect (ie, nonproportional hazards) was accommodated by including a term for the interaction of time (log-transformed) and treatment in the regression analysis.[25] According to graphical checks, the proportional hazards assumption was reasonable for the prognostic factors. Two-tailed P values for differences in the risk of treatment failure (in terms of either DFS or OS) were derived from the likelihood ratio test. Estimated hazard ratios (HRs) were reported with 95% CIs.

Cumulative incidence of relapse or death was estimated in patients who underwent transplantation accounting for competing risks (censoring second malignant neoplasms). The logistic regression model was used to analyze the influence of age, leukocyte count, and early response on the odds of nonresponse to induction therapy.

RESULTS

The estimates of EFS and OS of the 610 patients with Ph-positive ALL were 32.0% ± 2.0% and 44.9% ± 2.2% at 7 years after diagnosis, respectively.

Clinical and Laboratory Characteristics

Appendix Table A1 (online only) summarizes the presenting features of the 610 evaluable patients. The median age at diagnosis was 7.8 years (range, 0.7 to 17.65 years); 72 patients (12%) were less than 2 years of age and only 1 was younger than 1 year of age. The leukocyte count at diagnosis was at least 50,000/μL in approximately 43% of the patients and less than 10,000/μL in 23%. Despite the relatively high proportion of patients with hyperleukocytosis, leukemic involvement of the CNS at diagnosis was observed in only 6% of the patients. Nine patients had a T-cell lineage immunophenotype.

Early Responses to Chemotherapy

Early response to treatment as measured by prednisone response was available in 177 patients, 33 (19%) of whom had a poor response, a proportion approximately twice that of unselected patients with ALL. Among the 338 patients for whom early response was evaluated by bone marrow aspirates, 134 (40%) had poor response (Appendix Table A1), a proportion also higher than that of unselected patients with childhood ALL.[9]

Induction of Complete Remission

A total of 542 patients (89%) achieved a complete remission after induction therapy; the remaining patients either died during induction (n = 5) or failed to achieve remission (n = 63). The induction failure rate of 11% is much higher than the 2% to 3% induction failure rate seen among children with Ph-negative ALL. In a multivariate analysis, poor early response was the strongest predictor of induction failure (odds ratio, 13.3; 95% CI, 5.73 to 31.02; $P < .001$), although WBC count retained predictive value (odds ratio, 1.86; 95% CI, 1.04 to 3.32; $P = .04$ for $\geq v < 100{,}000/\mu L$). Of the 63 patients with induction failure, 45 patients subsequently underwent HSCT, and 11 patients were alive at last follow-up (nine patients after HSCT).

Patterns of Treatment Failure

Of the 542 patients who achieved a complete remission after induction chemotherapy, 252 (46%) experienced a relapse, including

Table 2. Estimated HRs Associated With Different Types of HSCT and Chemotherapy Alone in Patients With Ph-Positive Childhood Acute Lymphoblastic Leukemia Who Achieved Complete Remission After Initial Induction Therapy (n = 540*)

Variable	DFS			Survival		
	HR	95% CI	P	HR	95% CI	P
Treatment			< .001			.003
Chemotherapy alone	1.00			1.00		
HSCT						
At 0.5 years	1.34	0.94 to 1.90		1.37	0.81 to 2.31	
At 1 year	0.87	0.69 to 1.10		0.96	0.71 to 1.31	
At 5 years	0.32	0.20 to 0.52		0.42	0.25 to 0.70	
Age at diagnosis, years			.03			< .001
0-3	0.45	0.27 to 0.77		0.26	0.14 to 0.48	
3-6	0.65	0.41 to 1.01		0.45	0.28 to 0.73	
6-10	0.72	0.47 to 1.12		0.56	0.35 to 0.89	
10-15	0.77	0.50 to 1.19		0.62	0.39 to 0.98	
≥ 15	1.00			1.00		
Leukocyte count at diagnosis, per μL			< .001			.003
0-10	0.47	0.34 to 0.64		0.48	0.33 to 0.70	
10-25	0.55	0.40 to 0.76		0.67	0.46 to 0.96	
25-50	0.63	0.44 to 0.89		0.77	0.52 to 1.15	
50-100	0.62	0.44 to 0.88		0.81	0.55 to 1.17	
≥ 100	1.00			1.00		
Sex						
Male	1.10	0.88 to 1.38	.40	1.01	0.79 to 1.30	.93
Female	1.00			1.00		
Early response						
Good responders in PB or BM	1.00		.03	1.00		.007
Poor responders in PB	2.00	1.26 to 3.18		2.41	1.47 to 3.95	
Poor responders in BM	1.19	0.88 to 1.61		1.30	0.94 to 1.81	
Early response unknown	1.27	0.93 to 1.73		1.34	0.95 to 1.89	

Abbreviations: HR, hazard ratio; HSCT, hematopoietic stem-cell transplantation; Ph, Philadelphia chromosome; DFS, disease-free survival; PB, peripheral blood; BM, bone marrow.

*The model was fitted on 540 patients as a result of missing values in leukocyte count at diagnosis in two patients.

189 in the bone marrow (75%), 16 in the CNS (6%), 24 in bone marrow and another site(s) (10%), and seven in the testis (2% of 337 boys; Table 1). Of 146 relapses in the chemotherapy group, 33 (23%) were diagnosed within 6 months from complete response. In addition, 79 (15%) of these 542 patients died during first remission at a median of 0.83 years (range, 0.1 to 6.2 years) after remission was induced. The cause of death was related to HSCT in 54 patients, chemotherapy in 15 patients, and other factors in six patients and was unknown in four patients. Second malignant neoplasms developed in four patients (0.7%) as the first adverse event. Altogether, 207 (38%) of 542 patients were in continuous complete remission on the date of the last evaluation.

Impact of Postremission Therapy on Treatment Outcome

Of the 542 patients who achieved remission by the end of induction therapy, 217 were treated with chemotherapy only, whereas 325 underwent HSCT with different types of donors (Table 1). The Cox regression model was applied to assess the effect of different postremission treatments on DFS and OS, adjusting for relevant characteristics (ie, initial leukocyte count, age, sex, and early response), as shown in Table 2. The advantage of transplantation on DFS appeared during the second year of follow-up and became significantly more evident with each successive year, suggesting greater protection against late relapses with HSCT ($P < .001$). According to the Cox model, the hazard of failure (relapse or death in remission) at 5 years was reduced by two thirds by HSCT compared with chemotherapy alone (HR, 0.32; 95% CI, 0.20 to 0.52). According to univariate comparison of the DFS curves at the 5-year time point, the advantage of transplantation was borderline significant ($P = .049$; Fig 1A). Also for survival, HSCT improved the results compared with chemotherapy alone in the long term (according to the Cox model, $P = .003$; 5-year HR, 0.42; 95% CI, 0.25 to 0.70), but the advantage at 5 years was not significant in the univariate comparison ($P = .20$, Fig 1B).

Transplantation with a matched related donor was associated with a decrease in transplantation-related mortality over the years of this survey, with a cumulative incidence of 20% ± 5.5% and 11.7% ± 4.2% before and after year 2000, respectively. However, this did not result in a significantly better outcome, with 5-year DFS rates of 38.9% ± 6.6% and 41.1% ± 6.4% before and after year 2000, respectively ($P = .39$).

Patients who underwent transplantation with a matched unrelated donor in the same time intervals had 5-year DFS rates of 41.4% ± 6.5% and 55.8% ± 5.4% before and after 2000, respectively ($P = .07$; Fig 2). This significant improvement was explained by a better disease control, as illustrated by the cumulative incidence of relapse of 38.2% ± 6.4% before year 2000 and 21.4% ± 4.1% after 2000. Mortality remained similar in the two periods (19.7% ± 4.0% before 2000 *v* 19.0% ± 5.2% after 2000).

Impact of Prognostic Factors on Treatment Outcome

In the univariate analysis of the entire cohort of 610 patients with Ph-positive ALL, age, initial leukocyte count, and response to initial treatment had a significant impact on treatment outcome (Appendix

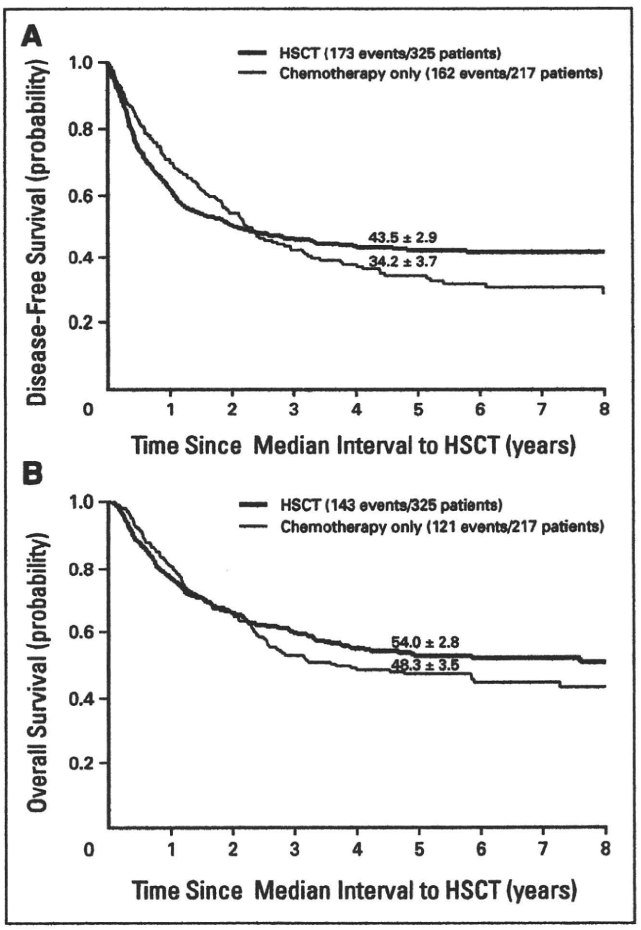

Fig 1. Estimates of (A) disease-free survival and (B) overall survival (± SE) in 542 patients treated with hematopoietic stem-cell transplantation (HSCT) or chemotherapy only. The curves have been adjusted for waiting time to transplantation, so that the zero on the time axis corresponds to the median time from first complete remission to transplantation (5.1 months); patients were assigned to this treatment group in a time-dependent fashion. Five-year estimates (from remission) are shown.

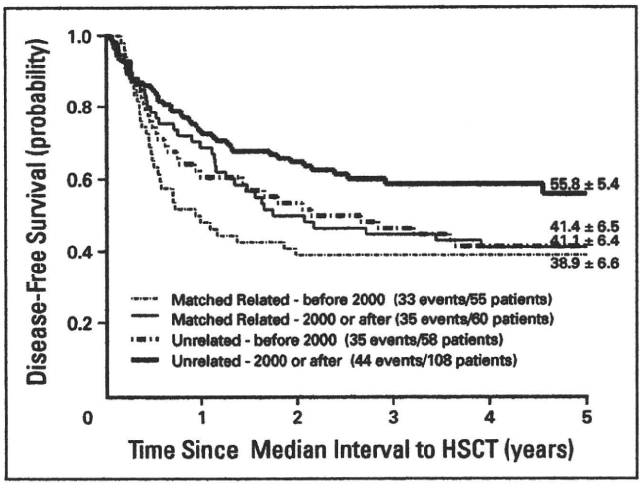

Fig 2. Estimates of disease-free survival (± SE) in 281 patients with Philadelphia chromosome–positive childhood acute lymphoblastic leukemia treated with hematopoietic stem-cell transplantation from HLA-matched related or unrelated donors before or after year 2000. Five-year estimates are shown.

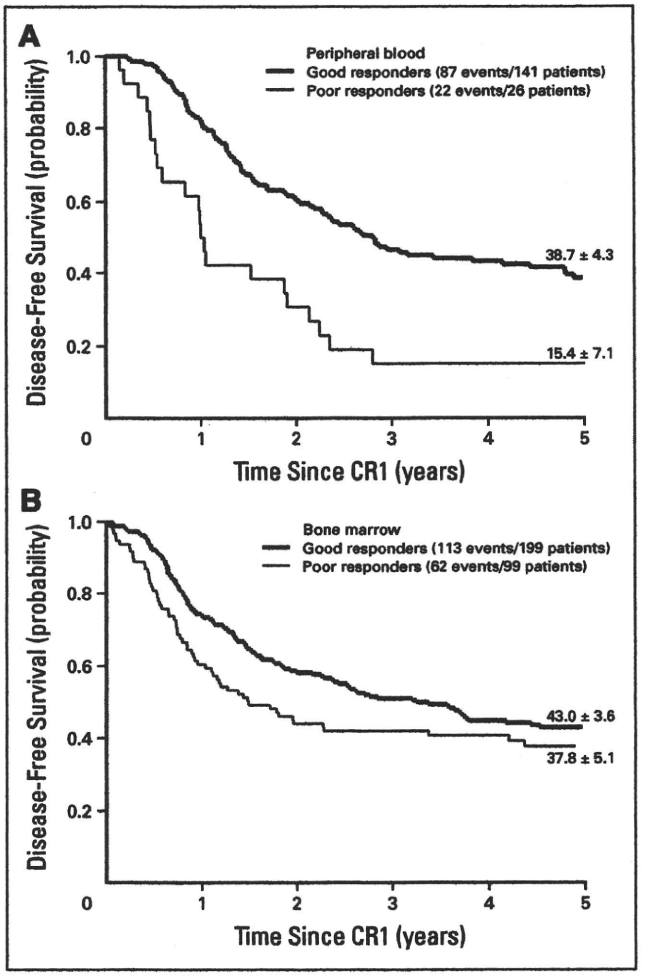

Fig 3. Estimates of disease-free survival (± SE) in good or poor responders as defined by (A) day 8 peripheral blood or (B) day 8 to 21 bone marrow evaluation. Five-year estimates are shown. CR1, first complete remission.

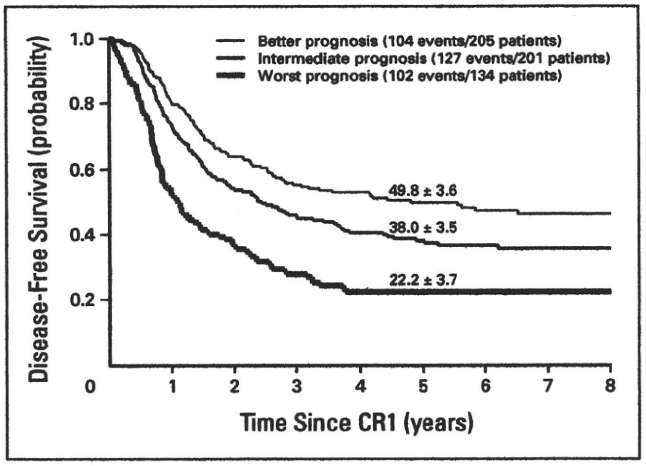

Fig 4. Estimates of disease-free survival (± SE) in 540 patients with Philadelphia chromosome–positive childhood acute lymphoblastic leukemia. The patients were classified according to modified Rome–National Cancer Institute criteria as follows: better prognosis (10 years of age or younger with a leukocyte count of < 50,000/μL), intermediate prognosis (intermediate-risk features), and worst prognosis (any age with a leukocyte count of > 100,000/μL). Five-year estimates are shown.

Table A1). On the basis of peripheral-blood blast cell count at day 8 or percent bone marrow blasts on day 8, 15, or 21 of remission induction (according to individual protocol), 348 (67.6%) of 515 evaluable patients were designated as good early responders; their 5-year EFS rate was 40.3% ± 2.7%, and 5-year DFS rate (n = 340) was 41.3% ± 2.8%. By contrast, the 5-years EFS and DFS rates for the 167 poor early responders were 24.6% ± 3.4% ($P < .001$) and 32.9% ± 4.4% ($P = .002$, n = 125), respectively.

Of the 33 patients with poor corticosteroid response, 26 achieved remission by the end of induction, but their 5-year DFS was only 15.4% ± 7.1%, a result that was inferior to the DFS rate of 38.7% ± 4.3% for the 141 good corticosteroid responders (Fig 3A; $P < .001$). Of the 134 poor responders based on the proportion of bone marrow blasts, 99 achieved remission and had a 5-year DFS rate of 37.8% ± 5.1%, compared with a 5-year DFS rate of 43.0% ± 3.6% for the 199 patients with good response who achieved remission (Fig 3B; $P = .06$).

Age and leukocyte count had prognostic significance on DFS and could be used to stratify patients into three distinct groups (Fig 4). Noticeably, within the subgroup of patients defined as better by the modified Rome-National Cancer Institute criteria (ie, ≤ 10 years of age with a leukocyte count ≤ 50,000/μL), a large subset of patients (149 of 205 patients) had a good early response and an overall 7-years DFS rate of 47.2% ± 4.5%. Their outcome by treatment is shown in Appendix Figure A1 (online only), where a nonsignificant advantage of HSCT versus chemotherapy is observed on DFS ($P = .12$) or on survival ($P = .72$). Patients with good early response but defined as intermediate or worst by modified Rome-National Cancer Institute criteria had overall 7-year DFS rates of 36.9% ± 4.4% (n = 133) and 21.4% ± 5.5% (n = 58), respectively. In the multivariate Cox regression models, treatment, age, leukocyte count, and early response retained independent prognostic significance (Table 2).

DISCUSSION

The outcome of Ph-positive ALL has steadily improved over the last three decades.[7-19,21] In this study, 45% of patients survived at 7 years, a result that compares favorably with the rate of 36% achieved in our previous cohort of 326 patients with Ph-positive ALL ($P = .017$).[21] As expected, given the large numbers, the characteristics of the patients in the two cohorts are extremely similar, with no significant difference in any of the presenting features (Appendix Table A2, online only), suggesting that there was no selection bias. As demonstrated also in our previous studies,[21,26] Ph-positive ALL represents a heterogeneous disease and can be stratified into distinct prognostic subgroups based on age, WBC count, and early treatment response. Early treatment response can be assessed by either peripheral-blood blast cell count after treatment with single-agent corticosteroid or by the percentage of bone marrow blasts after combination chemotherapy.[7-19,22,27-31] In this study, treatment response was shown to be a robust predictor of induction failure. Moreover, insufficient blast cell clearance from the peripheral blood on day 8 of single-agent prednisone treatment (poor prednisone response) was the most powerful adverse prognostic feature and was associated with a two-fold increase in the risk of failure after remission.

In our previous study, HSCT with matched related donor yielded a superior outcome compared with chemotherapy alone, but the advantage of HSCT did not extend to the use of matched unrelated donors.[21] In the present study, transplantation from matched unrelated donors produced similar outcomes to those attained with matched related donors. This finding could be attributed to improved supportive care and HLA typing, as well as to more potent graft-versus-leukemia effect on residual leukemia driven by the residual HLA disparity in unrelated donors.[32] The extended follow-up of the present cohort demonstrates that the advantage of transplantation over chemotherapy alone increased over time by preventing late relapses. The risk of failure (relapse or death) at 5 years was reduced to approximately one third for patients treated with transplantation compared with patients treated with chemotherapy alone. The significant result in the Cox model is strongly influenced by how the initial advantage of chemotherapy changes into a disadvantage in favor of transplantation as time increases. The univariate analysis, based on the single point comparison of the 5-year survival estimates, indicates that this model may overstate the late-term benefit of transplantation on survival. Our conservative interpretation is that results on survival are not so clear cut as results on DFS. Although both Cox model and survival curves agree on advantage of transplantation, this is not reflected in a fully similar measure by these two methods. We have to acknowledge that in a complex setting, such as the comparison between HSCT and chemotherapy, the Cox model and the univariate approach adjust in different ways for waiting time to transplantation and only the Cox model adjusts for patients characteristics, and this can in part explain this disagreement.

The results of the present study confirm and extend those of our former survey on patients treated a decade earlier.[21] However, although the improvements in outcome achieved in the 1996 to 2005 era were statistically significant, we observed only a small (10%) effect on OS. Treatment with either chemotherapy or HSCT in this era without tyrosine kinase inhibitor (at least during front-line treatment program) resulted in long-term survival rates of less than 50% for all groups analyzed. Overall, only 45% of children with Ph-positive ALL were alive 7 years after diagnosis, a result that remains unacceptable. Further optimization of chemotherapy or HSCT regimens is unlikely to lead to major improvements in outcome. Recent encouraging data from Children's Oncology Group study AALL0031[20] (albeit early and based on small numbers) show that outcomes for children with Ph-positive ALL were improved dramatically by incorporating a tyrosine kinase inhibitor (imatinib mesylate) into therapy. On the basis of these data and other data from adults, tyrosine kinase inhibitors are the cornerstone of therapy for children with Ph-positive ALL and should be incorporated in any future treatment schedule of childhood Ph-positive ALL. The high rates of induction failure observed with chemotherapy alone in this study emphasize the need to introduce tyrosine kinase inhibitors into treatment early during induction therapy. More study is needed to clearly define the relative roles of chemotherapy and HSCT in combination with tyrosine kinase inhibitor, and the present study will serve as a large, international historical reference for documenting any real future improvement.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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Appendix

Table A1. Outcome According to Demographics and Clinical Characteristics, Response to Induction Therapy, and Type of Postremission Therapy Among Patients With Ph-Positive Childhood Acute Lymphoblastic Leukemia (N = 610)

Characteristic	Induction Treatment Failure*		Chemotherapy Only		Stem-Cell Transplantation		All Patients		5-Year EFS			5-Year Survival		
	No. of Patients	%	No. of Patients	%	No. of Patients	%	No. of Patients	%	EFS Rate (%)	SE (%)	P†	Survival Rate (%) SE (%)		P†
Overall	68	100	217	100	325	100	610	100	34.1	2.0		47.7	2.1	
Sex											.43			.98
Male	39	57	141	65	196	60	376	62	32.8	2.5		48.2	2.6	
Female	29	43	76	35	129	40	234	38	36.2	3.2		46.7	3.4	
Age at diagnosis, years											.006			<.001
0-2	6	9	30	14	36	11	72	12	53.5	6.0		67.3	5.6	
3-5	8	12	52	24	82	25	142	23	39.2	4.3		53.7	4.3	
6-9	24	35	59	27	93	29	176	29	30.0	3.5		44.5	3.8	
10-14	26	38	60	28	94	29	180	30	28.7	3.5		41.9	3.8	
≥ 15	4	6	16	7	20	6	40	7	22.2	6.9		29.0	8.0	
Leukocyte count, per μL‡											<.001			<.001
< 10	5	7	62	29	72	22	139	23	45.6	4.4		62.8	4.2	
10-25	6	9	39	18	74	23	119	20	41.7	4.7		53.9	4.8	
25-50	8	12	31	14	45	14	84	14	35.0	5.3		45.7	5.5	
50-100	12	18	31	14	51	16	94	15	36.8	5.0		44.8	5.2	
≥ 100	36	54	53	25	82	25	171	28	18.0	3.0		32.7	3.8	
Immunophenotype‡														
B lineage	57	85	200	96	318	98	575	96	34.9	2.0		48.4	2.2	
T lineage	4	6	4	2	1	0.3	9	2						
Other	6	9	5	2	4	1	15	3						
Modified Rome-NCI criteria‡											<.001			<.001
Best prognosis	10	15	84	39	121	37	215	35	47.5	3.5		61.5	3.4	
Intermediate prognosis	22	33	80	37	121	37	223	37	34.5	3.3		45.2	3.4	
Worst prognosis	35	52	52	24	82	25	169	28	17.6	3.0		32.5	3.8	
CNS involvement§											.03			.36
Yes	2	3	15	7	18	6	35	6	20.0	6.8		42.9	8.4	
No	58	97	194	93	297	94	549	94	35.0	2.1		47.9	2.2	
Early response day 8 PB (corticosteroid)											<.001			<.001
Good	3	30	81	88	60	80	144	81	37.9	4.2		55.9	4.3	
Poor	7	70	11	12	15	20	33	19	12.1	5.7		18.0	7.3	
Early response day 8-21 BM											<.001			<.001
Good	5	13	67	68	132	66	204	60	41.9	3.5		55.9	3.6	
Poor	35	87	32	32	67	34	134	40	27.9	4.0		41.2	4.4	
Early response (any)											<.001			<.001
Good	8	12	148	68	192	59	348	57	40.3	2.7		55.9	2.7	
Poor	42	62	43	20	82	25	167	27	24.6	3.4		36.7	3.9	
Not known§	18	26	26	12	51	16	95	16	27.9	4.6		36.9	5.0	

Abbreviations: Ph, Philadelphia chromosome; EFS, event-free survival; NCI, National Cancer Institute; PB, peripheral blood; BM, bone marrow.
*Five deaths occurred during induction therapy, and there were 63 nonresponders to induction therapy.
†Univariate *P* value (log-rank test).
‡Data not known on WBC count at diagnosis and for NCI criteria for three patients, on immunophenotype for 11 patients, and on CNS involvement for 26 patients. The patients were classified according to modified Rome-NCI criteria as follows: best prognosis (10 years of age or younger with a leukocyte count of < 50,000/μL), intermediate prognosis (intermediate-risk features), and worst prognosis (any age with a leukocyte count of > 100,000/μL).
§This category includes patients with missing data and patients from protocols that did not evaluate early response.

Table A2. Distribution of Patient Demographics and Clinical Characteristics Between the Current Ph-Positive Childhood Acute Lymphoblastic Leukemia Cohort and the Historical Cohort*

Characteristic	Current Cohort (N = 610)		Historical Cohort (N = 326)		P†
	No. of Patients	%	No. of Patients	%	
Sex					.44
Male	376	62	210	64	
Female	234	38	116	36	
Age at diagnosis, years					.67
0-2	72	12	37	11	
3-5	142	23	83	25	
6-9	176	29	81	25	
10-14	180	30	99	30	
≥ 15	40	7	26	8	
Leukocyte count, per μL					.29
< 10	139	23	68	21	
10-25	119	20	59	18	
25-50	84	14	37	11	
50-100	94	15	48	15	
≥ 100	171	28	114	35	
Immunophenotype					.85
B lineage	575	98	300	98	
T lineage	9	2	6	2	
CNS involvement					.26
Yes	35	6	11	4	
No	549	94	271	96	

Abbreviation: Ph, Philadelphia chromosome.
*Historical cohort data from Aricò et al.[21]
†χ^2 test.

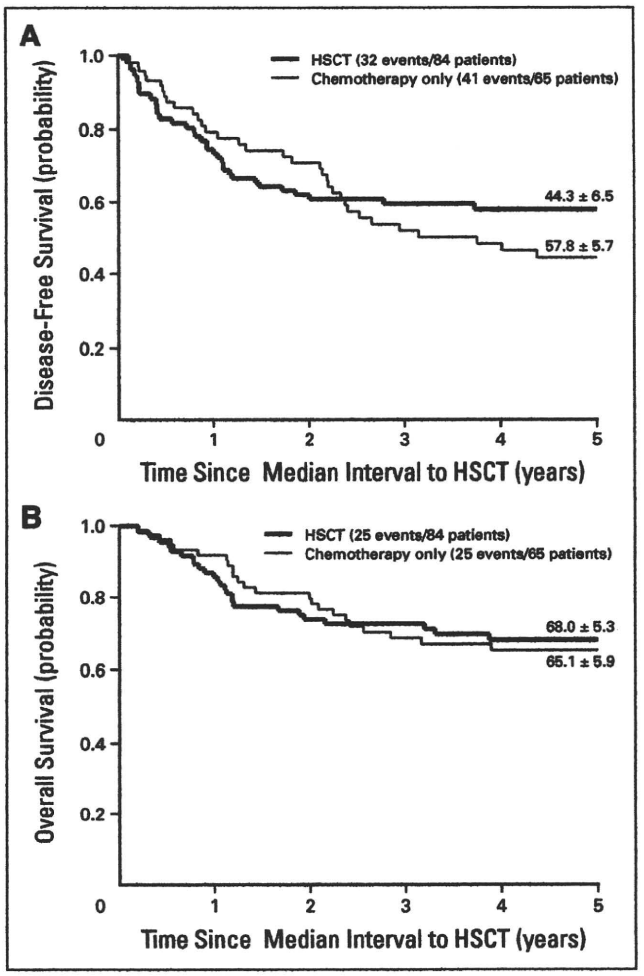

Fig A1. Estimates of (A) disease-free survival and (B) overall survival (± SE) in 149 patients with good early response and better modified Rome–National Cancer Institute criteria treated with hematopoietic stem-cell transplantation (HSCT) or chemotherapy only. The curves have been adjusted for waiting time to transplantation, so that the zero on the time axis corresponds to the median time from first complete remission to transplantation (5.1 months); patients were assigned to this treatment group in a time-dependent fashion. Five-year estimates (from remission) are shown.

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Nobuhiro Nishio · Seiji Kojima

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Abstract Dyskeratosis congenita (DC) is an inherited disease associated with nail dystrophy, abnormal skin pigmentation, oral leukoplakia, bone marrow failure and a predisposition to cancer. DC is a disease of defective telomere maintenance and patients with DC have very short telomeres. To date, mutations in six genes of telomerase and telomere components have been identified in patients with DC. Recently, mutations in telomerase and telomere components were also identified in patients with aplastic anemia, pulmonary fibrosis, and liver diseases who did not have mucocutaneous manifestations. These findings imply that defective telomere maintenance may cause not only classical DC but also a broad spectrum of diseases previously thought to be idiopathic, and have led to a new concept of diseases, termed "syndromes of telomere shortening". An understanding of the role of telomeres in these diseases is indispensable for diagnosis, genetic counseling and clinical management.

Keywords Dyskeratosis congenita · Telomere · Telomerase · Bone marrow failure

1 Introduction

Elizabeth Blackburn, Carol Greider, and Jack Szostak were awarded the 2009 Nobel Prize in Physiology or Medicine for their work describing telomeres and telomerase [1, 2]. Telomeres are DNA–protein structures that protect chromosome ends, which consist of a TTAGGG repeat bound by a cap protein, shelterin. Telomeres cannot be replicated by standard polymerase but only by a specialized transcriptase, called telomerase.

Dyskeratosis congenita (DC) is a rare inherited multisystem bone marrow failure syndrome characterized mainly by mucocutaneous abnormalities including nail dystrophy, mucosal leukoplakia, and abnormal skin pigmentation, along with a predisposition to cancer. Patients with DC have very short germ-line telomeres compared with normal individuals due to a defect of telomere maintenance. DC has been receiving increased attention because "telomere maintenance" is closely associated with life events, including aging and cancer predisposition. Recently, mutations in telomerase and telomere components were also identified in patients with aplastic anemia (AA), pulmonary fibrosis, and liver diseases who did not have mucocutaneous manifestations [3–13]. These findings implicate that defective telomere maintenance causes not only classical DC but also a broad spectrum of diseases previously thought to be idiopathic, and have lead to a new concept of diseases, termed "syndromes of telomere shortening".

In this review, we will discuss recent progress in the understanding of the pathophysiology of DC and other telomere diseases, as well as treatment for these diseases including stem cell transplantation.

2 Dyskeratosis congenita

The incidence of classic DC is approximately 1/1,000,000 individuals [14]. Classic DC presents with a triad of mucocutaneous abnormalities in around 80–90% of patients; abnormal skin pigmentation, nail dystrophy and oral leukoplakia [15]. Skin pigmentation and nail changes

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usually appear in childhood followed by oral leukoplakia and bone marrow failure, which develop by the age of 20 years. Other clinical manifestations, including non-mucocutaneous abnormalities, have also been reported. Non-mucocutaneous features such as bone marrow failure and pulmonary fibrosis occasionally precede mucocutaneous abnormalities, making it difficult to diagnose patients with DC based on clinical features alone. The diagnostic criteria for DC proposed by Vulliamy [16] include one or more of the three classic mucocutaneous features combined with hypoplastic bone marrow and at least two other somatic features known to occur in DC. The main causes of death in patients with DC are bone marrow failure/immunodeficiency (60–70%), pulmonary complications (10–15%), and malignancy (10%) [17, 18].

Until now, mutations in six genes involved in telomere maintenance have been identified in patients with DC. Figure 1 shows the schema of telomerase and shelterin complex. *DKC1* gene, encoding dyskerin, is the first gene identified in X-linked DC patients [19]. Dyskerin has a close association with the RNA component of telomerase (TERC), and mutations in dyskerin cause a reduction in accumulation of TERC and reduced telomere length [20]. In addition to its role in the biogenesis of telomerase RNA, dyskerin is involved in ribosomal RNA biogenesis. Dyskerin catalyzes uridine to pseudouridine, which is a critical step for ribosomal RNA maturation and function. These findings imply that both telomere and ribosomal defects may occur in patients with *DKC1* mutations. Subsequently, heterozygous *TERC* mutations were found in autosomal dominant DC patients [21]. Mutation screening demonstrated mutations of other components of telomerase complex including telomerase reverse transcriptase (*TERT*) [22, 23], *NOP10* [24], and *NHP2* [25] in patients with rare autosomal recessive DC. Mutations of *TERT* were also reported in the autosomal dominant family [8]. More recently, heterozygous mutations of *TINF2* encoding TIN2, main component of shelterin which protects telomeres, have been identified in ~11% of DC patients [5, 26].

3 Gene mutations of telomere maintenance in aplastic anemia and other bone marrow failure syndromes

Patients with DC have disease diversity in terms of age at onset, symptoms, and severity; this diversity occurs even among the patients with the same gene mutation. Bone marrow failure sometimes precedes mucocutaneous manifestations in patients with DC, and a substantial proportion of patients with AA have shorter telomeres compared with normal individuals [27, 28]. These observations prompted screening for gene mutations responsible for telomere maintenance in patients with AA and other bone marrow failure syndromes. This screening identified mutations in *TERC* and *TERT* in 3% of patients with AA [7, 9] (Table 1). We also identified *TERT* mutations in 2 of 96 Japanese children with AA, but no patient had a *TERC* mutation [6]. Patients with *TERC* or *TERT* mutations have very short telomeres in blood cells. Recently, Du et al. [4] found that 6 (5.5%) of 109 pediatric patients with severe AA had mutations of *TINF2*. We also screened for mutations of *TINF2*, but none of 96 pediatric patients with AA showed mutations of this gene (unpublished data).

Among three methods of measuring telomere length, including southern blot, real-time polymerase chain reaction, and flow cytometry and fluorescence in situ

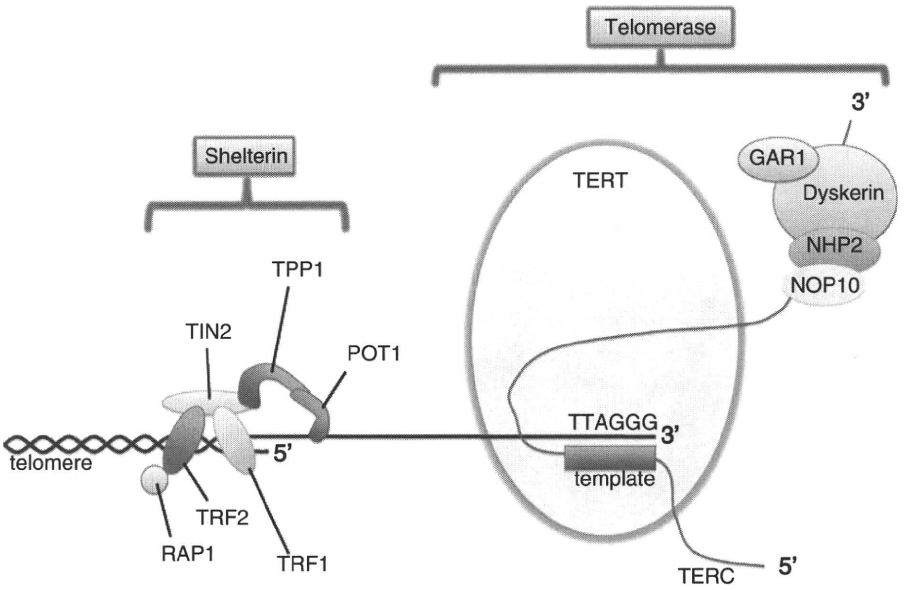

Fig. 1 Schema of telomerase and shelterin complex. Telomerase complex consists of the enzyme telomerase transcriptase (TERT), RNA component (TERC), and dyskerin protein complex (dyskerin, NOP10, NHP2, and GAR1). TERT adds new telomeres (TTAGGG repeats) onto the chromosome end by using the template provided by TERC. The shelterin complex consists of six proteins (TRF1, TRF2, RAP1, POT1, TPP1, and TIN2) and protects telomeres and regulates telomerase

Table 1 Mutations of genes associated with telomere maintenance identified in patients with aplastic anemia

References	Gene	Number of mutated and screened patients
Vulliamy et al. [10]	*TERC*	2/17 (12%)
Vulliamy et al. [8]	*TERT*	2/80 (2.5%)
Yamaguchi et al. [9]	*TERC*	2/150 (1.3%)
Yamaguchi et al. [7]	*TERT*	7/200 (3.5%)
Savage et al. [50]	*TERF1*	1/47 (2.1%)
	TERF2	1/47 (2.1%)
Liang et al. [6]	*TERT*	2/96 (2.1%)
Walne et al. [51]	*TINF2*	2/111 (1.8%)
Du et al. [3]	*TERT*	4/199 (2.0%)
Du et al. [4]	*TINF2*	6/109 (5.5%)

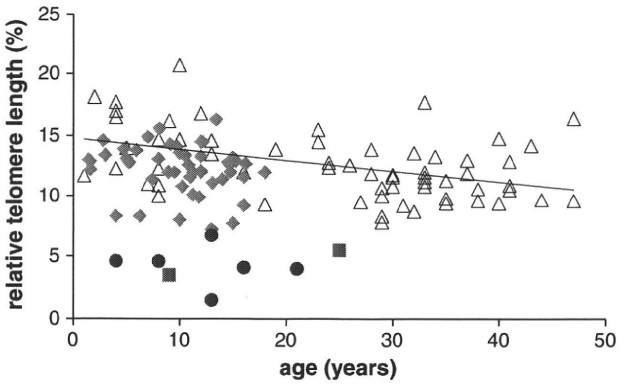

Fig. 2 Relative telomere length in peripheral blood lymphocytes from patients with dyskeratosis congenita (*filled circles*), patients with aplastic anemia harboring *TERT* mutations (*filled squares*), patients with idiopathic aplastic anemia (*filled argyles*) and normal individuals (*open triangles*). Telomere lengths were measured by flow cytometry-fluorescent in situ hybridization (flow-FISH). Relative telomere length was calculated as the ratio between the telomere signal of each sample and the telomere signal of the control cell line (cell line 1301). These data are from the Department of pediatrics, Nagoya University Graduate School of Medicine

hybridization (flow-FISH), flow-FISH is the most appropriate for "prospective" screening [29, 30]. As shown in Fig. 2, patients with DC and AA with the *TERT* mutation demonstrated very short telomeres as compared with idiopathic AA patients and normal individuals. Given the finding that a small subset of patients with apparently idiopathic AA carry telomere gene mutations and recognizing these patients is critical to treatment decisions, it is desirable to screen telomere gene mutations routinely in patients with AA before starting treatment. However, because screening of gene mutations is laborious and time-consuming, we have adopted screening of telomere length in blood cells instead of gene mutations.

It should be noted that short telomeres are not specific for patients with DC but are also seen in patients with bone marrow failure syndromes. Although short telomeres are also found in patients with other congenital bone marrow failure syndromes, such as Shwachman–Diamond syndrome and Fanconi anemia, telomere length in patients with DC is the shortest compared with other bone marrow failure syndromes. In fact, telomere length in most patients with DC is below the first percentile of telomere length found in healthy controls [31].

Family members of patients with DC should receive genetic counseling to rule out if they are silent carriers. In particular, genetic counseling is necessary during the proband search for a donor for hematopoietic stem cell transplantation. Sometimes, telomere length analysis in families with DC demonstrates that mutated carriers with clinical signs of bone marrow failure have the short telomeres. However, telomere length cannot predict the presence or absence of a mutation in family members with bone marrow failure. There are rare cases that show normal telomere length even though they harbor the same mutation as the proband, suggesting that mutation alone does not sufficiently shorten the telomeres [3].

4 Telomere diseases other than bone marrow failure syndromes

Clinical manifestations in patients with DC include not only bone marrow failure, but also other organ failures. Progressive pulmonary fibrosis develops in around 10–15% of patients with DC [17, 18], and is the second most common cause of death. Respiratory failure is also a common fatal complication after hematopoietic stem cell transplantation. Idiopathic pulmonary fibrosis (IPF) is an adult-onset, progressive scarring of the lung of unknown etiology that ultimately leads to respiratory failure. From 2 to 20% of patients with IPF have a family history of the disease that is inherited as an autosomal dominant trait with variable penetrance [12, 32]. Because some individuals in a pedigree of DC had the IPF phenotype, Armanios et al. [12] hypothesized that *TERC* or *TERT* may be candidate genes for familial IPF. They screened 73 probands of IPF and found 6 (8%) had heterozygous mutations in *TERT* or *TERC*. Tsakiri et al. [11] also independently found three missense mutations and one deletion of *TERT* genes in 44 probands of familial IPF and an additional single mutation in 44 sporadic cases of IPF. These mutant telomerase resulted in short telomeres. However, these patients did not show any classic mucocutaneous manifestations of DC.

Liver diseases have been also described as one of the clinical presentations in patients with DC. Some patients with DC develop severe liver complications after hematopoietic stem cell transplantation even if they have a normal liver function at the time of transplant [33]. In parallel with

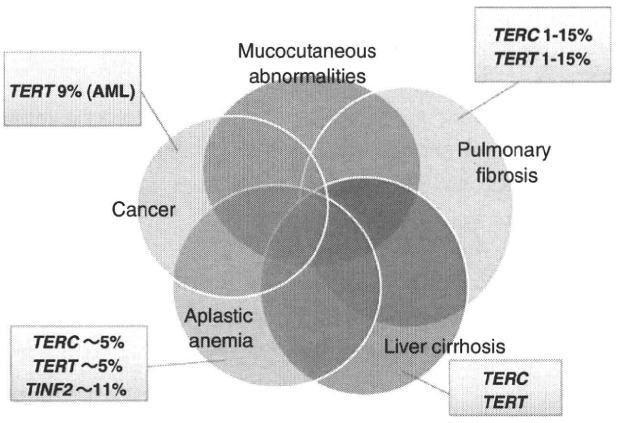

Fig. 3 Schema of phenotypic variations and identified gene mutations in defective telomere maintenance

reports of familial IPF, Calado et al. [13] reported that many relatives of patients with AA and a telomerase mutation had liver diseases, including pathologic fibrosis with inflammation and nodular regenerative hyperplasia. These patients did not present symptoms in childhood or display the characteristic physical abnormalities of DC, but had very short telomeres. These authors proposed that these disorders be collectively considered as "syndromes of telomere shortening". Figure 3 shows the schema of phenotypic variations and identified gene mutations in defective telomere maintenance.

5 Telomere shortening, chromosome instability and cancer predisposition

Patients with DC are prone to hematological malignancies and other solid tumors [17]. The defect of telomere maintenance and telomere attrition leads to chromosomal instability such as loss or gain of chromosomes and end-to-end fusion in in vitro studies and mouse models [34, 35]. Alter et al. recently reported that the expected cancer risk is 11-fold higher in patients with DC compared with the general population. The most frequent solid tumors were head and neck squamous cell carcinomas followed by skin and anorectal cancer [36].

Even outside DC, telomere attrition appears to cause chromosomal instability and cancer predisposition. Calado et al. [37] recently reported that patients with AA with shorter telomeres at diagnosis had a sixfold higher probability of developing clonal malignant disease following immunosuppressive therapy than patients with longer telomeres. They also showed that cultured bone marrow cells of patients with short telomeres in the presence of cytokines and high-dose granulocyte-colony stimulating factor (G-CSF) demonstrated increased telomere-free chromosomal ends and aneuploidy and translocations, including Robertsonian translocations.

Because patients with DC have been thought to be prone to myeloid malignancy, a screening for *TERT* and *TERC* mutations in patients with acute myeloid leukemia (AML) was conducted by the NIH group [38]. The authors found constitutional *TERT* mutations in 9% of patients with AML and a strong association of *TERT* mutations with the risk of cytogenetic abnormalities including trisomy 8 and inversion 16. None of the AML patients with *TERT* mutations had physical abnormalities that led to a suspicion of DC.

In addition, short telomeres have been linked to tumorigenesis of several solid tumors, including esophageal cancer, colorectal cancer, gastric cancer [39], and lung cancer [40]. Recent genome-wide studies demonstrated a higher frequency of *TERT* gene polymorphism in these patients than in normal individuals [41, 42].

6 Treatment of bone marrow failure

Bone marrow failure and immune deficiency are the most common causes of death in up to 60–70% of patients with DC. Androgen (e.g. oxymetholone) has been used to improve cytopenia in patients with DC since the 1960s. However, the mechanism of action of androgen has not been well understood until recently. Calado et al. [43] showed that in vitro exposure of normal peripheral blood cells to androgen produce higher *TERT* mRNA levels, and cells from patients who had heterozygous mutation of telomerase restored their low baseline telomerase activity to normal levels. As telomere shortening is closely associated with malignant disease, androgen therapy might prevent or postpone the development of various types of cancers. Erythropoietin and/or G-CSF combined with androgen has occasionally provided transient hematopoietic recovery to poor responders to androgen alone [44]. However, this combination should be used with caution because severe splenic peliosis and fatal rupture have been reported in two patients with DC who received simultaneous administration of androgen and G-CSF [45].

Allogeneic hematopoietic stem cell transplantation is the only curative treatment for bone marrow failure in patients with DC. However, the outcome in previous reports has been disappointing because of unacceptable transplant-related toxicities, including severe pulmonary/liver complications, especially in transplants from an alternative donor [36, 46]. To avoid these complications, non-myeloablative conditioning regimens have been recently used in several cases. Dietz et al. [47] reported encouraging results of six patients with DC who received a fludarabine-based non-myeloablative regimen. Four patients are alive, three of whom were recipients of unrelated grafts. Non-myeloablative transplants are expected to provide improvement in short-term survival. However,

longer-term follow-up is necessary because the late effects of conditioning agents and allogeneic immune responses within the recipient's organs, such as the lung and liver, remain to be clarified.

7 Future direction

Since the review article concerning DC was published by Walne et al. [14] in 2005 in this journal, many advances have occurred in the understanding of DC; however, many unsolved issues remain. Six causative genes have been identified, but mutations of these genes have been found in only half of patients with DC. Telomere-related gene mutations have been identified in patients with not only DC but also in patients with idiopathic AA, pulmonary fibrosis, and liver disease. These findings indicate that telomere-related diseases have a broad spectrum and may represent a new disease entity. A recent study demonstrated that exogenous expression of *TERC* alone can increase telomere activity and create growth potential and longevity in both *TERC* mutant and *DKC1* mutant cells [48]. More recently, Agarwal et al. [49] established induced pluripotent stem cells derived from a patient with DC and showed that the reprogrammed DC cells overcome a critical limitation in TERC levels to restore telomere maintenance and self-renewal. These findings indicate that drugs or gene therapy that can upregulate TERC activity have attractive therapeutic potential in patients with DC. Multicenter prospective studies are warranted to establish appropriate conditioning regimens aimed at reducing transplant-related mortality. We should improve not only short-term outcomes, such as hematological recovery, but also long-term effects on vital organs, especially the lungs and liver, following stem cell transplantation.

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Congenital dyserythropoietic anemia

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Abstract Congenital dyserythropoietic anemias (CDAs) are a heterogeneous group of rare hereditary disorders of erythropoiesis characterized by morphologic abnormal erythroblasts in the bone marrow. Three types of the disease are known as type I, II and III, and the variant type of CDA and several minor subgroups of CDA have been also reported since the first classification. Recently, responsible genes for type I (*CDAN1*) and type II (*SEC23B*) have been identified and the molecular pathogenesis of the disease is currently being explored. Although CDAs rarely transform to myelodysplastic syndrome or leukemia, the disease is important to understand the mechanism of hemopoiesis in humans.

Keywords Congenital dyserythropoietic anemia · Inherited bone marrow failure

1 Introduction

Congenital dyserythropoietic anemias (CDAs) are a heterogeneous group of rare hereditary disorders of erythropoiesis characterized by morphologic abnormal erythroblasts in the bone marrow. Non-erythroid hematopoietic cell lineages are morphologically normal. The term congenital dyserythropoietic anemia was first used by Crookston et al. in 1967 [1]. Subsequently, Heimpel et al. [2] proposed the classification of CDAs into three major types (type I, II and III), based on the morphological abnormalities affecting the erythroblasts and, in the case of CDA type II, also on serological characteristics. The variant type of CDA and several minor subgroups of CDA have been reported since the first classification [3].

Dyserythropoiesis is the major cause of anemia in patients with CDA, although a shortened half-life of the mature cells in circulation may also contribute to anemia. The definitions of ineffective erythropoiesis and dyserythropoiesis are shown as follows [4]:

- *Ineffective erythropoiesis* Failure to mature or cell death of red blood cell precursors in intact, stimulated bone marrow before delivery to the circulation as erythrocytes.
- *Dyserythropoiesis* Qualitative defect in red cells or red cell precursors (or both). Dyserythropoiesis often leads to ineffective erythropoiesis with intramedullary destruction of red cells and their precursors and often a decreased half-life of circulating red blood cells.

There have been some advances in our knowledge of the responsible gene since the first report by Dgany et al. [5] showing the disease genes for CDA type I (CDAN1).

Inheritance is autosomal recessive in the majority of cases. Diagnosis of CDAs are sometimes delayed because of the rarity and lack of information, especially in non-severe cases [6, 7].

In general, diagnosis of the CDAs requires the presence of all of the following four criteria:

1. evidence of congenital anemia/jaundice or of heredity;
2. evidence of ineffective erythropoiesis;
3. typical morphological appearance of bone marrow erythroblasts (Figs. 1, 2, 3);
4. exclusion of congenital anemias, which fulfill criteria one and two but have been classified according to the underlying defect, such as the thalassemia syndromes,

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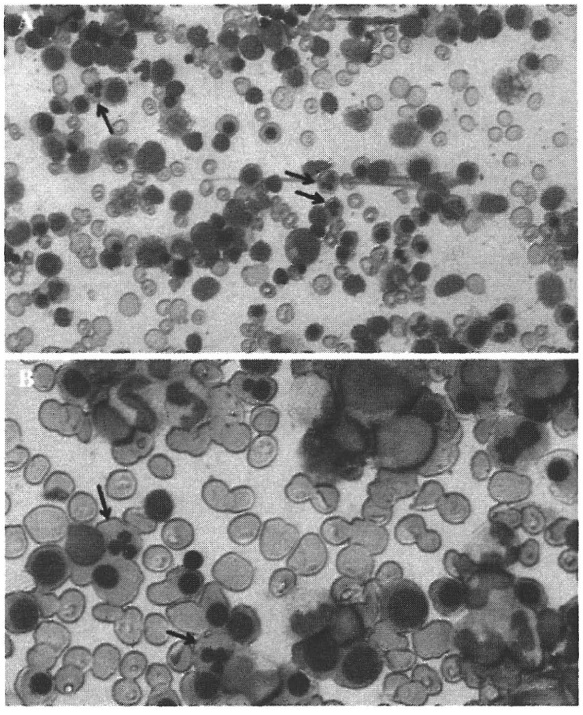

Fig. 1 **a** Light microscopy of bone marrow erythroblasts obtained by aspiration (×200). *Arrows* show trinuclearity as observed in all CDA types. **b** Light microscopy of bone marrow erythroblasts obtained by aspiration (×1000). *Arrows* show trinuclearity as observed in all CDA types

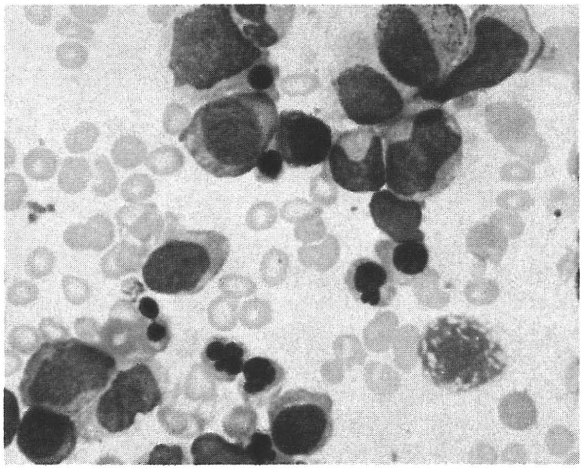

Fig. 2 Light microscopy of bone marrow (×1000). Multinucleated erythroblasts and intra-nuclear bridging is seen (courtesy of Dr. Takashi Taga)

some types of pathological hemoglobins or hereditary sideroblastic anemias [8].

CDA should be considered when the reticulocyte response is suboptimal for the degree of anemia in a patient

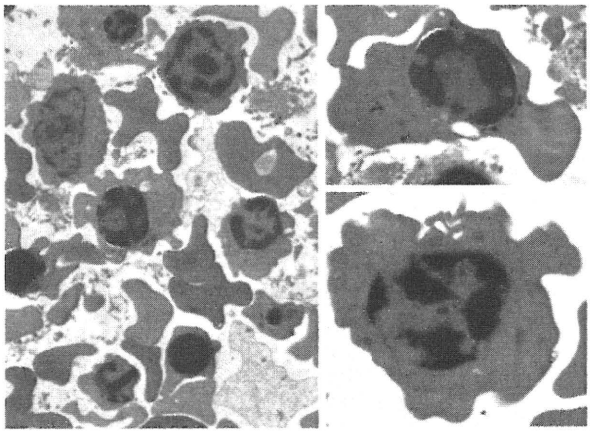

Fig. 3 Electron microscopy (bone marrow). Early polychromatic erythroblasts from CDA type I showing the "Swiss-cheese" abnormality of the heterochromatin (courtesy of Dr. Takashi Taga)

with erythroid hyperplasia or when there is unexplained hyperbilirubinemia or iron overload. Acquired types of anemia with ineffective erythropoiesis, such as megaloblastic anemia due to vitamin (B12 or folate) deficiency or myelodysplastic syndrome (MDS), need to be excluded. In MDS and AML-M6, morphologic abnormalities may mimic CDA.

Treatment is essentially symptomatic. Prevention of tissue damage secondary to iron overload is important [10]. Patients with CDA type I show hematologic improvement and a reduction in iron overload in response to recombinant interferon alpha [11–15]. Splenectomy is effective in some patients with CDA type II [10], whereas the benefit of splenectomy in other forms of CDA is controversial. Successful treatment with HLA-matched allogenic HSCT has been reported in severely affected cases of CDA [16–18].

Table 1 shows the main features of each type of CDA [3, 4, 8, 9].

2 CDA type I

CDA type I is inherited as an autosomal recessive disease and some cases have consanguineous parents. Obligate heterozygotes have normal hematologic indices, peripheral blood and bone marrow morphology. The incidence of CDA type I is about 1 per 100000 births per year, and more than 150 patients have been described mainly Western Europeans, people from the Middle East, Indians and Japanese. Diagnosis may be made rarely in utero, but also at any time between the neonatal period and late adulthood; in most patients the diagnosis is made during childhood and adolescence [19–22]. There is no gender preference.

The signs for neonatal presentation include intrauterine growth retardation, hepatosplenomegaly, early jaundice