Research Article

Statistics in Medicine

Received 15 January 2010, Accepted 27 May 2010 Published online 18 April 2011 in Wiley Online Library

(wileyonlinelibrary.com) DOI: 10.1002/sim.3999

Application of the continual reassessment method to a phase I dose-finding trial in Japanese patients: East meets West

Satoshi Morita*†

After cancer-related phase I dose-finding trials are completed in Western countries, further phase I trials are often conducted to determine recommended doses (RDS) for Japanese patients. This may be due to concerns about possible differences in treatment tolerability between Caucasians and Japanese. In most of these, a conventional '3+3' cohort study design is used in making dose escalation decisions, possibly due to its relatively easy implementation. Since its proposal by O'Quigley *et al.* (1990; *Biometrics*, 46:33–48), the continual reassessment method (CRM) has been used increasingly in cancer-related phase I dose-finding studies as an alternative to '3+3' designs. One of the principal advantages of applying a Bayesian CRM may be the utilization of all available prior information to estimate RDS through prior distributions that are assumed for model parameters representing the dose–toxicity relationship. In this paper, we present an application of the Bayesian CRM to a phase I dose-finding study in Japanese patients with advanced breast cancer using an informative prior elicited from clinical investigators. In some settings, it may be appropriate to use an informative prior that reflects the accurate and comprehensive previous knowledge of clinical investigators. On the other hand, for a model-based Bayesian outcome-adaptive clinical trial, it is necessary to establish sufficiently vague priors so that accumulating data dominate decisions as the amount of observed data increases. Thus, we retrospectively investigated the relative strength of the prior using a recently proposed method to compute a prior effective sample size. Copyright © 2011 John Wiley & Sons, Ltd.

Keywords: continual reassessment method; dose-finding; phase I trial; prior distribution; prior effective sample size

1. Introduction

After cancer-related phase I dose-finding trials are completed in Western countries, Japanese investigators often conduct trials using the same regimens in Japan to find the optimal doses for Japanese patients. This may be because of concerns about possible differences in treatment tolerability between Caucasians and Japanese. In many cases, recommended doses (RDs) of treatments have been set at higher levels in Caucasians than in Japanese. For example, a phase I study of Taxotere (docetaxel) monotherapy was undertaken in Caucasians to test dose levels from 5 to 115 mg/m^2 [1]. This study identified 100 mg/m^2 as the RD. A subsequent phase I study in Japan tested dose levels from 20 to 90 mg/m^2, and determined that 60 mg/m^2 was the RD for Japanese patients [2].

Japanese clinical investigators develop phase I trial study designs using observed toxicity data and RD levels identified in Western trials as pre-study information. For example, they test a smaller number of dose levels than the original study at doses that account for the RDs in Caucasian patients. In most of these Japanese phase I trials, a conventional '3+3' cohort design is used for making dose escalation decisions, possibly due to its relatively easy implementation and statistical simplicity and the fact that clinical investigators are in general quite familiar with it.

Since its proposal by O'Quigley *et al.* [3], the continual reassessment method (CRM) has been increasingly used in phase I dose-finding studies in cancer patients as an alternative to the '3+3'

Department of Biostatistics and Epidemiology, Yokohama City University Medical Center, 4-57 Urafune-cho, Minami-ku, Yokohama 232-0024, Japan

**Correspondence to: Satoshi Morita, Department of Biostatistics and Epidemiology, Yokohama City University Medical Center, 4-57 Urafune-cho, Minami-ku, Yokohama 232-0024 Japan.*

†E-mail: smorita@urahp.yokohama-cu.ac.jp

design. The CRM, based on a Bayesian parametric model that includes a logistic and a power model [3, 4] is characterized by one or more model parameters representing the dose–toxicity relationship. Although two-parameter models are flexible, they generally require a larger number of patients to estimate two model parameters, e.g. intercept and slope. One-parameter models that analyze one aspect of the dose–toxicity curve (in many cases, the slope) may not be flexible enough to accurately estimate the entire dose–toxicity curve. However, because a one-parameter model in the CRM has proven to be robust in determining a RD [3], it may be reasonable to use a one-parameter model for dose-finding in a cancer phase I trial.

The prior distributions assumed for model parameters are derived from pre-study information and are updated based on accumulated toxicity data observed in consecutive patient cohorts. The prior distribution of the model parameter should reasonably represent clinical investigators' uncertainty about the dose–toxicity relationship before starting the study, sometimes based on historical data from previous clinical studies. A Bayesian approach that formally uses historical/external data to establish such a prior distribution has not yet been fully developed. However, the integration of any available prior information into the estimation of RD levels for Japanese patients may be one of the major advantages of applying Bayesian CRM.

In some settings, it may be appropriate to use an informative prior that reflects the accurate and comprehensive knowledge that clinical investigators already possess. On the other hand, in other cases one may need to avoid excessively informative priors that may unduly influence posterior inferences. In particular, for clinical trials with a model-based Bayesian outcome-adaptive design, it is necessary to establish sufficiently vague priors so that accumulating data dominate decisions as the amount of observed data increases. After completing a Japanese phase I trial, we were concerned about the strength of the established prior distribution relative to the observed data in the trial in which 16 patients were enrolled in total. Thus, we retrospectively investigated the relative strength of the prior using a recently proposed method to compute a prior effective sample size (ESS) [5]. In this paper, we present an application of the CRM to a phase I dose-finding study in Japanese patients with advanced breast cancer using an informative prior elicited from Japanese clinical investigators.

Section 2 provides a motivating example. In Section 3, we describe the application of the CRM to a Japanese phase I study. We discuss establishment of a prior assumed for a dose–toxicity relationship in Section 4. We close with a discussion in Section 5.

2. A motivating example

Although chemotherapy regimens utilizing infusional 5-FU, e.g. the CEF-infu regimen (cyclophosphamide, epirubicin, and infusional 5-FU) [6], have been shown to have high antitumor activity, such regimens require central venous access and pumps. To avoid these inconveniences, a research team from the European Organization for Research and Treatment of Cancer (EORTC) conducted a phase I dose-finding study to develop a new combination regimen substituting the infusional 5-FU in CEF-infu with capecitabine [7]. Capecitabine (Xeloda®) is a novel oral 5-FU prodrug with high single-agent antitumor activity in metastatic breast cancer [8, 9], and also represents an attractive combination partner for the other CEF-infu chemotherapeutic agents [10–12]. The primary objective of the EORTC study was to determine the RD of capecitabine in combination with epirubicin and cyclophosphamide (CEX) in patients with advanced breast cancer. In the EORTC CEX study, four dose levels were planned for capecitabine in combination with fixed doses of epirubicin and CEX (100 and 600 mg/m^2, day 1, every 3 weeks), as summarized in Table I. Capecitabine was escalated from 750 to 1250 mg/m^2 twice daily for three weeks in four dose levels. A conventional '3+3' cohort design was used when making dose escalation decisions. That is, escalation to the next dose level was permitted if zero out of three (0/3) or one out of six (1/6) patients experienced dose-limiting toxicity (DLT). DLT is usually defined as the occurrence of grade 4 hematologic toxicity and grade 3 or 4 non-hematologic toxicity. If more than one patient developed a DLT, the maximum toxic dose (MTD) was reached, and the previous dose level was defined as the RD for phase II studies. In this study, 11 patients received CEX at four dose levels. While defining the MTD, three, three, three, and two patients were entered at dose levels 1, 2, 3, and 4, respectively, as shown in Table I. No DLTs occurred at dose levels 1, 2, and 3. At dose level 4, two out of two patients experienced DLTs. In addition, a high rate of capecitabine treatment modification (interruption and/or reduction) was required at dose level 3. Thus, the EORTC CEX study concluded

Table I. Dose levels of epirubicin and capecitabine studied in the Japanese and EORTC CEX studies and incidence of dose-limiting toxicities (DLTs) observed in the EORTC CEX study. The dose level of cyclophosphamide was fixed at 600 mg/m^2 on day 1 in both studies.

	Dose level	Epirubicin (mg/m^2, day 1 q21d)	Capecitabine (mg/m^2 twice daily, days 1–14 q21d)	Incidence of DLTs*
Japanese CEX	4	100	900	—
	3	90	900	$\frac{2}{6}$
	2	90	829	$\frac{0}{3}$
	1	75	829	$\frac{1}{4}$
	0	75	628	$\frac{0}{3}$
EORTC CEX	4	100	1250	$\frac{2}{2}$
	3		1050	$\frac{0}{3}$
	2		900	$\frac{0}{3}$
	1		750	$\frac{0}{3}$

*The number of patients experiencing any DLT/the number of evaluable patients.

that the recommended CEX regimen be limited to dose level 2 and consist of capecitabine 900 mg/m^2 twice daily, epirubicin 100 mg/m^2, and CEX 600 mg/m^2.

Although the EORTC study identified a recommended CEX regimen in this way, concern was raised over possible differences in CEX tolerability between Caucasians and Japanese [6, 13]. To answer this question, we conducted a phase I dose-finding trial using the CRM to determine the RDs of the CEX combination in Japanese patients with advanced breast cancer [14, 15]. Based on data from the EORTC CEX study and assuming that the RD of CEX in Japanese patients should not be higher than that in Caucasians, five dose levels (0–4) were planned in the Japanese CEX study, as summarized in Table I. Treatment consisted of a fixed dose of CEX (600 mg/m^2 on day 1) in combination with three doses of epirubicin and three doses of capecitabine. Dose level 4, the highest in our study, corresponded to the CEX RD as determined in the EORTC CEX analysis. The European and Japanese CEX studies employed the same DLT definitions.

3. The CRM in the Japanese cex trial

3.1. Study design using the CRM

3.1.1. Dose–toxicity model. In the CRM we used numerical dose levels X_j for $j=0,\ldots,4$, to reduce the dimension of the dose levels for the CEX treatment consisting of the three anti-cancer agents. The numerical values of X_j were specified using 'backward fitting' [16] as described below, instead of the actual dose levels for the CEX treatment in Table I. This dimension reduction allows a dose–toxicity model to suitably fit the pre-study estimates of the proportion of patients who would experience a DLT at the dose levels. The outcome variable is the indicator $Y_i=1$ if a patient i suffers a DLT, 0 if not. A one-parameter logistic regression model,

$$\pi(X_i,\beta)=\Pr(Y_i=1|X_i,\beta)=\frac{\exp(\beta_0+\beta_1 X_i)}{1+\exp(\beta_0+\beta_1 X_i)} \tag{1}$$

with the intercept b_0 fixed at 3 and a slope parameter b_1, is assumed. The likelihood for n patients is

$$f(\mathbf{Y}_n|\mathbf{X}_n,\beta)=\prod_{i=1}^{n}\pi(X_i,\beta)^{Y_i}\{1-\pi(X_i,\beta)\}^{1-Y_i}. \tag{2}$$

3.1.2. Setting up the CRM. Before starting the study, we conducted a preliminary study among participating clinical oncologists to obtain necessary reference information for implementing the CRM. We set up the CRM design using the following five steps:

(i) In step 1, we identified the target DLT probability as 0.33 and obtained the prior estimates of the proportion of patients who would experience a DLT at each dose level from 0 to 4 as 0.05, 0.10, 0.25, 0.40, and 0.60, respectively.

(ii) In step 2, we predetermined the model's intercept b_0 at 3, as discussed in Section 3.1.3.
(iii) In step 3, we specified a prior distribution function of the slope b_1. Letting $Ga(a,b)$ denote the gamma distribution with mean a/b and variance a/b^2, we assumed $Ga(a,b)$ for b_1 in order to constrain the slope b_1 to be positive and for computational convenience. This constraint implies an assumption that a higher dose level increases the probability of DLT.
(iv) In step 4, we specified numerical values of X_j for $j=0,\ldots,4$ using backward fitting as follows. We added a constraint $E(b_1)=1$ that corresponds to an equation $a=b$ in the gamma prior distribution to make the *a priori* dose–toxicity curve exactly reflect the prior estimate of DLT occurrence probabilities regardless of the degree of clinical uncertainty [17]. Under the dose–toxicity model with the slope b_1 fixed at 1, we computed each X_j to match $\Pr(Y=1|X_j,\beta_0=3,\beta_1=1)$ with the prior probability estimate of DLT occurrence at dose level j for $j=0,\ldots,4$. As a result, $\{X_0,X_1,X_2,X_3,X_4\}=\{-5.94,-5.20,-4.10,-3.41,-2.60\}$.
(v) In step 5, we specified the hyperparameters of the prior $p(b_1|a,b)$ as $a=b=5$. Details of this step are described in Section 4.

3.1.3. Specification of the intercept b_0. Under $a=b=5$ and $b_0=3$, the prior dose-toxicity curve with a 90 per cent credible interval is given in Figure 1(a). This prior dose-toxicity curve may reflect the oncologist's greater confidence in higher rather than lower dose levels. That is, taking into account that dose level 4 in the Japanese CEX study corresponds to the RD identified in the EORTC CEX study, $b_0=3$ may be a reasonable choice. In contrast, if we use a negative value for the intercept, i.e. $b_0=-5$, $\{X_0,X_1,X_2,X_3,X_4\}$ is computed as $\{2.06, 2.80, 3.90, 4.59, 5.41\}$ using backward fitting. In this setting, the prior dose–toxicity curve represents greater uncertainty in higher rather than lower dose levels (Figure 1(b)) and therefore should be considered that the specification $b_0=-5$ contradicts the pre-study information.

3.1.4. Dose escalation/de-escalation rule. Our study plan involved treating up to 22 patients. The starting dose was level 1, which was given to the first enrolled patient. The CRM then ran sequentially with three patients per cohort. Each cohort was treated at the dose level X_j with an estimated probability of DLT $\pi\{X_j, E(\beta_1|\text{data})\}$ closest to 0.33 and not exceeding 0.40. If the computed probability of the suggested dose level was greater than 0.40, the cohort was treated at the preceding dose level. Untried doses were not skipped when escalating dose level. The trial was stopped if level 0 was considered too toxic to be administered, e.g. $\pi\{X_0, E(\beta_1|\text{data})\}>0.40$. The posterior distribution of the slope parameter b_1 and each posterior estimate $\pi\{X_j, E(\beta_1|\text{data})\}$ along with its 90 per cent credible

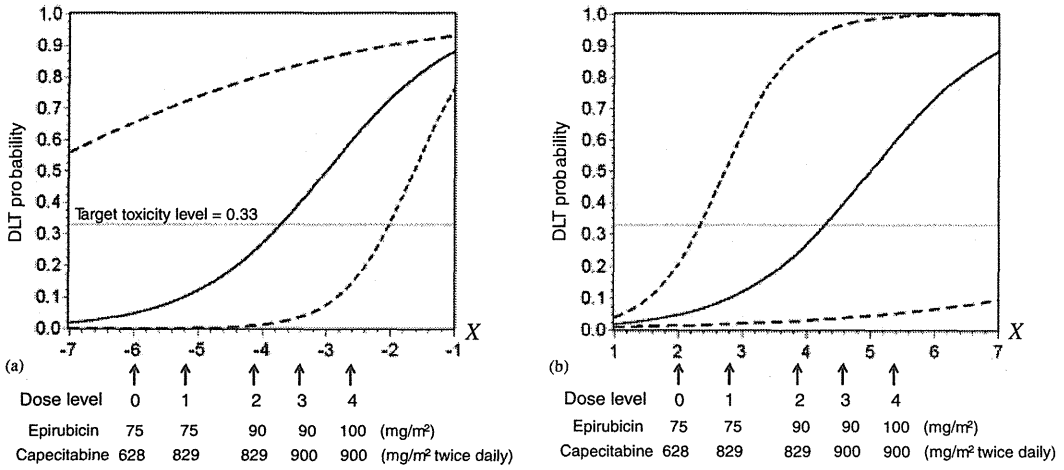

Figure 1. (a) Prior dose–toxicity curve (solid line) and its 90 per cent credible intervals (dashed lines) with the intercept $b_0=3$ under the gamma prior distribution, Ga (5,5). The horizontal axis X denotes the dose levels. The five values of $\{X1, X2, X3, X4, X5\}=\{-5.94,-5.20,-4.10,-3.41,-2.60\}$ used in the CRM computation are indicated by arrows. *The actual dose levels of epirubicin and capecitabine are also shown.* The horizontal straight line indicates the target DLT level (0.33) and (b) Prior dose–toxicity curve and its 90 per cent credible intervals with the intercept $b_0=-3$.

Table II. Incidence of dose-limiting toxicities (DLTs).

	Cohort 1	Cohort 2	Cohort 3	Cohort 4	Cohort 5	Cohort 6
No. of evaluable patients	1	3	3	3	3	3
Doselevel*	1	0	1	2	3	3
Epirubicin (mg/m^2, day 1 q21d)	75	75	75	90	90	90
Capecitabine (mg/m^2 twice daily, days 1–14 q21d)	829	628	829	829	900	900
No. of patients experiencing any DLT	1	0	0	0	1	1
Grade 3 HFS†	1	—	—	—	—	—
Grade 3 anorexia	—	—	—	—	1	—
Grade 3 mucositis	—	—	—	—	—	1

*The dose level of CEX was fixed at 600 mg/m^2 on day 1 every 3 weeks.
†HFS, hand-foot syndrome.

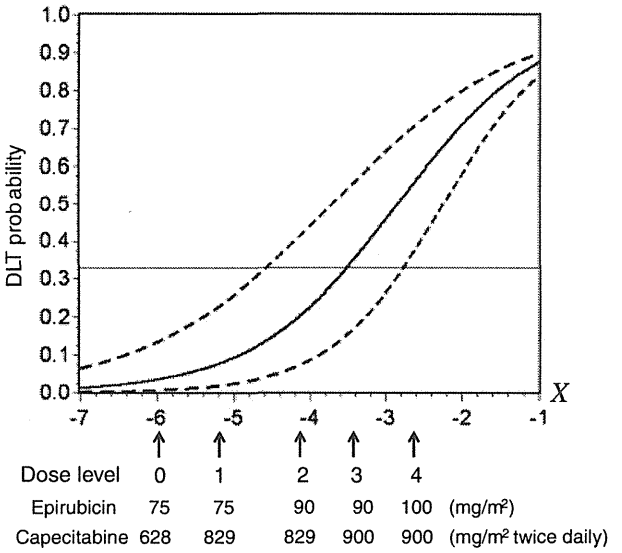

Figure 2. The posterior mean dose–toxicity curve (solid line) and its 90 per cent credible intervals (dashed lines) after updating with the toxicity data from all 16 patients.

interval were computed using numerical integration. An Independent Data and Safety Monitoring Committee (IDSMC) reviewed the interim analyses and was assigned the responsibility of making any recommendations to stop the trial on both clinical and statistical perspectives.

3.2. Implementation of the CRM

Because the results of the Japanese CEX trial were reported in detail in Saji *et al.* [14] and Morita *et al.* [15], we report here in brief. DLTs observed at each dose level and the dose escalation/de-escalation history throughout the study are shown in Tables I and II, respectively. The first patient treated at level 1 experienced a DLT (grade 3 hand-foot syndrome). The dose level was then de-escalated to level 0 for the second cohort. No DLTs were identified in the second, third (level 1), and fourth (level 2) cohorts. One of three patients in cohort 5 treated at level 3 experienced DLT (grade 3 anorexia). In the next cohort treated at level 3, one patient experienced DLT (grade 3 mucositis). Figure 2 shows the updated dose–toxicity curve including toxicity data from these 16 patients. The estimated DLT occurrence probability at level 3 was 0.354 (90 per cent credible interval: 0.174–0.560). With respect to efficacy data, one complete response and three partial responses were observed in six patients at level 3. Taking these CRM computations and the encouraging efficacy data into account, the DSMC recommended that the study be stopped. Therefore, we terminated the study and recommended that dose level 3 be further evaluated in a phase II trial.

4. Establishing a prior

In clinical trials with Bayesian model-based study designs, the prior should reasonably represent the physician's uncertainty. We established the prior distribution used in the Japanese CEX study based on the knowledge and experience of the participating clinical oncologists with regards to the CEX regimen. As described in Section 3, we assumed a gamma distribution Ga(a, b) for the prior distribution of the slope parameter b_1. Subject to $a=b$, the hyperparameter a determines the credible interval of the prior dose–toxicity curve under the gamma prior Ga(a, b). Thus, we determined that the hyperparameter a appropriately depicted the pre-study perceptions of the surveyed oncologists regarding the dose–toxicity relationship. By adjusting the hyperparameter a, i.e. $a=2$, 8, 20, 40, in addition to $a=5$ (Figure 1(a)) we created several graphical presentation patterns as shown in Figure 3. The clinical oncologists consulted in this study came to the consensus that the DLT probability at dose level 1 would be unlikely to be higher than 0.7 (more than double the target DLT level of 0.33) and the DLT probability at dose level 4 would be at least higher than 0.15 (around half of the target DLT level). The oncologists also concurred that the prior dose–toxicity curve and its credible interval constructed at $a=5$ reasonably reflected their knowledge and contained a sufficiently large degree of clinical uncertainty.

Although we determined the hyperparameters of the prior of b_1 based on an extensive discussion of the previous data using meticulous graphical presentations, our choice of the hyperparameters was arbitrary. If an established prior is overly informative, the prior may unduly influence posterior inferences and decisions, particularly early in the trial. Since dose levels must be selected sequentially in phase I dose-finding trials based on very small amounts of data, it may be important to quantify information contained in the chosen priors. These concerns may be addressed by quantifying the prior information

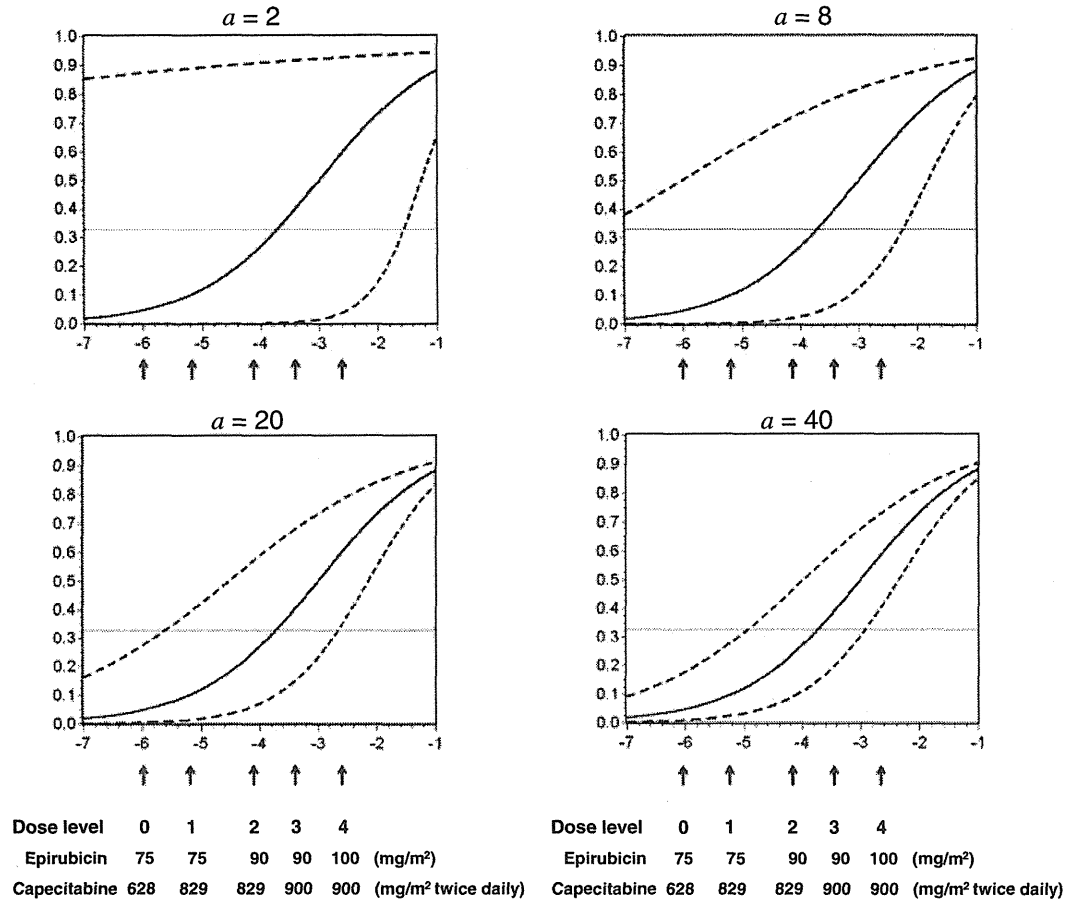

Figure 3. *Prior dose–toxicity curves with hyperparameters a=2, 8, 20, and 40.* Dashed lines indicate its 90 per cent credible intervals.

in terms of an equivalent number of hypothetical patients, i.e. a prior ESS. Such a summary would allow one to judge the relative contributions of the prior and the data to the decisions. We applied an ESS method proposed recently by Morita *et al.* [5] to the Japanese CEX trial in a retrospective fashion. The prior ESS computed at $a=5$ was 2.1. Thus, after enrolling three patients, the information from the likelihood started to dominate the prior, as desired. In addition, under Ga(5,5), the coefficient of variation (=standard deviation/mean) of the slope parameter b_1 was approximately 0.45, which might indicate some uncertainty in the slope parameter. Hence the prior specified in the Japanese CEX trial seemed quite reasonable.

As for the sensitivity analysis of the prior, the prior ESS values computed at $a=2$, 6, 7, 8, 20, and 40 are 0.86, 2.6, 3.0, 3.4, 8.6, and 17.1, respectively. It appears that $a<7$ may be needed to ensure an ESS<3. The prior with $a=40$ has ESS$=17.1$, so that it has impact roughly equal to that of the data on the posterior inference, as suggested by comparing Figures 2 and 3. In addition, under $a=40$, the *a priori* 90 per cent credible interval for the increase in the odds of a DLT occurrence, e.g. for the dose escalation from level 1 to level 2, is computed as 2.3–4.1, which may be excessively narrow compared with the 90 per cent credible interval of 1.5–7.5 computed under $a=5$. Thus, given the limited amount of information available during the design stage of the Japanese CEX study, the prior with $a=40$ may be criticized as being overly informative.

5. Discussion

When designing a phase I dose-finding study using a Bayesian CRM, certain choices must be made regarding details involved in a dose–toxicity model, numerical values of dose levels, prior distributions of model parameters, etc., and these should be sensible and plausible. If a one-parameter logistic model is chosen for modeling a dose–toxicity relationship, as was our approach in the Japanese CEX study, the intercept has to be specified at a certain real value. The actual dose levels of the combination therapy planned in the Japanese CEX study were based on information from the identical regimen conducted earlier in Caucasian patients, the EORTC CEX trial. In order to reduce the dimension of the dose levels, we specified the numerical values of the dose levels in the dose–toxicity formulation using backward fitting. In addition, we established the prior distribution of the slope parameter in the Japanese phase I trial by eliciting pre-study perceptions regarding the dose–toxicity relationship from Japanese clinical investigators.

So far, in many cases Japanese clinical investigators have conducted phase I studies assuming that a RD in Japanese patients should be lower than in Caucasian patients, based on results of clinical trials conducted in Western countries. That is, a large amount of historical data based on numerous studies has been integrated to design Japanese phase I trials. The Japanese CEX study, however, did not take full advantage of the pre-study information on dose–toxicity relationships derived from the EORTC CEX study to formally establish the prior distribution of the model parameter in the CRM.

Differences in RDs may be caused by specific differences between the abilities of Japanese and Caucasian populations to tolerate particular toxicities. These interracial differences can be regarded as patient prognostic covariates, but unfortunately such covariates have not yet been identified. Extensions of methods to find RDs for ordered prognostic subgroups have been proposed by O'Quigley and Paoletti [18], Yuan and Chappell [19], and Ivanova and Wang [20]. These methods may be applied to identifying RDs within racial subgroups in the setting of a multinational phase I study. Thall *et al.* [21] have proposed a Bayesian sequential phase I/II dose-finding design accounting for patient covariates and dose–covariate interactions. This method may also prove useful in modeling the Japanese–Caucasian association in a multinational study setting. It may be a significant challenge, however, to construct informative prior(s) on such an interracial difference in dose–toxicity curves [22].

In the context of Bayesian clinical trial design, well-chosen priors are important to ensure that posterior-based decision rules have good study operating characteristics. Some appropriate criteria for calibrating priors may be desired to obtain sensible prior distributions. A prior ESS quantifying the prior information in terms of the number of hypothetical patients may provide a useful tool for understanding the impact of prior-related assumptions. A useful property of prior ESS is that it is readily interpretable by clinical investigators who are involved in designing a clinical trial. ESS_RegressionCalculator.R, a computer program used to calculate the ESS for a normal linear or logistic regression model, is available from the website http://biostatistics.mdanderson.org/SoftwareDownload.

Acknowledgements

Satoshi Morita's research was supported in part by Grant H20-CLINRES-G-009 from the Ministry of Health, Labour and Welfare of Japan. He is grateful to Dr Masakazu Toi and Dr Junichi Sakamoto for providing important advice and suggestions. The author thanks the associate editor and the two referees for their constructive and helpful comments and suggestions.

References

1. Extra JM, Rousseau F, Bruno R, Clavel M, Le Bail N, Marty M. Phase I and pharmacokinetic study of taxotere (RP 56976; NSC 628503) given as a short intravenous infusion. *Cancer Research* 1993; **53**:1037–1042.
2. Taguchi T, Furue H, Niitani H, Ishitani K, Kanamaru R, Hasegawa K, Ariyoshi Y, Noda K, Furuse K, Fukuoka M, Yakushiji M, Kashimura M. Phase I clinical trial of RP 56976 (docetaxel) a new anticancer drug. *Japanese Journal of Cancer and Chemotherapy* (*Gan To Kagaku Ryoho*) 1994; **21**:1997–2005 (in Japanese).
3. O'Quigley J, Pepe M, Fisher L. Continual reassessment method: a practical design for phase I clinical trials in cancer. *Biometrics* 1990; **46**:33–48.
4. O'Quigley J, Shen LZ. Continual reassessment method: a likelihood approach. *Biometrics* 1996; **52**:673–684.
5. Morita S, Thall PF, Müller P. Determining the effective sample size of a parametric prior. *Biometrics* 2008; **64**:595–602.
6. Bonnefoi H, Biganzoli L, Cufer T, Mauriac L, Hamilton A, Schaefer P, Piccart M. An EORTC phase I study of epirubicin in combination with fixed doses of cyclophosphamide and infusional 5-fu (CEF-infu) as primary treatment of large operable or locally advanced/inflammatory breast cancer. *Breast Cancer Research and Treatment* 2001; **70**:55–63.
7. Bonnefoi H, Biganzoli L, Mauriac L, Cufer T, Schaefer P, Atalay G, Piccart M. An EORTC phase I study of capecitabine (Xeloda) in combination with fixed doses of cyclophosphamide and epirubicin (CEX) as primary treatment for large operable or locally advanced/inflammatory breast cancer. *European Journal of Cancer* 2003; **39**:277–283.
8. Blum JL, Jones SE, Buzdar AU, Lo Russo PM, Kuter I, Vogel C, Osterwalder B, Burger HU, Brown CS, Griffin T. Multicenter phase II study of capecitabine in paclitaxel-refractory metastatic breast cancer. *Journal of Clinical Oncology* 1999; **17**:485–493.
9. Blum JL, Dieras V, Lo Russo PM, Horton J, Rutman O, Buzdar A, Osterwalder B. Multicenter, phase II study of capecitabine in taxane-pretreated metastatic breast carcinoma patients. *Cancer* 2001; **92**:1759–1768.
10. O'Shaughnessy J, Miles D, Vukelja S, Moiseyenko V, Ayoub JP, Cervantes G, Fumoleau P, Jones S, Lui WY, Mauriac L, Twelves C, Van Hazel G, Verma S, Leonard R. Superior survival with capecitabine plus docetaxel combination therapy in anthracycline-pretreated patients with advanced breast cancer: phase III trial results. *Journal of Clinical Oncology* 2002; **20**:2812–2823.
11. Blum JL, Dees EC, Chacko A, Doane L, Ethirajan S, Hopkins J, McMahon R, Merten S, Negron A, Neubauer M, Ilegbodu D, Boehm KA, Asmar L, O'Shaughnessy JA. Phase II trial of capecitabine and weekly paclitaxel as first-line therapy for metastatic breast cancer. *Journal of Clinical Oncology* 2006; **24**:4384–4390.
12. Gradishar WJ, Meza LA, Amin B, Samid D, Hill T, Chen M. Capecitabine plus paclitaxel as front-line combination therapy for metastatic breast cancer: a multicenter phase II study. *Journal of Clinical Oncology* 2004; **22**:2321–2327.
13. Iwata H, Nakamura S, Toi M, Shin E, Masuda N, Ohno S, Takatsuka Y, Hisamatsu K, Yamazaki K, Kusama M, Kaise H, Sato Y, Kuroi K, Akiyama F, Tsuda H, Kurosumi M. Interim analysis of a phase II trial of cyclophosphamide, epirubicin and 5-fluorouracil (CEF) followed by docetaxel as preoperative chemotherapy for early stage breast carcinoma. *Breast Cancer* 2005; **12**:99–103.
14. Saji S, Toi M, Morita S, Iwata H, Ito Y, Ohno S, Kobayashi T, Hozumi Y, Sakamoto J. Dose-finding phase I and pharmacokinetic study of capecitabine (xeloda) in combination with epirubicin and cyclophosphamide (CEX) in patients with inoperable or metastatic breast cancer. *Oncology* 2007; **72**:330–337.
15. Morita S, Toi M, Saji S, Iwata H, Ohno S, Ito Y, Kobayashi T, Hozumi Y, Sakamoto J. Practical application of the continual reassessment method to a phase I dose-finding trial in advanced breast cancer. *Drug Information Journal* 2007; **41**:691–700.
16. Garrett-Mayer E. The continual reassessment method for dose-finding studies: a tutorial. *Clinical Trials* 2006; **3**:57–71.
17. Ishizuka N, Morita S. Practical implementation of the continual reassessment method. In *Handbook of Statistics in Clinical Oncology* (2nd edn), Crowley J (ed.). CRC Press: New York, 2005; 97–116.
18. O'Quigley J, Paoletti X. *Continual reassessment method for ordered groups. Biometrics* 2003; **59**:430–440.
19. Yuan Z, Chappell R. Isotonic designs for phase I cancer clinical trials with multiple risk groups. *Clinical Trials* 2004; **1**:499–508.
20. Ivanova A, Wang K. Bivariate isotonic design for dose-finding with ordered groups. *Statistics in Medicine* 2006; **25**:2018–2026.
21. Thall PF, Nguyen HQ, Estey EH. Patient-specific dose finding based on bivariate outcomes and covariates. *Biometrics* 2008; **64**:1126–1136.
22. Garthwaite PH, Kadane JB, O'Hagan A. Statistical methods for eliciting probability distributions. *Journal of the American Statistical Association* 2005; **100**:680–701.

Annals of Oncology 25: 435–441, 2014
doi:10.1093/annonc/mdt558
Published online 7 January 2014

Continuing increased risk of oral/esophageal cancer after allogeneic hematopoietic stem cell transplantation in adults in association with chronic graft-versus-host disease

Y. Atsuta[1,*], R. Suzuki[1], T. Yamashita[2], T. Fukuda[2], K. Miyamura[3], S. Taniguchi[4], H. Iida[5], T. Uchida[6], K. Ikegame[7], S. Takahashi[8], K. Kato[9], K. Kawa[10], T. Nagamura-Inoue[11], Y. Morishima[12], H. Sakamaki[13] & Y. Kodera[14], for the Japan Society for Hematopoietic Cell Transplantation

[1]Department of HSCT Data Management and Biostatistics, Nagoya University Graduate School of Medicine, Nagoya; [2]Hematopoietic Stem Cell Transplantation Unit, National Cancer Center Hospital, Tokyo; [3]BMT Center, Japanese Red Cross Nagoya First Hospital, Nagoya; [4]Department of Hematology, Toranomon Hospital, Tokyo; [5]Department of Hematology, Meitetsu Hospital, Nagoya; [6]Department of Hematology and Oncology, Nagoya Daini Red Cross Hospital, Nagoya; [7]Division of Hematology, Department of Internal Medicine, Hyogo College of Medicine, Nishinomiya; [8]Department of Molecular Therapy, The Institute of Medical Science, The University of Tokyo, Tokyo; [9]Department of Pediatrics, Japanese Red Cross Nagoya First Hospital, Nagoya; [10]Osaka Medical Center and Research Institute for Maternal and Child Health, Izumi; [11]Department of Cell Processing and Transfusion, Research Hospital, The Institute of Medical Science, The University of Tokyo, and Tokyo Cord Blood Bank, Tokyo; [12]Division of Epidemiology and Prevention, Aichi Cancer Center Research Institute, Nagoya; [13]Division of Hematology, Tokyo Metropolitan Cancer and Infectious Diseases Center, Komagome Hospital, Tokyo; [14]Department of Promotion for Blood and Marrow Transplantation, Aichi Medical University, School of Medicine, Nagakute, Japan

Received 2 August 2013; revised 6 November 2013; accepted 12 November 2013

Background: The number of long-term survivors after hematopoietic stem cell transplantation (HSCT) showed steady increase in the past two decades. Second malignancies after HSCT are a devastating late complication. We analyzed the incidence of, risk compared with that in the general population, and risk factors for secondary solid cancers.

Patients and methods: Patients were 17 545 adult recipients of a first allogeneic stem cell transplantation between 1990 and 2007 in Japan. Risks of developing secondary solid tumors were compared with general population by using standard incidence ratios (SIRs).

Results: Two-hundred sixty-nine secondary solid cancers were identified. The cumulative incidence was 0.7% [95% confidence interval (CI), 0.6%–0.9%] at 5 years and 1.7% (95% CI, 1.4%–1.9%) at 10 years after transplant. The risk was significantly higher than that in the general population (SIR = 1.8, 95% CI, 1.5–2.0). Risk was higher for oral cancer (SIR = 15.7, 95% CI, 12.1–20.1), esophageal cancer (SIR = 8.5, 95% CI, 6.1–11.5), colon cancer (SIR = 1.9, 95% CI, 1.2–2.7), skin cancer (SIR = 7.2, 95% CI, 3.9–12.4), and brain/nervous system cancer (SIR = 4.1, 95% CI, 1.6–8.4). The risk of developing oral, esophageal, or skin cancer was higher at all times after 1-year post-transplant. Extensive-type chronic graft-versus-host disease (GVHD) was a significant risk factor for the development of all solid tumors (RR = 1.8, $P < 0.001$), as well as for oral (RR = 2.9, $P < 0.001$) and esophageal (RR = 5.3, $P < 0.001$) cancers. Limited-type chronic GVHD was an independent risk factor for skin cancers (RR = 5.8, $P = 0.016$).

Conclusion: Recipients of allogeneic HSCT had a significantly higher ~2-fold risk of developing secondary solid cancers than the general population. Lifelong screening for high-risk organ sites, especially oral or esophageal cancers, is important for recipients with active, or a history of, chronic GVHD.

Key words: secondary solid cancers, late effect, hematopoietic stem cell transplantation

introduction

Hematopoietic stem cell transplantation (HSCT) is a curative treatment of choice for malignant and non-malignant hematological disorders [1]. The annual number of allogeneic HSCT has increased steadily over the past three decades worldwide [2–6]. Progress in transplant procedures in addition to this steady increase in the number of HSCT procedures worldwide has contributed to an increase in the number of long-term survivors.

Secondary malignancies, including new solid cancers, are an important cause of late mortality. Several studies have reported that survivors of HSCT have a 2–3-fold increased risk of

*Correspondence to: Dr Yoshiko Atsuta, Department of Hematopoietic Stem Cell Transplantation Data Management and Biostatistics, Nagoya University Graduate School of Medicine, 1-1-20 Daiko-Minami, Higashi-ku, Nagoya 461-0047, Japan. Tel: +81-52-719-1973; Fax: +81-52-719-1973; E-mail: y-atsuta@med.nagoya-u.ac.jp

developing new solid cancers compared with an age-, sex-, region-, and calendar-year-adjusted population and the risk among long-term survivors ranges from 1% to 6% at 10 years after transplantation [7–14]. Identified risk factors include exposure to radiation as a part of the conditioning regimen and chronic graft-versus-host disease (GVHD), and the latter has been shown to be strongly correlated with the development of squamous cell carcinoma [8, 10, 12, 15–17]. However, a recent long-term follow-up analysis of patients who were transplanted after myeloablative doses of busulfan and cyclophosphamide without total body irradiation (TBI) found a similar increased incidence of 0.6% at 5 years and 1.2% at 10 years after transplantation [13]. We conducted a nationwide, retrospective cohort study with a large and different cohort from those used in previous reports from North America and Europe, to determine the incidence and risks of developing secondary solid cancers.

methods

data source and collection of data

The recipient clinical data were collected by the Japan Society for Hematopoietic Cell Transplantation (JSHCT) using the Transplant Registry Unified Management Program, as described previously [18]. The JSHCT collect recipients' baseline, disease, transplant, and transplant outcome information who received HSCT in the previous year. Patient information regarding survival, disease status, and long-term complications including chronic GVHD and second malignancies are renewed annually. This study was approved by the data management committee of the JSHCT, as well as the institutional review board of Nagoya University Graduate School of Medicine.

patients

Adult patients (at least 16 years of age) who received a first HSCT between 1990 and 2007 were considered as subjects for the present study. Those who were inherently susceptible to developing cancer [Fanconi anemia ($N = 3$) and congenital immunodeficiency ($N = 12$)] were excluded. Three-hundred five recipients (1.7%) were excluded because of insufficient follow-up data. The study included 17 545 recipients; 5358 recipients of related bone marrow, 3587 recipients of related peripheral blood stem cells (including 134 bone marrow and peripheral blood stem cells combined), 6508 recipients of unrelated bone marrow, and 2092 recipients of unrelated cord blood.

statistical analysis

Standard incidence ratios (SIRs) were calculated to determine whether the number of recipients in the present cohort who developed secondary solid tumor after receiving a HSCT was different than that in the general population (supplementary method, available at *Annals of Oncology* online). Cumulative incidences of solid cancer or GVHD were estimated by taking into account the competing risk of death among patients who did not develop a second malignancy or GVHD [19]. The influence of potential risk factors was estimated by using the Cox proportional hazard model [20]. A stepwise multivariate approach was used to identify the most important predictor with respect to the development of secondary solid cancers. The variables considered were age at transplant, patient sex, donor-type (related versus unrelated), graft source, TBI as part of the conditioning regimen, reduced-intensity conditioning, grade 2–4 acute GVHD, and chronic GVHD. The model was stratified into four categories according to the primary disease; acute myeloid leukemia, acute lymphoblastic leukemia, chronic myeloid leukemia, and others. Acute and chronic GVHD were considered as time-dependent covariates. TBI and chronic GVHD were frequent risk factors and were always kept in the model. Risk factors for high-risk cancer sites with adequate numbers of events for analyses were also analyzed; oral cavity/pharynx, esophagus, colon, and skin. The models for high-risk cancer sites were stratified according to the primary disease as described, and patient age at transplantation (<19, 20–29, 30–39, 40–49, 50–59, and >60), and also adjusted by patient age as a continuous variable. All *P*-values were two-sided.

results

patient and transplant characteristics

Table 1 shows the patient characteristics, their disease, and transplant regimens for 17 545 recipients of a first HSCT. The cumulative incidences of grade 2–4 acute GVHD at 150 days and chronic GVHD at 2 years post-transplant were 35% [95% confidence interval (CI), 35%–36%] and 41% (95% CI, 40%–41%), respectively. The observation period reached 69 465 person-years among the subjects for analyses. Of the 17 545 recipients, 5864 had survived for 5 or more years, and 2192 recipients had survived 10 or more years at the time of analysis (Table 2).

incidence and types of secondary solid cancers

The cumulative incidence of solid cancers was 0.7% (95% CI, 0.6–0.9) at 5 years, 1.7% (95% CI, 1.4–1.9) at 10 years, and 2.9% (95% CI, 2.5–3.4) at 15 years after transplantation (Figure 1). Two-hundred sixty-nine solid cancers were identified. Multiple solid cancers were observed in 11 patients. Nineteen recipients were diagnosed within 1-year post-transplantation (Table 2).

risk compared with the general population

HSCT recipients had a 1.8-fold higher risk of invasive solid cancers compared with the general population (95% CI, 1.5–2.0). SIR was significantly higher for cancers of the oral cavity/pharynx (SIR = 15.7), esophagus (SIR = 8.5), colon (SIR = 1.9), skin (SIR = 7.2), and brain/nervous system (SIR = 4.1; Table 2). The risks of developing secondary cancers of the oral cavity/pharynx, esophagus, and skin were significantly higher than those in the general population throughout all periods after 1 year (Figure 2). The risk for developing colon cancer was elevated during the period of 1–4 years (SIR = 2.7), whereas the risks for developing cancer of the pancreas (SIR = 4.5) were elevated during the period of 5–9 years. Recipients were at higher risk of developing cancers of the rectum (SIR = 3.6) and the brain/nervous system (SIR = 19.1) after 10 years post-transplantation. The risk of developing secondary solid cancers of all types compared with the general population increased with the time since transplantation. This trend was observed for oral/pharynx and esophageal cancer (Table 2; Figure 2).

recipients' age at transplantation and risks for developing secondary solid cancers

SIRs were also analyzed according to the recipient's age at transplantation (Table 3). Compared with the general population in Japan, the SIRs were significantly increased for all solid cancers, oral/pharynx, esophagus, liver, bronchus/lung, and brain/nervous system for recipients who were 16–19 years of age at transplant, all solid cancers, oral/pharynx, and esophagus for recipients who

Table 1. Patient, disease, and transplant characteristics

Characteristics	Number	Percent
Total number	17 545	
Year of transplant		
1990–1994	1630	9
1995–1999	3750	21
2000–2004	7078	40
2005–2007	5087	29
Patient sex		
Male	10 386	59
Female	7149	41
Missing	10	<1
Patient age		
Median (range)	40 (16–85)	
16–19	1399	8
20–29	3506	20
30–39	3787	22
40–49	4167	24
50–59	3549	20
≥60	1137	6
Diagnosis		
Acute myeloid leukemia	6096	35
Acute lymphoblastic leukemia	3334	19
Chronic myeloid leukemia	2514	14
Myelodysplastic syndromes	1716	10
Adult T-cell leukemia	591	3
Other leukemia	130	1
Myeloproliferative disorders	224	1
Non-Hodikin's lymphoma	1652	9
Hodikin's lymphoma	46	<1
Other lymphoma/type missing	54	<1
Multiple myeloma	210	1
Aplastic anemia	745	4
Pure red cell aplasia	4	<1
Paroxysmal nocturnal hemoglobinuria	20	<1
Solid tumor	109	1
Others	86	<1
Data missing	14	<1
Donor		
Related, siblings	7825	45
Related, other relatives	941	5
Related, data missing	179	1
Unrelated	8600	49
Stem cell source		
Bone marrow	11 866	68
Peripheral blood	3453	20
Bone marrow and peripheral blood	134	1
Cord blood	2092	12
Conditioning regimen		
Myeloablative		
Cyclophosphamide + TBI ± other	8298	47
Other TBI regimen	1321	8
Busulfan + cyclophosphamide ± other	2798	16
Other non-TBI regimen	778	4
Reduced intensity		
Fludarabine + busulfan ± other	1527	9
Fludarabine + cyclophosphamide ± other	503	3
Fludarabine + melphalan ± other	1480	8
Other RIST	631	4
Data missing	209	1
GVHD prophylaxis		
No	85	<1
Cyclosporine A + sMTX	10 091	58
Cyclosporine A ± other	1175	7
Tacrolimus + sMTX	4682	27
Tacrolimus ± other	876	5
Other	323	2
Data missing	312	2

TBI, total body irradiation; sMTX, short-term methotrexate.

were 20–29 years of age at transplant, all solid cancers, oral/pharynx, esophagus, and gallbladder for recipients who were 30–39 years of age at transplant, all solid cancers, oral/pharynx, esophagus, and skin for recipients who were 40–49 years of age at transplant, all solid cancers, oral/pharynx, esophagus, colon, and skin for recipients who were 50–59 years of age at transplant (Table 3).

risk factors for the development of secondary solid cancers

Extensive-type chronic GVHD and age at transplantation were important risk factors for the development of secondary solid cancers (Table 4). The risk was not increased in recipients who received TBI for conditioning. The results were similar when subjects were limited to those who received myeloablative conditioning (RR = 1.5, $P = 0.069$ for limited-type chronic GVHD, RR = 1.9, $P < 0.001$ for extensive-type chronic GVHD, and RR = 0.9, $P = 0.751$ for TBI). Risk factor analyses for high-risk organs with more than 10 cancer cases revealed that extensive-type chronic GVHD was an independent risk factor for cancers in the oral cavity/pharynx and esophagus. Limited-type chronic GVHD was a risk factor for cancers of skin (Table 4). For secondary cancers which developed within 1-year post-transplant, the only risk factor identified was older age at transplant (age 60 years or older; supplementary Table, available at *Annals of Oncology* online).

discussion

Our main objective was to determine the incidence of, the risk compared with the general population, and risk factors for secondary solid tumors after allogeneic stem cell transplantation in a large cohort of adult recipients. Allogeneic HCT recipients were at higher risk of developing cancers of the oral cavity, esophagus, colon, and skin. The incidence and SIR of developing all solid cancers continued to increase with follow-up, which suggested a continuous increase as follow-up progressed. Our data are important since we included a large number of subjects and person-years of follow-up, in a transplant cohort that is different from those in previously reported large studies.

Table 2. Standard incidence ratio, ratio of observed versus expected number of secondary solid cancers according to duration post-transplant

	Time since transplantation (years)								Total		
	<1		1–4		5–9		10 or longer				
Number of recipients	17 545		10 210		5864		2192			17 545	
Person-years at risk	12 803		30 599		18 845		7218			69 465	
Secondary cancer sites	O	SIR	O	SIR	O	SIR	O	SIR	O/E	SIR	95% CI
All solid cancers	19	0.7	97	1.5*	90	2.0*	63	3.1*	269/153.6	1.8*	1.5–2.0
Oral/pharynx	0	0.0	16	9.5*	27	23.4*	21	38.5*	64/4.1	15.7*	12.1–20.1
Esophagus	0	0.0	13	6.5*	17	12.6*	11	16.8*	41/4.8	8.5*	6.1–11.5
Stomach	2	0.4	7	0.6	6	0.8	1	0.3	16/26.0	0.6	0.4–1.0
Colon	2	0.8	16	2.7*	5	1.2	4	2.2	27/14.3	1.9*	1.2–2.7
Rectum	0	0.0	1	0.2	0	0.0	5	3.6*	6/10.7	0.6	0.2–1.2
Liver	1	0.6	5	1.4	0	0.0	2	1.8	8/8.6	0.9	0.4–1.8
Gallbladder	2	5.1	2	2.1	2	3.0	0	0.0	6/2.3	2.6	1.0–5.7
Pancreas	0	0.0	2	1.0	6	4.5*	1	1.6	9/4.7	1.9	0.9–3.7
Broncus/lung	3	1.2	4	0.6	9	2.1	3	1.5	19/15.1	1.3	0.8–2.0
Skin	2	7.0	6	8.1*	3	5.7*	2	8.4*	13/1.8	7.2*	3.9–12.4
Female breast	0	0.0	3	0.3	1	0.1	3	0.9	7/24.5	0.3	0.1–0.6
Cervix uteri	1	1.3	4	2.0	1	0.7	1	1.6	7/4.8	1.5	0.6–3.0
Corpus uteri	2	3.7	1	0.7	2	1.8	0	0.0	5/3.6	1.4	0.4–3.2
Ovary	0	0.0	1	0.7	1	1.0	1	2.2	3/3.6	0.8	0.2–2.4
Prostate	1	1.2	0	0.0	1	0.6	1	1.4	3/5.4	0.6	0.1–1.6
Bladder	1	1.9	3	2.4	0	0.0	0	0.0	4/2.9	1.4	0.4–3.5
Kidney	0	0.0	1	0.6	1	0.9	0	0.0	2/4.1	0.5	0.1–1.8
Brain/nervous system	1	3.4	1	1.4	1	2.1	4	19.1*	7/1.7	4.1*	1.6–8.5
Thyroid	0	0.0	2	1.1	2	1.5	0	0.0	4/4.5	0.9	0.2–2.3
Other[a]	1		9		4		3		17		

[a]Other sites included two testicular cancers, four connective tissue cancers, four bone cancers, one larynx cancer, one malignant salivary gland tumor, one duodenum papilla cancer, one germ cell tumor, one carcinomatous pleurisy of origin unknown, and two squamous cell carcinomas of unknown origin.
*$P < 0.05$.

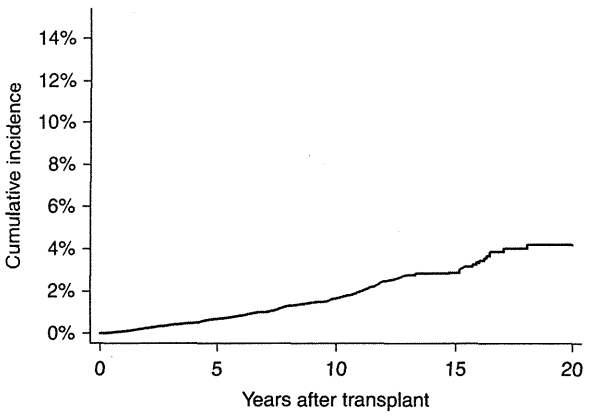

Figure 1. Cumulative incidence of developing a secondary solid cancer. The cumulative incidence of solid cancers was 0.7% [95% confidence interval (CI), 0.6–0.9] at 5 years, 1.7% (95% CI, 1.4–1.9) at 10 years, and 2.9% (95% CI, 2.5–3.4) at 15 years after transplantation.

Extensive-type chronic GVHD has repeatedly been shown to be a significant risk factor for the development of secondary solid tumor and is highly correlated with squamous cell carcinoma [8, 9, 12, 15, 16]. Extensive-type chronic GVHD was also shown to be a significant risk factor for oral cancer in our study. Extensive-type chronic GVHD was shown to be a significant risk factor for esophageal cancer, which was found to be increased in recipients compared with the general population in our study as well as in two other smaller Japanese cohorts in previous studies [11, 14]. Subjects were shown to be at a higher risk for the development of cancers of the oral cavity or esophagus at all time periods after 1 year. Data were not obtained for affected organ sites of chronic GVHD in JSHCT data collection prior to transplants in 2006. Therefore, we could not investigate whether oral or esophageal cancers were related to the chronic GVHD of the same organ. However, results of risk factor analyses for cancer sites of oral, esophagus, colon, and skin which showed high associations of extensive-type chronic GVHD and oral or esophagus cancer, limited-type chronic GVHD, and skin cancer showed that development of secondary solid tumors were likely to be influenced by GVHD-affected sites. Lifelong screening for oral, pharynx, or esophageal cancers for recipients with active or resolved chronic GVHD is important after 1-year post-transplant. The prognosis of solid cancers is highly influenced by the stage of the cancers when they are first detected. Our findings support recently published recommended screening guidelines [21, 22].

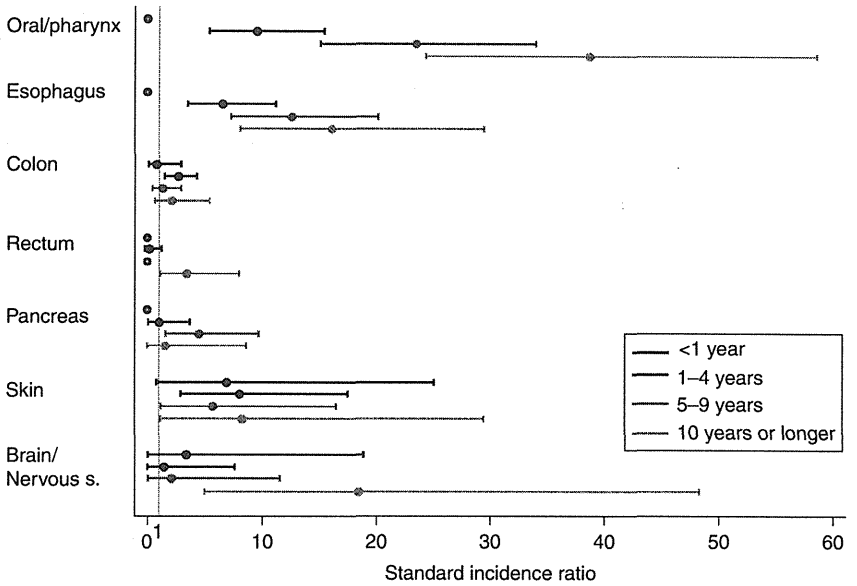

Figure 2. Trends of standard incidence ratios (SIRs) and its 95% confidence intervals (CIs) of high-risk secondary solid cancer sites according to time since transplant. The SIR and 95% CIs for <1, 1–4, 5–9, and 10 years or longer post-transplant were 0.0, 9.5 (5.4–15.4), 23.4 (15.4–34.0), and 38.5 (23.8–58.9) for oral/pharynx cancer, 0.0, 6.5 (3.5–11.2), 12.6 (7.3–20.2), and 16.8 (8.4–30.1) for esophageal cancer, 0.8 (0.1–2.9), 2.7 (1.5–4.3), 1.2 (0.4–2.9), and 2.2 (0.6–5.7) for colon cancer, 0.0, 0.2 (0.0–1.3), 0.0, and 3.6 (1.2–8.4) for rectum cancer, 0.0, 1.0 (0.1–3.7), 4.5 (1.6–9.7), and 1.6 (0.0–8.9) for pancreatic cancer, 7.0 (0.8–25.1), 8.1 (3.0–17.5), 5.7 (1.2–16.7), and 8.4 (1.0–30.3) for skin cancer, and 3.4 (0.1–19.0), 1.4 (0.0–7.7), 2.1 (0.1–11.6), and 19.1 (5.2–49.0) for cancers of brain/nervous system, respectively.

Table 3. Standard incidence ratio according to recipient's age at transplant

	Recipient's age at transplantation											
	16–19		20–29		30–39		40–49		50–59		60 or older	
Number-of-recipients	1399		3506		3787		4167		3549		1137	
Person-years at risk	7083		17 912		17 303		16 198		9126		1843	
Secondary cancer sites	O	SIR	O	SIR	O	SIR	O	SIR	O	SIR	O	SIR
All solid cancers	18	17.0*	28	4.1*	51	2.4*	71	1.4*	79	1.5*	22	1.0
Oral/pharynx	7	140.0*	11	50.7*	19	36.5*	13	10.1*	12	8.1*	2	3.9
Esophagus	1	350.0*	3	131.0*	13	48.5*	10	7.0*	13	5.9*	1	1.1
Stomach	1	13.3	0	0.0	1	0.3	7	0.8	5	0.5	2	0.5
Colon	0	0.0	0	0.0	3	2.0	6	1.3	12	2.1*	6	2.6
Rectum	1	33.1	0	0.0	0	0.0	1	0.3	4	0.9	0	0.0
Liver	1	66.4*	1	8.1	0	0.0	2	0.8	3	0.8	1	0.6
Gallbladder	0	0.0	0	0.0	2	12.0*	1	1.5	2	2.1	1	2.0
Pancreas	0	0.0	0	0.0	2	5.5	1	0.7	4	2.0	2	2.3
Broncus/lung	1	44.3*	0	0.0	2	1.6	7	1.6	7	1.1	2	0.7
Skin	1	28.6	1	6.3	0	0.0	6	11.6*	4	7.4*	1	4.0
Female breast	0	0.0	1	0.7	1	0.2	1	0.1	3	0.5	1	0.9
Cervix uteri	0	0.0	1	1.2	3	1.9	2	1.4	1	1.4	0	0.0
Corpus uteri	0	0.0	1	5.2	0	0.0	2	1.4	2	1.6	0	0.0
Ovary	0	0.0	1	3.2	0	0.0	1	0.7	0	0.0	1	6.4
Prostate	0	0.0	0	0.0	0	0.0	2	2.4	0	0.0	1	0.5
Bladder	0	0.0	0	0.0	0	0.0	2	2.3	2	1.7	0	0.0
Kidney	0	0.0	0	0.0	0	0.0	2	1.4	0	0.0	0	0.0
Brain/nervous system	2	23.9*	1	3.8	1	2.7	1	2.0	1	2.6	1	9.1
Thyroid	0	0.0	2	3.9	0	0.0	1	0.7	1	0.9	0	0.0

*$P < 0.05$.

Table 4. Risk factors for second solid cancers among >1 year survivors after hematopoietic stem cell transplantation

Solid cancer	Risk factor	Number of patients with second cancer	RR	95% CI	*P*-value
All second solid cancers[a]		249			
	Total body irradiation	151	0.9	0.7–1.1	0.294
	Chronic GVHD				
	Limited type	45	1.4	1.0–1.9	0.087
	Extensive type	93	1.8	1.4–2.4	<0.001
	Age at transplant (years)				
	16–29	45	1.0		
	30–39	46	1.6	1.0–2.4	0.042
	40–49	68	2.5	1.7–3.7	<0.001
	50–59	71	5.5	3.7–8.2	<0.001
	60 or older	19	7.9	4.4–14.1	<0.001
Oral cancer[b]		64			
	Total body irradiation	38	1.0	0.8–1.3	0.957
	Chronic GVHD				
	Limited type	10	1.4	0.6–2.9	0.440
	Extensive type	29	2.9	1.6–5.1	<0.001
Esophageal cancer[b]		41			
	Total body irradiation	22	0.6	0.3–1.1	0.108
	Chronic GVHD				
	Limited type	7	2.1	0.8–5.9	0.151
	Extensive type	25	5.3	2.4–11.8	<0.001
Colon cancer[b]		26			
	Total body irradiation	12	0.5	0.2–1.2	0.144
	Chronic GVHD				
	Limited type	6	1.7	0.6–4.9	0.353
	Extensive type	10	1.6	0.6–4.2	0.329
	Grade 2–4 acute GVHD	12	2.0	0.9–4.4	0.101
Skin cancer[b]		13			
	Total body irradiation	12	1.2	0.8–1.6	0.377
	Chronic GVHD				
	Limited type	6	5.8	1.4–23.9	0.016
	Extensive type	2	1.8	0.3–8.9	0.500

RR, relative risk; CI, confidence interval; TBI, total body irradiation; GVHD, graft-versus-host disease.
[a]Stratified for primary disease (acute myeloid leukemia, acute lymphoblastic leukemia, chronic myeloid leukemia, and other).
[b]Stratified for primary disease (acute myeloid leukemia, acute lymphoblastic leukemia, chronic myeloid leukemia, and other) and patient age groups (<19, 20–29, 30–39, 40–49, 50–59, and >60). Adjusted for patient age as a continuous variable.

The incidence of secondary solid tumors in our study was similar to those in previously reported large studies [8, 9, 12, 13]. Rizzo et al. [12] reported that the incidence of secondary solid cancers among 28 874 transplant recipients and 85 583 person-years at risk was 1% at 10 years and 2.2% at 15 years, which were very similar to our results using the same statistical method for cumulative incidence, while treating death before secondary solid tumor as a competing risk. Majhail et al. [13] reported that the incidence of secondary solid cancers after HSCT using non-TBI, busulfan-cyclophosphamide conditioning was also ~1.2% at 10 years. The oral cavity was the most prominent high-risk cancer site compared with the general population, as in previous reports [8, 9, 12, 13]. Despite regional and racial differences in cancer incidence and cancer sites in the general population, the impact of HSCT on secondary cancer was similar.

In previous studies, TBI was reported to be a significant risk factor for the development of secondary cancer, but significant differences were not found in our study [7, 8, 10, 12, 23]. The subjects in this study were adult recipients, which may explain the different findings. Conditioning with radiation was reported to be associated with the development of secondary solid cancer in recipients at a younger age at transplant [12]. Moreover, a recent long-term follow-up analysis of patients who were transplanted after myeloablative doses of busulfan and cyclophosphamide without TBI found a similar increased incidence of secondary solid cancers as previous reports [13].

An older recipient age at transplant was a significant risk factor for the development of secondary solid tumor, as in previous studies [9, 13]. This result was not surprising since it is also the case in the general population. However, it is important to note that older patients are at higher risk of developing

secondary cancer and to promote patient education and preventive practices, since there has been a dramatic increase in the number of transplant recipients who are more than 50 years of age at transplant over the past decade. In comparison with the general population, younger patients were at a higher risk of developing a solid tumor. Several high-risk cancer sites (esophagus, liver, and bronchus/lung) in younger group did have only one observed cases, therefore, these results should not be emphasized and need to be confirmed in other studies. These sites were found to be significant because the expected numbers in general population for these sites were extremely small.

Although this study included a large number of recipients and a large number of person-years of follow-up, there are limitations. The follow-up years for older recipients were still limited, and therefore we may find a higher incidence of and risk of secondary solid cancers among recipients who are 50 years of age or older at transplant in the future. Second limitation involves possible under-reporting by recipients to transplant centers or by transplant centers to the registry. Until recently, transplant recipients have received only limited information regarding screening or the prevention of secondary solid cancers. Another limitation of this analysis was lack of central pathology review for secondary solid tumors. JSHCT data collection does not include the submission of specimen or pathology report. Since this study included transplants from 1990, central pathology review was difficult to perform at the time of analyses. In addition, limiting secondary tumors to centrally diagnosed tumors would decrease the number of identified secondary tumors; therefore, secondary solid tumors were identified as reported from transplant centers.

In conclusion, recipients of allogeneic hematopoietic stem cell transplant had a significantly higher risk of developing secondary solid cancers than the general population. Older recipients are at higher risk of developing secondary solid tumors, as in the general population. Lifelong screening is important for high-risk organ sites, especially for oral, pharynx, and esophageal cancers in recipients with active, or a history of, chronic GVHD.

acknowledgements

The authors are grateful for the assistance and cooperation of the staff members of the collaborating institutes of the Japan Cord Blood Bank Network, the Japan Marrow Donor Program, and the Japan Society for Hematopoietic Cell Transplantation.

funding

This work was supported by Grants-in-Aid for Scientific Research for Young Scientists B (19790714 and 23791077) from the Japanese Ministry of Education, Culture, Sports, Science and Technology.

disclosure

The authors have declared no conflicts of interest.

references

1. Copelan EA. Hematopoietic stem-cell transplantation. N Engl J Med 2006; 354: 1813–1826.
2. Gratwohl A, Baldomero H, Aljurf M et al. Hematopoietic stem cell transplantation: a global perspective. JAMA 2010; 303: 1617–1624.
3. Yoshimi A, Suzuki R, Atsuta Y et al. Hematopoietic SCT activity in Asia: a report from the Asia-Pacific Blood and Marrow Transplantation Group. Bone Marrow Transplant 2010; 45: 1682–1691.
4. Pasquini MC, Wang Z, Horowitz MM et al. 2010 report from the Center for International Blood and Marrow Transplant Research (CIBMTR): current uses and outcomes of hematopoietic cell transplants for blood and bone marrow disorders. Clin Transpl 2010; 87–105.
5. Passweg JR, Baldomero H, Gratwohl A et al. The EBMT activity survey: 1990–2010. Bone Marrow Transplant 2012; 47: 906–923.
6. Ahmed SO, Ghavamzadeh A, Zaidi SZ et al. Trends of hematopoietic stem cell transplantation in the Eastern Mediterranean region, 1984–2007. Biol Blood Marrow Transplant 2011; 17: 1352–1361.
7. Bhatia S, Ramsay NK, Steinbuch M et al. Malignant neoplasms following bone marrow transplantation. Blood 1996; 87: 3633–3639.
8. Curtis RE, Rowlings PA, Deeg HJ et al. Solid cancers after bone marrow transplantation. N Engl J Med 1997; 336: 897–904.
9. Kolb HJ, Socie G, Duell T et al. Malignant neoplasms in long-term survivors of bone marrow transplantation. Late Effects Working Party of the European Cooperative Group for Blood and Marrow Transplantation and the European Late Effect Project Group. Ann Intern Med 1999; 131: 738–744.
10. Bhatia S, Louie AD, Bhatia R et al. Solid cancers after bone marrow transplantation. J Clin Oncol 2001; 19: 464–471.
11. Shimada K, Yokozawa T, Atsuta Y et al. Solid tumors after hematopoietic stem cell transplantation in Japan: incidence, risk factors and prognosis. Bone Marrow Transplant 2005; 36: 115–121.
12. Rizzo JD, Curtis RE, Socie G et al. Solid cancers after allogeneic hematopoietic cell transplantation. Blood 2009; 113: 1175–1183.
13. Majhail NS, Brazauskas R, Rizzo JD et al. Secondary solid cancers after allogeneic hematopoietic cell transplantation using busulfan-cyclophosphamide conditioning. Blood 2011; 117: 316–322.
14. Yokota A, Ozawa S, Masanori T et al. Secondary solid tumors after allogeneic hematopoietic SCT in Japan. Bone Marrow Transplant 2012; 47: 95–100.
15. Curtis RE, Metayer C, Rizzo JD et al. Impact of chronic GVHD therapy on the development of squamous-cell cancers after hematopoietic stem-cell transplantation: an international case-control study. Blood 2005; 105: 3802–3811.
16. Leisenring W, Friedman DL, Flowers ME et al. Nonmelanoma skin and mucosal cancers after hematopoietic cell transplantation. J Clin Oncol 2006; 24: 1119–1126.
17. Friedman DL, Rovo A, Leisenring W et al. Increased risk of breast cancer among survivors of allogeneic hematopoietic cell transplantation: a report from the FHCRC and the EBMT-Late Effect Working Party. Blood 2008; 111: 939–944.
18. Atsuta Y, Suzuki R, Yoshimi A et al. Unification of hematopoietic stem cell transplantation registries in Japan and establishment of the TRUMP System. Int J Hematol 2007; 86: 269–274.
19. Gooley TA, Leisenring W, Crowley J et al. Estimation of failure probabilities in the presence of competing risks: new representations of old estimators. Stat Med 1999; 18: 695–706.
20. Cox DR. Regression model and life tables. J R Stat Soc B 1972; 34: 187–200.
21. Majhail NS, Rizzo JD, Lee SJ et al. Recommended screening and preventive practices for long-term survivors after hematopoietic cell transplantation. Bone Marrow Transplant 2012; 47: 337–341.
22. Majhail NS, Rizzo JD, Lee SJ et al. Recommended screening and preventive practices for long-term survivors after hematopoietic cell transplantation. Biol Blood Marrow Transplant 2012; 18: 348–371.
23. Baker KS, DeFor TE, Burns LJ et al. New malignancies after blood or marrow stem-cell transplantation in children and adults: incidence and risk factors. J Clin Oncol 2003; 21: 1352–1358.

Different effects of HLA disparity on transplant outcomes after single-unit cord blood transplantation between pediatric and adult patients with leukemia

Yoshiko Atsuta,[1] Junya Kanda,[2] Minoko Takanashi,[3] Yasuo Morishima,[4] Shuichi Taniguchi,[5] Satoshi Takahashi,[6] Hiroyasu Ogawa,[7] Kazuteru Ohashi,[8] Yuju Ohno,[9] Yasushi Onishi,[10] Nobuyuki Aotsuka,[11] Tokiko Nagamura-Inoue,[12] Koji Kato,[13] and Yoshinobu Kanda,[2] on behalf of the HLA Working Group of the Japan Society for Hematopoietic Cell Transplantation

[1]Department of Hematopoietic Stem Cell Transplantation Data Management / Biostatistics, Nagoya University Graduate School of Medicine, Nagoya; [2]Division of Hematology, Saitama Medical Center, Jichi Medical University, Saitama; [3]The Japanese Red Cross Tokyo Blood Center, Tokyo; [4]Division of Epidemiology and Prevention, Aichi Cancer Center Research Institute, Nagoya; [5]Department of Hematology, Toranomon Hospital, Tokyo; [6]Department of Molecular Therapy, The Institute of Medical Science, The University of Tokyo, Tokyo; [7]Division of Hematology, Department of Internal Medicine, Hyogo College of Medicine, Hyogo; [8]Hematology Division, Tokyo Metropolitan Cancer and Infectious Diseases Center, Komagome Hospital, Tokyo; [9]Department of Internal Medicine, Kitakyushu Municipal Medical Center, Kitakyushu; [10]Department of Hematology and Rheumatology, Tohoku University Hospital, Sendai; [11]Department of Hematology and Oncology, Japanese Red Cross Narita Hospital, Narita; [12]Department of Cell Processing and Transfusion, Research Hospital, The Institute of Medical Science, The University of Tokyo, and Tokyo Cord Blood Bank, Tokyo; and [13]Department of Pediatrics, Japanese Red Cross Nagoya First Hospital, Nagoya, Japan

ABSTRACT

Recent advances in unrelated cord blood transplantation have increased chances and options available in allogeneic stem cell transplantation. The effect of HLA disparity on outcomes after cord blood transplantation was studied recently in mainly pediatric populations. Results showed that HLA matching in combination with total nucleated cell dose positively affects survival. The effect of HLA disparity after single-unit cord blood transplantation may be different in adults because their total nucleated cell dose is much lower compared to pediatric patients. We investigated the effect of HLA disparity on the outcome of single-unit unrelated cord blood transplantation separately in 498 children aged 15 years or under (HLA-A, HLA-B low-resolution, and HLA-DRB1 high-resolution matched [6/6], n=82, and one locus- [5/6], n=222, two loci- [4/6], n=158, three loci- [3/6] mismatched, n=36) and 1,880 adults (6/6, n=71; 5/6, n=309; 4/6, n=1,025; 3/6, n=475) with leukemia. With adjusted analyses, in children, 4/6 showed significantly increased risks of overall mortality (relative risk [RR]=1.61, P=0.042) and transplant-related mortality (RR=3.55, P=0.005) compared to 6/6. The risk of grade 2 to 4 acute GVHD was increased in 5/6 (RR=2.13, P=0.004) and 4/6 (RR=2.65, P<0.001). In adults, the risk of mortality did not increase with the number of mismatched loci (RR=0.99, P=0.944 for 5/6; RR=0.88, P=0.436 for 4/6). The risk of relapse was significantly decreased in 4/6 (RR=0.67, P=0.034). The risk of transplant-related mortality (TRM) or acute GVHD was not increased in 5/6 or 4/6. The effect of HLA disparity on transplant outcome differed between children and adults. In children, an increased number of mismatched HLA loci correlated with an increased risk of mortality. In adults, there was no increase in mortality with an increase in the number of mismatched HLA loci.

Introduction

Recent advances in unrelated cord blood transplantation (UCBT) have provided increased opportunities for patients with hematologic malignancies to receive hematopoietic stem cell transplantation (HSCT). This has led to an increased number of UCBT procedures over the past decade.[1,2] Clinical comparison studies of cord blood and bone marrow from unrelated donors have shown comparable results, which indicates that cord blood is a reasonable alternative donor / stem cell source.[3-12] These studies support the use of HLA-A, HLA-B, low-resolution and HLA-DRB1 zero- to two-loci-mismatched UCB for patients with leukemia in the absence of an HLA-A, HLA-B, HLA-C, and HLA-DRB1 allele matched unrelated adult donor, and the use of UCB as a first-line option when a transplant is urgently required.

The effect of HLA mismatches after bone marrow transplantation from unrelated donors (UBMT) has been well studied, and HLA-A, HLA-B, HLA-C, and HLA-DRB1 allele matched bone marrow is currently the first alternative for HLA-identical sibling donors.[13-16] An increase in the number of HLA mismatches, antigen-level, or high-resolution, at HLA-A, HLA-B, HLA-C, or HLA-DRB1 loci from 8/8 to 7/8, or 7/8 to 6/8 was associated with higher mortality with an approximately 10% reduction in survival in UBM recipients.[12,13,15] Since HLA mismatches are better tolerated after UCB with a lower incidence of severe graft-*versus*-host disease (GVHD), up to two HLA antigen mismatches of HLA-A, HLA-B, low resolution and HLA-DRB1 high resolution are considered in the current CB selection algorithm. Several reports have recently described the effect of HLA disparity on the transplant outcomes after UCBT.[9,17,18] Eapen *et al.* reported the pos-

 doi:10.3324/haematol.2012.076042
The online version of this article has a Supplementary Appendix.
Manuscript received on August 17, 2012. Manuscript accepted on January 9, 2013.
Correspondence: y-atsuta@med.nagoya-u.ac.jp

sibility of a better outcome in HLA 6/6 matched UCB in 35 recipients, and Barker *et al.* confirmed these results with a larger number of UCB recipients.[9,18] However, these studies, which assessed the effect of HLA disparity on the outcome of single-unit CBT, were mainly conducted in pediatric populations in which the infused cell dose is much greater than that in adult recipients.

The aim of this study was to assess the effect of HLA disparity on the transplant outcomes after single-unit UCBT in pediatric and adult recipients. The accumulation of single-unit CBT in adult recipients has enabled us to assess separately the effect of HLA disparity on CBT outcomes in children and adults.

Design and Methods

Study design and data source

For this retrospective observational study, recipients' clinical data were provided by the Japan Cord Blood Bank Network (JCBBN). All 11 cord blood banks in Japan are affiliated with the JCBBN. JCBBN collected the recipients' clinical information at 100 days post-transplant through the Transplant Registry Unified Management Program (TRUMP) of the Japan Society of Hematopoietic Cell Transplantation (JSHCT).[19] Information on survival, disease status, and long-term complications including chronic graft-*versus*-host disease and second malignancies is renewed annually. Patient consent is not required for TRUMP registration of the JSHCT for the registry data consists of anonymized clinical information. This study was approved by the data management committees of the JSHCT and the JCBBN, and by the institutional review boards of Saitama Medical Center, Jichi Medical University and Nagoya University Graduate School of Medicine, Japan.

Patients

The subjects were patients with acute myeloid leukemia (AML), acute lymphoblastic leukemia (ALL), chronic myeloid leukemia (CML), or myelodysplastic syndrome (MDS), who were recipients of their first UCBT between January 2000 and December 2009. Among 2,461 recipients of single-unit UCB with complete HLA-A, HLA-B, low-resolution and HLA-DRB1 high-resolution data, 51 recipients with 4 HLA mismatches were excluded. Thirty recipients who did not receive GVHD prophylaxis and 2 recipients for whom information regarding the conditioning regimen was missing were excluded. A total of 2378 single-unit UCB recipients (498 children aged 15 years or under at transplant, and 1880 adults aged 16 years or over at transplant) were subjects for analysis.

HLA typing

Histocompatibility data for low-resolution typing for the HLA-A, HLA-B, and HLA-DR loci and high-resolution typing for HLA-DRB1 were obtained from the TRUMP database which includes HLA information provided by cord blood banks or transplant centers. The level of HLA typing in the present study was HLA-A, HLA-B, low-resolution, and HLA-DRB1 high-resolution, as in other studies in Europe and North America. However, according to current practice in Japan, mismatches in HLA-DR loci were counted at the low-resolution level at UCB unit selection. Therefore, results regarding the effect of HLA mismatches in HLA-A, HLA-B, and HLA-DR low-resolution are also provided (*Online Supplementary Table S1*). Analyses from the Japan Marrow Donor Program (JMDP) showed better survival in HLA class II mismatched recipients compared to HLA class I mismatched recipients. Thus, in Japan, a single-DRB1-mismatched UBM donor is preferred over a single-A-mismatched UBM or single-B-mismatched UBM donor.[15,20] This background affected HLA typing strategy of HLA-DR low-resolution typing instead of high-resolution typing for selection of cord blood units in Japan. This observation may explain the fact that the frequency of 4/6 grafts is higher in this cohort than in cohorts in Europe and the USA.

Definitions

The primary outcome of the analyses was overall survival, defined as time from transplant to death from any cause. Several secondary end points were also analyzed. Neutrophil recovery was defined as an absolute neutrophil count of at least 0.5x10^9/L cells per cubic millimeter for three consecutive points; platelet recovery was defined as a count of at least 50x10^9 platelets per cubic millimeter without transfusion support. The recipients of reduced-intensity conditioning were also defined with the criteria above, according to the previous report that confirmed complete donor chimeras of all engrafted patients after CBT with reduced-intensity conditioning.[21] Diagnosis and clinical grading of acute GVHD were performed according to the established criteria.[22,23] Relapse was defined as the recurrence of underlying hematologic malignant diseases. Transplant-related death was defined as death during a continuous remission.

Statistical analysis

Descriptive statistical analysis was performed to assess patient baseline characteristics, diagnosis, disease status at conditioning, donor-patient ABO mismatches, preparative regimen, and GVHD prophylaxis. Medians and ranges are provided for continuous variables and percentages are shown for categorical variables. Cumulative incidence curves were used in a competing-risks setting to calculate the probability of acute and chronic GVHD, relapse and transplant-related mortality (TRM).[24] Gray's test was used for group comparisons of cumulative incidences.[25] An adjusted comparison of the groups with regard to overall survival (OS) was performed with the use of the Cox's proportional-hazards regression model.[26] For other outcomes with competing risks, Fine and Gray's proportional-hazards model for the subdistribution of a competing risk was used.[27] For neutrophil and platelet recovery, death before neutrophil or platelet recovery was the competing event. For GVHD, death without GVHD and relapse were competing events. For relapse, death without relapse was the competing event, and for transplant-related mortality (TRM), relapse was the competing event.[28] For acute GVHD, subjects were limited to those who engrafted, and for chronic GVHD, subjects were limited to those who engrafted and survived at least 100 days after transplantation.

The variables considered were the patient's age at transplant (5 years or over *vs.* under 5 years for pediatric recipients, and 50 years or over *vs.* under 50 years for adult recipients; cut-off points were around the median in each group), patient's sex, donor-patient sex mismatch (matched *vs.* male to female *vs.* female to male), donor-patient ABO mismatch (major mismatch *vs.* matched or minor mismatch), diagnosis (AML, ALL, CML or MDS), disease status at conditioning (first or second complete remission (CR) of AML, 1CR of ALL, first chronic phase of CML, and refractory anemia or refractory anemia with ringed sideroblasts as standard-risk diseases *vs.* advanced for all others), the conditioning regimen (reduced-intensity conditioning *vs.* myeloablative conditioning), and the type of prophylaxis against GVHD (tacrolimus-based *vs.* cyclosporine-based). Conditioning regimens were classified as myeloablative if total-body irradiation >8 Gy, oral busulfan ≥9 mg/kg, intravenous busulfan ≥7.2 mg/kg, or melphalan >140 mg/m^2 was used based on the report from the Center for International Blood and Marrow Transplant Research.[29,30] We cat-

egorized patients for whom there was insufficient information regarding the doses of agents or radiation used for the conditioning regimen according to information on the conditioning intensity (i.e. whether or not the conditioning regimen was intended to be myeloablative) as reported by the treating clinicians. The cryopreserved total nucleated cell dose was categorized as >10.0x10^7/kg, 5.0-9.9 x 10^7/kg, 2.5-4.9x10^7/kg, or <2.5 x 10^7/kg for children, and >3.0x10^7/kg, 2.5-2.9x10^7/kg, 2.0-2.4x10^7/kg, or <2.0x10^7/kg for adults. HLA disparity and nucleated cell dose were maintained in the model. Since patient age was highly correlated with the total nucleated cell dose in children, age was excluded from multivariate analyses for pediatric recipients. Other variables were selected in a backward stepwise manner with a variable retention criterion of P<0.05. Interaction between HLA disparity and adult (patient age at transplant 16 years or over) or child (patient age at transplant 15 years or under) was tested for overall survival by using a Cox's proportional-hazards regression model adjusted by other significant covariates in the final model for adult and pediatric recipients except for patient age. All P values were two-sided.

Results

Patients' characteristics

Table 1 shows patients' characteristics, their disease, and transplant regimens. Median age at transplant was five years (range 0-15) in 498 pediatric and 49 years (range 16-82) in 1880 adult recipients of single-unit CBT. The proportion of females was 45% in both children and adults. Among children, the proportion of patients with ALL was greatest (58%) followed by that of patients with AML (34%). Among adults, the most frequent disease was AML (59%), followed by ALL (22%) and MDS (13%). The median number of cryopreserved total nucleated cells received in children was 5.30 x 10^7/kg, which was significantly greater (approximately double) than the number of nucleated cells received in adult patients (2.52 x 10^7/kg). In adults, only 33 patients (2%) received CB with a total nucleated cell dose greater than or equal to 5.0 x 10^7/kg. In children, 82 patients (16%) received HLA-matched (6/6) UCB, 222 (45%) received one-locus-mismatched (5/6), 158 (32%) received two-loci-mismatched (4/6), and 36 (7%) received three-loci-mismatched (3/6) UCB. For adults, the numbers and proportions of recipients were 71 (4%) for 6/6, 309 (16%) for 5/6, 1025 (55%) for 4/6, and 475 (25%) for 3/6. Among those who received 3/6 UCB, only 2 pediatric and 11 adult patients received three HLA-A, HLA-B, HLA-DR low-resolution mismatched UCB. Eighty-eight percent (TBI regimen 62%, non-TBI regimen 26%) and 62% (TBI regimen 56%, non-TBI regimen 6%) of children and adults, respectively, received myeloablative conditioning. Fludarabine-based reduced-intensity conditioning was given to 34% of adult recipients. T-cell depletion *in vivo* with antithymocyte globulin or antilymphocyte globulin was performed in only 6 (2%) child recipients and 26 (1%) adult recipients. The median follow-up period for survivors was 2.4 years (range 0.1-9.5) for pediatric recipients and 2.1 (range 0.1-9.0) years for adult recipients.

Outcome

Overall survival, relapse, and transplant-related mortality: among children, overall mortality in 4/6 UCB recipients was significantly higher than that in 6/6 UCB recipients (RR=1.61, 95% confidence interval [CI], 1.02-2.56, P=0.042) (Table 2). Overall mortality increased with the number of mismatched loci in children (P for trend 0.043). The increased mortality in 4/6 UCB recipients was mainly affected by increased transplant-related mortality (TRM) (RR=3.55, 95%CI: 1.47-8.58, P=0.005) (P for trend 0.002) but not by the risk of relapse (RR=0.77, 95%CI: 0.48-1.24, P=0.392) in children. Among children, there were no differences in the risks of mortality and relapse between 5/6 UCB recipients (RR=1.07, P=0.765 for overall mortality; RR=1.06, P=0.794 for relapse; and RR=1.29, P=0.58 for TRM) and 6/6 UCB recipients (Table 2).

In adults, the number of HLA mismatches was not significantly associated with increased mortality (for overall mortality: RR=0.99, P=0.944 for 5/6; RR=0.88, P=0.436 for 4/6; RR=0.95, P=0.751 for 3/6; for TRM, RR=1.41, P=0.205 for 5/6; RR=1.24, P=0.408 for 4/6; RR=1.29, P=0.339 for 3/6). A two-loci mismatch was associated with a decreased risk of relapse in adult recipients (RR=0.70, P=0.075 for 5/6; RR=0.67, P=0.034 for 4/6; RR=0.70, P=0.07 for 3/6) (Table 2). The risks of mortality were similar when subjects were limited to those with standard risk disease status or to those with advanced risk disease status at transplant, to those who received myeloablative conditioning or to those who received reduced-intensity conditioning (*Online Supplementary Table S2*). A decreased risk of relapse was more prominent in patients with acute myeloid leukemia, and those who received reduced-intensity conditioning (*Online Supplementary Table S2*).)

Figure 1 shows unadjusted overall survival curves in children and adults. In children, the unadjusted probabilities of survival at three years post-transplant were 66% for 6/6, 62% for 5/6, 45% for 4/6, and 62% for 3/6 (P=0.032) (Figure 1A). In adults, the survival probabilities in all of the HLA disparity groups were similar (38% for 6/6, 37% for 5/6, 39% for 4/6, and 40% for 3/6 at three years post-transplant, P=0.567) (Figure 1B). A similar trend was seen when subjects were limited to standard-risk disease status at transplant (81% for 6/6, 76% for 5/6, 57% for 4/6, and 81% for 3/6 at three years post-transplant, P=0.035, for children; 51% for 6/6, 57% for 5/6, 58% for 4/6, and 55% for 3/6 at three years post-transplant, P=0.375, for adults) (*Online Supplementary Figure S1*).

A test of the interaction between HLA disparity and age (adult *vs.* child) revealed that the effect of HLA disparity on overall survival differed significantly between the pediatric and adult patient groups (P=0.009 for HLA disparity of 0-1 mismatches *vs.* 2-3 mismatches).

Hematologic recovery

The cryopreserved total nucleated cell dose significantly affected neutrophil and platelet recovery in children and neutrophil recovery in adults (Table 3). HLA disparity did not significantly affect neutrophil or platelet recovery in adults or children for neutrophil recovery: RR=1.03, P=0.823 for 5/6; RR=0.96, P=0.799 for 4/6; RR=0.67, P=0.068 for 3/6 in children; RR=0.89, P=0.436 for 5/6; RR=0.92, P=0.576 for 4/6; RR=0.84, P=0.243 for 3/6 in adults; for platelet recovery: RR=0.89, P=0.438 for 5/6; RR=0.75, P=0.09 for 4/6; RR=0.71, P=0.164 for 3/6 in children; RR=1.05, P=0.775 for 5/6; RR=1.05, P=0.791 for 4/6; RR=0.99, P=0.951 in 3/6 in adults (Table 3).

Table 1. Patients', disease, and transplant characteristics of pediatric and adult recipients of single-unit cord blood.

Characteristics	Children (age<16) N.	(%)	Adult (age>16) N.	(%)
N. of transplants	498		1880	
Patient age at transplant				
Median (range)	5 (0-15)		49 (16-82)	
0-9 years	378	(76)		
10-19 years	120	(24)	88	(5)
20-29 years			236	(13)
30-39 years			317	(17)
40-49 years			351	(19)
50-59 years			492	(26)
≥60 years or older			396	(21)
Patient sex				
Male	275	(55)	1039	(55)
Female	223	(45)	841	(45)
Sex matching				
Matched	207	(42)	696	(37)
Male to female	114	(23)	391	(21)
Female to male	125	(25)	485	(26)
Unknown	52	(10)	308	(16)
Diagnosis				
AML	170	(34)	1115	(59)
ALL	290	(58)	418	(22)
CML	7	(1)	106	(6)
MDS	31	(6)	241	(13)
Disease status				
Standard	247	(50)	673	(36)
Advanced	236	(47)	1127	(60)
Unknown	15	(3)	80	(4)
ABO matching				
Matched	182	(37)	602	(32)
Minor mismatch	127	(26)	522	(28)
Major mismatch	113	(23)	451	(24)
Bidirectional	75	(15)	301	(16)
Unknown	1	(<1)	4	(<1)
HLA mismatched number				
Matched (6/6)	82	(16)	71	(4)
One locus mismatched (5/6)	222	(45)	309	(16)
Two loci mismatched (4/6)	158	(32)	1025	(55)
Three loci mismatched (3/6)	36	(7)	475	(25)
N. of cryopreserved nucleated cells (x10⁷/kg)				
Median	5.30		2.52	
Range	0.81-38.7		0.71-9.98	
N. of cryopreserved CD34-positive cells (x10⁵/kg)				
Median	1.68		0.83	
Range	0.072-65.66		0.07-14.02	
Preparative regimen*				
MAST				
CY+TBI	216	(43)	891	(47)
Other TBI regimen	93	(19)	162	(9)
BU+CY	86	(17)	65	(3)
Other non-TBI regimen	41	(8)	47	(3)
RIST				
FL+BU+other	6	(1)	172	(9)
FL+CY+other	12	(2)	119	(6)
FL+Mel+other	21	(4)	357	(19)
Other RIST	23	(5)	67	(4)
T-cell depletion *in vivo***				
ATG or ALG use	9	(2)	26	(1)
GVHD prophylaxis***				
Cyclosporine A + sMTX	157	(32)	748	(40)
Cyclosporine A + MMF/steroid	37	(7)	99	(5)
Cyclosporine A alone	31	(6)	142	(8)
Tacrolimus + sMTX	216	(43)	434	(23)
Tacrolimus + MMF/steroid	24	(5)	132	(7)
Tacrolimus alone	20	(4)	304	(16)
Others	13	(3)	21	(1)

continued on the next page

continued from the previous page

**CY: cyclophosphamide; CA: citarabine; BU: busulfan; TBI: total body irradiation; FL: fludarabine; Mel: melphalan, **ATG: antithymocyte globulin; ALG: antilymphocyte globulin; ***sMTX: short-term methotrexate; MMF: mycophenolate mofetil.*

Acute and chronic graft-versus-host disease

The risk of grade 2 to 4 acute GVHD was significantly higher in HLA-mismatched UCB pediatric recipients (RR=2.13, *P*=0.004 for 5/6; RR=2.65, *P*<0.001 for 4/6; RR=2.39, *P*=0.0015 for 3/6; P for trend 0.001) (Table 4). The risk of chronic GVHD and extensive-type chronic GVHD was also significantly higher in 4/6 UCB recipients (RR=2.99, *P*=0.005 for chronic GVHD, and RR=7.62, *P*=0.047 for extensive-type chronic GVHD), and the risks increased according to the number of mismatches (P for trend, 0.002 for chronic GVHD, 0.005 for extensive-type chronic GVHD). In adults, in contrast to the results in children, there were no differences in the risks of grade 2 to 4 acute GVHD in 5/6 and 4/6 UCB recipients (for grade 2 to 4 acute GVHD, RR=1.03, *P*=0.916 for 5/6, RR=1.27, *P*=0.276 for 4/6). The risk of grade 2 to 4 acute GVHD was higher for 3/6 (RR=1.72, *P*=0.017). In adult recipients, the risk of chronic GVHD was increased in recipients of 4/6 UCB (RR=1.90, *P*=0.04), however, there were no differences in the risk of extensive-type chronic GVHD (RR=1.15, *P*=0.758 for 5/6; RR=1.62, *P*=0.253 for 4/6; RR=1.28, *P*=0.574 for 3/6) (Table 4).

Effect of total nucleated cell dose on outcome

An increase in the cryopreserved total nucleated cell dose increased the incidence of neutrophil recovery in both children and adults, as well as the incidence of platelet recovery in children (Table 3). The cumulative incidences of neutrophil recovery were 94% for >10 x 10^7/kg, 88% for 5.0-9.9 x 10^7/kg, 82% for 2.5-4.9 x 10^7/kg, and 86% for <2.5 x 10^7/kg in children (*P*<0.001) (Figure 2A). The cell dose was significantly correlated with the recipient's age at transplant in children (the median ages were one year for >10 x 10^7/kg, 3 years for 5.0-9.9 x 10^7/kg, 8 years for 2.5-4.9 x 10^7/kg, and 12 years for <2.5 x 10^7/kg). The cumulative incidences of neutrophil recovery were 76% for >2.5 x 10^7/kg and 74% for <2.5 x 10^7/kg in adults (*P*=0.007) (Figure 2B). The cumulative incidences of TRM at three years post-transplant were 13% for >10 x 10^7/kg, 14% for 5.0-9.9 x 10^7/kg, 14% for 2.5-4.9 x 10^7/kg, and 14% for <2.5x10^7/kg in children (*P*=0.98) and 29% for >2.5x10^7/kg and 28% for <2.5 x 10^7/kg in adults (*P*=0.77) (*Online Supplementary Figure S2*). The probabilities of overall survival at three years post-transplant were 68% for >10x10^7/kg, 53% for 5.0-9.9 x 10^7/kg, 57% for 2.5-4.9 x 10^7/kg, and 55% for <2.5x10^7/kg in children (*P*=0.30) and 36% for >2.5 x 107/kg and 41% for <2.5x10^7/kg in adults (*P*=0.13). A lower total nucleated cell dose was neither associated with increased mortality in children or adults in multivariate analyses (Table 2). Thus, there was no combined effect of HLA disparity and total nucleated cell dose on mortality neither in children nor in adults (cumulative

incidence of TRM at three years post-transplant, 8% for 6/6, 11% for 5/6 and >5 x 10^7/kg, 11% for 5/6 and 2.5-4.9 x 10^7/kg, 0% for 5/6 and <2.5 x 10^7/kg, 23% for 4/6 and >5 x 10^7/kg, 24% for 4/6 and 2.5-4.9 x 10^7/kg, 25% for 4/6 and <2.5 x 10^7/kg in children, and 23% for 6/6, 29% for 5/6 and >2.5 x 107/kg, 30% for 5/6 and <2.5 x 10^7/kg, 27% for 4/6 and >2.5 x 10^7/kg, 27% for 4/6 and <2.5 x 10^7/kg in adults (*Online Supplementary Figure S3*).

Association of outcomes with the type of HLA mismatches for 4/6 adult recipients

The large number of adult recipients of 4/6 CB enabled us to analyze association of outcomes with the type of HLA mismatches in this population. The number of recipients were 7 for HLA-A double mismatch, 170 for HLA-A and HLA-B mismatch, 190 for HLA-A and HLA-DRB1 mismatch, 36 for HLA-B double mismatch, 581 for HLA-B and HLA-DRB1 mismatch, and 41 for HLA-DRB1 double mismatch. With adjusted analyses, adjusted with same variables in the final model of all adult recipients, there was no significant effect of HLA mismatch types on overall mortality with HLA-A and HLA-B mismatch as the reference (*Online Supplementary Table S3*). The risk of relapse was significantly decreased in HLA-A and HLA-DRB1

Table 2. Multivariate analyses of overall survival, relapse, and transplant-related mortality.

Outcome	N.	Overall mortality RR	95%CI	P	Relapse RR	95%CI	P	Transplant-related mortality RR	95%CI	P
Children 15 years or younger										
HLA disparity										
Matched (6/6)	82	1.00			1.00			1.00		
5/6	222	1.07	(0.68-1.69)	0.765	1.06	(0.68-1.65)	0.794	1.29	(0.52-3.23)	0.58
4/6	158	1.61	(1.02-2.56)	0.042	0.77	(0.48-1.24)	0.282	3.55	(1.47-8.58)	0.005
3/6	36	1.25	(0.65-2.42)	0.498	0.91	(0.45-1.86)	0.802	1.56	(0.43-5.63)	0.497
Total nucleated cell dose										
≥10.0x10^7/kg	85	1.00			1.00			1.00		
5.0-9.9x10^7/kg	169	1.14	(0.72-1.79)	0.579	1.10	(0.69-1.75)	0.684	0.82	(0.40-1.68)	0.592
2.5-4.9x10^7/kg	190	0.92	(0.58-1.45)	0.707	0.90	(0.56-1.44)	0.651	0.90	(0.45-1.80)	0.77
<2.5x10^7/kg	43	0.88	(0.47-1.67)	0.701	0.98	(0.53-1.83)	0.961	0.67	(0.24-1.88)	0.443
Adults 16 years or older										
HLA disparity										
Matched (6/6)	71	1.00			1.00			1.00		
5/6	309	0.99	(0.71-1.38)	0.944	0.70	(0.47-1.04)	0.075	1.41	(0.83-2.41)	0.205
4/6	1025	0.88	(0.65-1.21)	0.436	0.67	(0.47-0.97)	0.034	1.24	(0.75-2.04)	0.408
3/6	475	0.95	(0.69-1.31)	0.751	0.70	(0.48-1.03)	0.07	1.29	(0.77-2.16)	0.339
Total nucleated cell dose										
≥3.0x10^7/kg	439	1.00			1.00			1.00		
2.5-2.9x10^7/kg	492	0.99	(0.83-1.17)	0.876	0.86	(0.70-1.06)	0.167	1.10	(0.86-1.42)	0.445
2.0-2.4x10^7/kg	705	0.86	(0.72-1.01)	0.06	0.79	(0.65-0.97)	0.021	1.05	(0.83-1.33)	0.694
<2.0x10^7/kg	183	0.93	(0.73-1.18)	0.562	0.79	(0.59-1.07)	0.126	1.00	(0.70-1.45)	0.983

For overall mortality, other predictive variables were advanced disease status at transplant in children, and age at transplant over 50 years, male sex, advanced disease status at transplant, chronic myeloid leukemia (associated with a lower risk of mortality), and reduced-intensity conditioning in adults. For relapse, other predictive variables were advanced disease status at transplant, and acute lymphoblastic leukemia or myelodysplastic syndrome (associated with a lower risk of relapse) in children, and advanced disease status at transplant and myelodysplastic syndrome (associated with a lower risk of relapse) in adults. For transplant-related mortality, there was no other predictive variable in children. Other predictive variables for adults were age at transplant over 50 years and female to male donor-recipient sex mismatch.

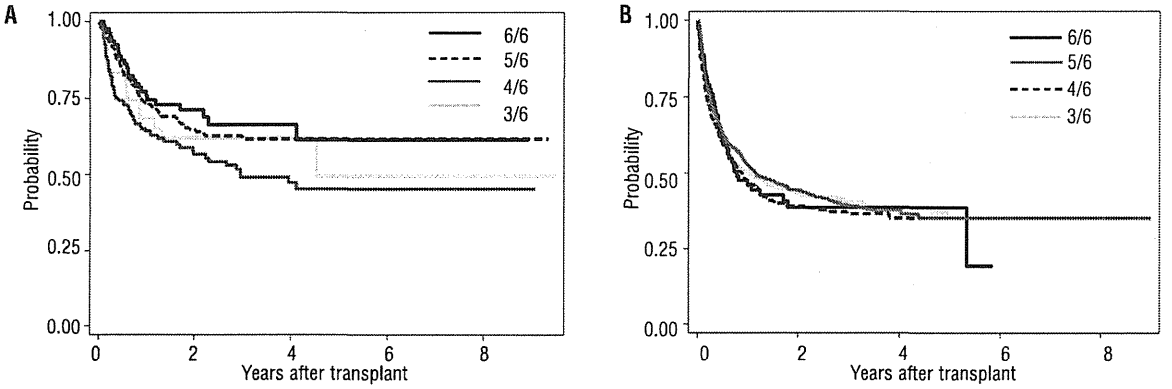

Figure 1. Unadjusted probabilities of overall survival in HLA disparity groups for pediatric (A) and adult (B) recipients with leukemia. (A) In children, the unadjusted probabilities of survival at three years post-transplant were 66% for recipients of HLA matched (6/6), 62% for one-locus-mismatched (5/6), 45% for two-loci-mismatched (4/6), and 62% for three-loci-mismatched (3/6) single-unit unrelated cord blood (*P*=0.032). (B) In adults, these probabilities were 38% 37%, 39%, and 40% respectively (*P*=0.567) (B).