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ORIGINAL PAPER

Risk factors for CAR-T cell manufacturing failure among DLBCL patients: A nationwide survey in Japan

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Summary
For successful chimeric antigen receptor T (CAR-T) cell therapy, CAR-T cells must be manufactured without failure caused by suboptimal expansion. In order to determine risk factors for CAR-T cell manufacturing failure, we performed a nationwide cohort study in Japan and analysed patients with diffuse large B-cell lymphoma (DLBCL) who underwent tisagenlecleucel production. We compared clinical factors between 30 cases that failed (7.4%) with those that succeeded ($n = 378$). Among the failures, the proportion of patients previously treated with bendamustine (43.3% vs. 14.8%; $p < 0.001$) was significantly higher, and their platelet counts (12.0 vs. $17.0 \times 10^4/\mu L$; $p = 0.01$) and CD4/CD8 T-cell ratio (0.30 vs. 0.56; $p < 0.01$) in peripheral blood at apheresis were significantly lower than in the successful group. Multivariate analysis revealed that repeated bendamustine use with short washout periods prior to apheresis (odds ratio [OR], 5.52; $p = 0.013$ for ≥6 cycles with washout period of 3–24 months; OR, 57.09; $p = 0.005$ for ≥3 cycles with washout period of <3 months), low platelet counts (OR, 0.495 per $10^5/\mu L$; $p = 0.022$) or low CD4/CD8 ratios (<one third) (OR, 3.249; $p = 0.011$) in peripheral blood at apheresis increased the risk of manufacturing failure. Manufacturing failure remains an obstacle to CAR-T cell therapy for DLBCL patients. Avoiding risk factors, such as repeated bendamustine administration without sufficient washout, and risk-adapted strategies may help to optimize CAR-T cell therapy for DLBCL patients.

KEYWORDS
chimeric antigen receptor T cell therapy, manufacturing failure, tisagenlecleucel

INTRODUCTION

Since chimeric antigen receptor T (CAR-T) cell therapy targeting CD19 has shown good outcomes in patients with relapsed or refractory (r/r) mature B-cell lymphomas,[1–3] the number of eligible patients is increasing.

Among patients who have sought CAR-T cell therapy in clinical trials, 1–13% reportedly experience manufacturing failure due to suboptimal expansion of CAR-T cells during the manufacturing process.[3,4] Because manufacturing failure not only requires re-apheresis and treatment delays, but can also sometimes deprive patients of the chance to receive this treatment, especially in cases with highly aggressive disease, it is essential to be able to estimate the risk of manufacturing failure, in order to reduce it.

Manufacturers of CAR-T pharmaceuticals specify washout periods for anti-tumour drugs prior to apheresis. These are calculated from pharmacokinetic data for each drug,[5] but it is often difficult to determine whether using certain drugs before apheresis is tolerable in clinical practice, because effects of these drugs on CAR-T cell manufacturing outcomes are mostly unknown.

Therefore, we performed a nationwide cohort study in Japan to determine risk factors for CAR-T cell manufacturing failure in a clinical setting. Our findings provide valuable information for treatment decision-making during the periapheresis period, so as to optimize CAR-T cell therapy.

PATIENTS AND METHODS

Patients and data collection

This retrospective study, conducted by the CAR-T cell therapy taskforce established by the Japan Society of Transfusion Medicine and Cell Therapy (JSTMCT), enrolled patients with diffuse large B-cell lymphoma (DLBCL) who underwent CAR-T cell production for tisagenlecleucel (tisa-cel) using T cells harvested at Japanese institutions from October 2019 to March 2022. Compassionate use and clinical trial cases were excluded. We reviewed medical records and extracted clinical factors, including diagnosis and treatment history of underlying disease, laboratory tests at apheresis and factors related to apheresis procedures, as well as results of CAR-T cell production. Disease status at apheresis was assessed using the revised response criteria for malignant lymphoma.[6] The washout period of bendamustine was defined as the time from the last bendamustine cycle to apheresis. Cell counts in peripheral blood at apheresis were obtained within 2 days before apheresis. Our protocol complied with the declaration of Helsinki and was approved by the

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institutional review boards of participating institutions or the central review board at Kyoto University Hospital.

Cell collection and tisagenlecleucel manufacturing

Autologous peripheral blood mononuclear cell (PBMNC) concentrates were collected using Spectra Optia (Terumo BCT) with either the MNC or cMNC program, COM.TEC (Fresenius Kabi), or Fenwal Amicus (Fresenius Kabi), as determined by the institution. PBMNC concentrates were cryopreserved, and shipped to manufacturing facilities for CAR-T cell production. $CD3^+$ cell counts in peripheral blood and in the collection bag were measured by flow cytometry after staining with fluorochrome-conjugated anti-CD3 antibody at each facility. All processes from cell collection to shipment were performed according to procedural guidelines provided by Novartis. Tisa-cel was manufactured and production results were judged by Novartis, using several parameters, including cell doses and CAR expression.

Statistical analyses

Categorical and continuous variables were compared between groups using Fisher's exact test and two-tailed, unpaired Student's *t*-test respectively. In comparisons between groups, CD4/CD8 ratios were log-transformed to normalize skewed distributions. We evaluated the effect of each factor on the manufacturing outcome using a logistic regression model. We first performed univariate logistic regression to examine potential predictors of manufacturing failure. In the multivariate analysis, all variables with $p<0.1$ in univariate analyses were included and multiple regression with backward stepwise elimination (significance level = 0.05) was performed. p values <0.05 were considered statistically significant. Statistical analyses were performed using Stata, version 17 (Stata Corp.).

RESULTS

Patient characteristics

We analysed 408 cases with DLBCL from 22 institutes. The median age at apheresis was 60 years (range, 11–87) (Supplemental Table S1). Cases included 226 male patients (55.4%) and 182 female patients (44.6%). Disease status was progressive disease at apheresis in 137 patients (33.9%). Median time between diagnosis and apheresis was 21.5 months (2.5–361.2). Median numbers of chemotherapy regimens, chemotherapy cycles and anti-CD20 cycles prior to apheresis were 3 (1–12), 11 (2–44) and 10 (0–43) respectively. 69 patients (16.9%) received chemotherapy regimens that included bendamustine before apheresis, 54 of whom received three or more cycles of bendamustine. The median time from last chemotherapy to apheresis was 42 days (0–1463). 163 patients (40.0%) had received high-dose chemotherapy with autologous stem cell transplantation (auto-SCT), and 111 (27.9%) had received radiation therapy before apheresis. Cell counts in peripheral blood at apheresis were as follows: platelets, 16.5×10^4/μL (2.3–82.3); lymphocytes, 804/μL (38–4023); $CD3^+$ cells, 648/μL (36–3515); CD4/CD8 T-cell ratios, 0.53 (0.02–5.60). A median of 3.2×10^9 (0.7–15.0) and 2.9 (0.7–13.5) $CD3^+$ cells was harvested and shipped for CAR-T cell production respectively. 30 cases experienced manufacturing failures (7.4%).

Preapheresis factors predictive of manufacturing failure

Then, we compared clinical factors between cases that failed ($n=30$) and those that succeeded ($n=378$; Table 1). The number of chemotherapy regimens (median 4 vs. 3; $p=0.038$), total chemotherapy cycles (13 vs. 10; $p=0.005$), anti-CD20 therapy cycles (13 vs. 10; $p=0.020$) prior to apheresis and the proportion of patients previously treated with bendamustine (43.3 vs. 14.4%; $p<0.001$), especially those with three or more cycles (43.3 vs. 10.9%; $p<0.001$) were significantly higher in the failed group than the successful group. Among patients with histories of auto-SCT, the time between auto-SCT and apheresis was shorter (118 vs. 351 days; $p=0.049$) in the failed group. Platelet counts (12.0 vs. 17.0×10^4/μL; $p=0.006$) and CD4/CD8 ratios (0.30 vs. 0.56; $p<0.001$) were significantly lower in the failed group (Figure 1 and Table 1). Serum lactate dehydrogenase (LDH) level was higher (239 vs. 232 IU/L; $p=0.036$) in the failed group. There were no significant differences in failure rates related to apheresis device type or collection program. There were no significant differences between groups in the total number of $CD3^+$ cells (3.1 vs. 2.9×10^9; $p=0.509$) or the proportion of $CD3^+$ cells to total nucleated cells (55.6 vs. 46.4%; $p=0.142$) shipped for tisa-cel production, whereas numbers of total nucleated cells, lymphocytes and $CD3^+$ cells collected by apheresis were slightly but significantly higher in the failed group. There was no significant difference in the year in which apheresis was performed.

We performed univariate and multivariate logistic regression analysis for manufacturing failure (Table 2). Multivariate analysis revealed that ≥3 cycles of bendamustine therapy prior to apheresis, lower platelet counts and CD4/CD8 ratios less than one third in peripheral blood at apheresis constituted risk factors for manufacturing failure. Meanwhile, the total number of chemotherapy cycles or lymphocyte counts in peripheral blood at apheresis were not associated with manufacturing failure.

Effects of bendamustine use prior to apheresis on manufacturing failure probability

Because our data indicated that bendamustine use before apheresis increased the risk of manufacturing failure, bendamustine use was analysed in greater detail. We compared

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TABLE 1 Patient characteristics.

		Failed	Successful	
		N = 30	*N* = 378	*p* value
Patient background				
Age at apheresis	Years	62 (33–75)	60 (11–87)	0.454
Sex	Male	14 (46.7%)	212 (56.1%)	0.344
	Female	16 (53.3%)	166 (43.9%)	
Disease status at apheresis	CR/PR/SD	19 (63.3%)	248 (66.3%)	0.841
	PD	11 (36.7%)	126 (33.7%)	
Primary refractory	Yes	16 (59.3%)	145 (43.8%)	0.159
Time between diagnosis to apheresis	Months	25.6 (6.9–225.7)	20.7 (2.5–361.2)	0.377
Number of chemotherapy regimens	Regimens	4 (2–10)	3 (1–12)	0.038*
Number of chemotherapy cycles	Cycles	13 (6–33)	10 (2–44)	0.005*
Number of anti-CD20 cycles	Cycles	13 (4–35)	10 (0–43)	0.020*
History of bendamustine	Yes	13 (43.3%)	56 (14.8%)	<0.001*
History of bendamustine (≥3 cycles)	Yes	13 (43.3%)	41 (10.8%)	<0.001*
Cycles of bendamustine	Cycles	6 (3–10)	4 (1–12)	0.051
Time between last chemo to apheresis	Days	43 (16–456)	42 (0–1463)	0.200
History of auto-SCT	Yes	15 (50.0%)	148 (39.3%)	0.253
Time between auto-SCT to apheresis	Days	118 (42–811)	351 (27–5885)	0.049*
History of irradiation	Yes	9 (34.6%)	102 (27.4%)	0.509
Dose of irradiation	Gy	43 (22–80)	40 (4–138)	0.304
Time between radiation to apheresis	Days	89 (47–948)	115 (0–7195)	0.462
Laboratory data at apheresis				
Hb	g/dL	9.5 (7.6–15.0)	9.9 (6.7–16.4)	0.557
Plt	10^4/μL	12.0 (3.8–39.1)	17.0 (2.3–82.3)	0.006*
WBC	/μL	3150 (1400-7800)	3935 (900–18 640)	0.101
Lymphocyte count	/μL	1134 (63–3416)	780 (38–4023)	0.066
Monocyte count	/μL	343 (78–1068)	435 (0–2842)	0.202
$CD3^+$ cell count	/μL	731 (60–2550)	640 (36–3515)	0.326
$CD4^+$ cell count	/μL	170 (19–1175)	216 (0–1156)	0.807
$CD8^+$ cell count	/μL	491 (9–2781)	401 (0–3311)	0.017*
$CD56^+$ cell count	/μL	85 (3–422)	82 (1–1666)	0.670
$CD19^+$ cell count	/μL	0 (0–380)	0 (0–832)	0.348
CD4/CD8 ratio		0.30 (0.02–5.60)	0.56 (0.09–3.80)	<0.001*
LDH	IU/L	239 (138–3579)	232 (72–1919)	0.036*
Apheresis factors				
Device	Spectra Optia	30 (100.0%)	373 (98.7%)	1.000
	Others	0 (0.0%)	5 (1.3%)	
Program	cMNC	23 (76.7%)	333 (88.1%)	0.085
	MNC	7 (23.3%)	45 (11.9%)	
Body weight	kg	49.3 (36.2–92.6)	58.0 (29.5–103.0)	0.063
Blood volume, processed	L	8.0 (3.3–30.0)	8.2 (1.7–39.6)	0.782
Run time of apheresis	min	170 (69–591)	179 (57–716)	0.781
TNC yield	$/10^9$	8.6 (1.6–59.6)	7.3 (1.9–43.7)	<0.001*
Lymphocyte yield	$/10^9$	5.4 (0.9–17.7)	4.3 (0.6–15.9)	0.043*
$CD3^+$ cell yield	$/10^9$	3.9 (0.8–14.8)	3.2 (0.7–15.0)	0.020*

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TABLE 1 (Continued)

		Failed	Successful	
		N = 30	*N* = 378	*p* value
$CD3^+$ cell shipped	$/10^9$	3.1 (0.8–6.9)	2.9 (0.7–13.5)	0.509
Year of apheresis	2019–2020	14 (46.7%)	134 (35.4%)	0.239
	2021–2022	16 (53.3%)	244 (64.6%)	
Factory	Morris Plains	24 (80.0%)	255 (67.5%)	0.220
	Kobe	6 (20.0%)	123 (32.5%)	

Abbreviations: CR, complete response; Hb, haemoglobin; LDH, lactate dehydrogenase; PD, progressive disease; Plt, platelet; PR, partial response; SCT, stem cell transplantation; SD, stable disease; TNC, total nucleated cell; WBC, white blood cell.

*indicates $p < 0.05$.

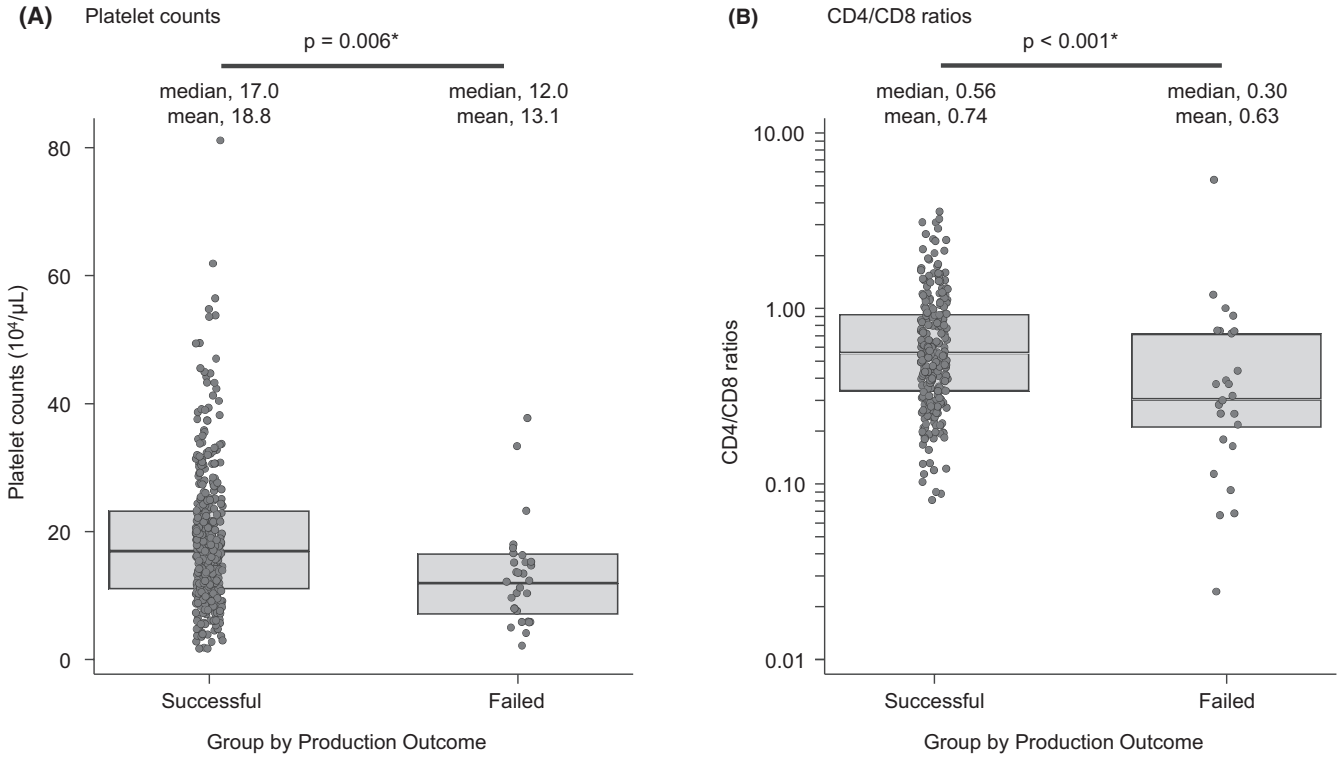

FIGURE 1 Comparison of platelet counts and CD4/CD8 ratios in peripheral blood at apheresis between the failed and successful groups. (A) Platelet count. (B) CD4/CD8 ratio of T cells. * indicates $p < 0.05$.

numbers of bendamustine administration cycles and subsequent washout periods between the successful and failed groups (Figure 2, and Supplemental Table S2). In the successful group, the proportion of patients who had undergone apheresis after a washout period ≥24 months was higher, whereas the proportion of those who had undergone apheresis after a washout period <3 months was higher in the failed group. There were patients in the successful group who received ≤2 bendamustine administration cycles (26.8%), but not in the failed group. These results suggested that both a larger number of treatment cycles and a shorter washout period for bendamustine were associated with an increased risk of manufacturing failure, whereas ≤2 cycles, or a washout period ≥24 months had little adverse effect on CAR-T manufacturing. Therefore, by combining the number of bendamustine cycles and washout periods, bendamustine risk categories were defined as follows: high risk (washout <3 months and ≥3 cycles), intermediate risk (washout of 3–24 months and ≥6 cycles) and low risk (others, including patients with no history of bendamustine). Univariate logistic analysis showed that these bendamustine risk categories stratify risk of CAR-T manufacturing failure (Supplemental Table S3).

Then, in order to quantify impact of bendamustine use and other factors on risk of manufacturing failure in combination with other predictive factors, we refined the multivariate logistic analysis (Table 3). We found that repetitive bendamustine therapy without sufficient washout (OR, 5.520; 95% CI, 1.053–9.991; $p = 0.040$ for intermediate bendamustine risk; OR, 57.088; 95% CI, 3.370–966.966; $p = 0.005$ for high bendamustine risk), lower platelet counts (OR, 0.495 per $10^5/\mu L$; 95% CI, 0.271–0.903; $p = 0.022$) and low CD4/CD8 ratios (<one third) (OR, 3.249; 95% CI 1.314–8.036;

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TABLE 2 Preapheresis and apheresis factors associated with manufacturing failure.

		Univariate			Multivariate		
		Odds ratio	**95% CI**	***p* value**	**Odds ratio**	**95% CI**	***p* value**
Patient background							
Age at apheresis	/year	0.988	0.957–1.020	0.453			
Sex	Female versus male	1.460	0.693–3.076	0.320			
Disease status at apheresis	PD versus CR/PR/SD	1.140	0.526–2.468	0.741			
Primary refractory	Yes versus no	1.866	0.840–4.143	0.125			
Time between diagnosis to apheresis	/month	1.003	0.996–1.010	0.379			
Number of chemotherapy regimens	/regimen	1.256	1.009–1.565	0.041*			
Number of chemotherapy cycles	/cycle	1.084	1.021–1.151	0.008*	1.002	0.922–1.089	0.961
Number of anti-CD20 cycles	/cycle	1.070	1.008–1.136	0.026*			
History of bendamustine	Yes versus no	4.397	2.024–9.553	<0.001*			
History of bendamustine (≥3 cycles)	Yes versus no	6.286	2.848–13.871	<0.001*	3.244	1.053–9.991	0.040*
Cycles of bendamustine	/cycle	1.322	1.164–1.502	<0.001*			
Time between last chemo to apheresis	/day	0.998	0.995–1.001	0.221			
History of auto-SCT	Yes versus no	1.547	0.735–3.259	0.251			
Time between auto-SCT to apheresis	/day	0.997	0.994–1.000	0.031*			
History of irradiation	Yes versus no	1.324	0.576–3.041	0.509			
Dose of irradiation	/Gy	1.016	0.985–1.048	0.313			
Time between radiation to apheresis	/day	1.000	0.998–1.001	0.477			
Laboratory data at apheresis							
Hb	/g/dL	0.932	0.738–1.178	0.556			
Plt	/10^5/μL	0.474	0.881–0.978	0.005*	0.519	0.281–0.960	0.037*
WBC	/10^3/μL	0.841	0.685–1.033	0.099			
Lymphocyte count	/10^3/μL	1.491	0.968–2.297	0.070	1.442	0.841–2.473	0.184
Monocyte count	/10^3/μL	0.384	0.089–1.664	0.201			
$CD3^+$ cell count	/10^3/μL	1.327	0.754–2.335	0.327			
$CD4^+$ cell count	/10^3/μL	0.785	0.114–5.403	0.806			
$CD8^+$ cell count	/10^3/μL	2.071	1.108–3.873	0.023*			
$CD56^+$ cell count	/10^3/μL	0.439	0.010–18.916	0.668			
$CD19^+$ cell count	/10^3/μL	5.834	0.129–263.407	0.364			
CD4/CD8 ratio	/log	0.422	0.248–0.716	0.001*			
CD4/CD8 ratio	<1/3 versus >=1/3	3.982	1.719–9.223	0.001*	3.039	1.223–7.548	0.017*
LDH	/IU/L	1.001	1.000–1.002	0.070			
Apheresis factors							
Program	MNC versus cMNC	2.252	0.914–5.548	0.078			
Body weight	/kg	0.969	0.938–1.002	0.065			
Blood volume, processed	/L	0.989	0.912–1.072	0.782			
Run time of apheresis	/min	1.001	0.996–1.005	0.781			
TNC yield	/10^9	1.083	1.026–1.143	0.004*			

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TABLE 2 (Continued)

		Univariate			Multivariate		
		Odds ratio	95% CI	*p* value	Odds ratio	95% CI	*p* value
Lymphocyte yield	$/10^9$	1.142	1.001–1.303	0.049*			
$CD3^+$ cell yield	$/10^9$	1.196	1.023–1.398	0.025*			
$CD3^+$ cell shipped	$/10^9$	1.087	0.849–1.391	0.508			
Year of apheresis	2021–22 versus 2019–20	0.628	0.297–1.326	0.222			
Factory	Kobe versus Morris Plains	0.518	0.207–1.301	0.162			

Abbreviations: CI, confidence interval; other abbreviations are shown in Table 1.
*indicates $p < 0.05$.

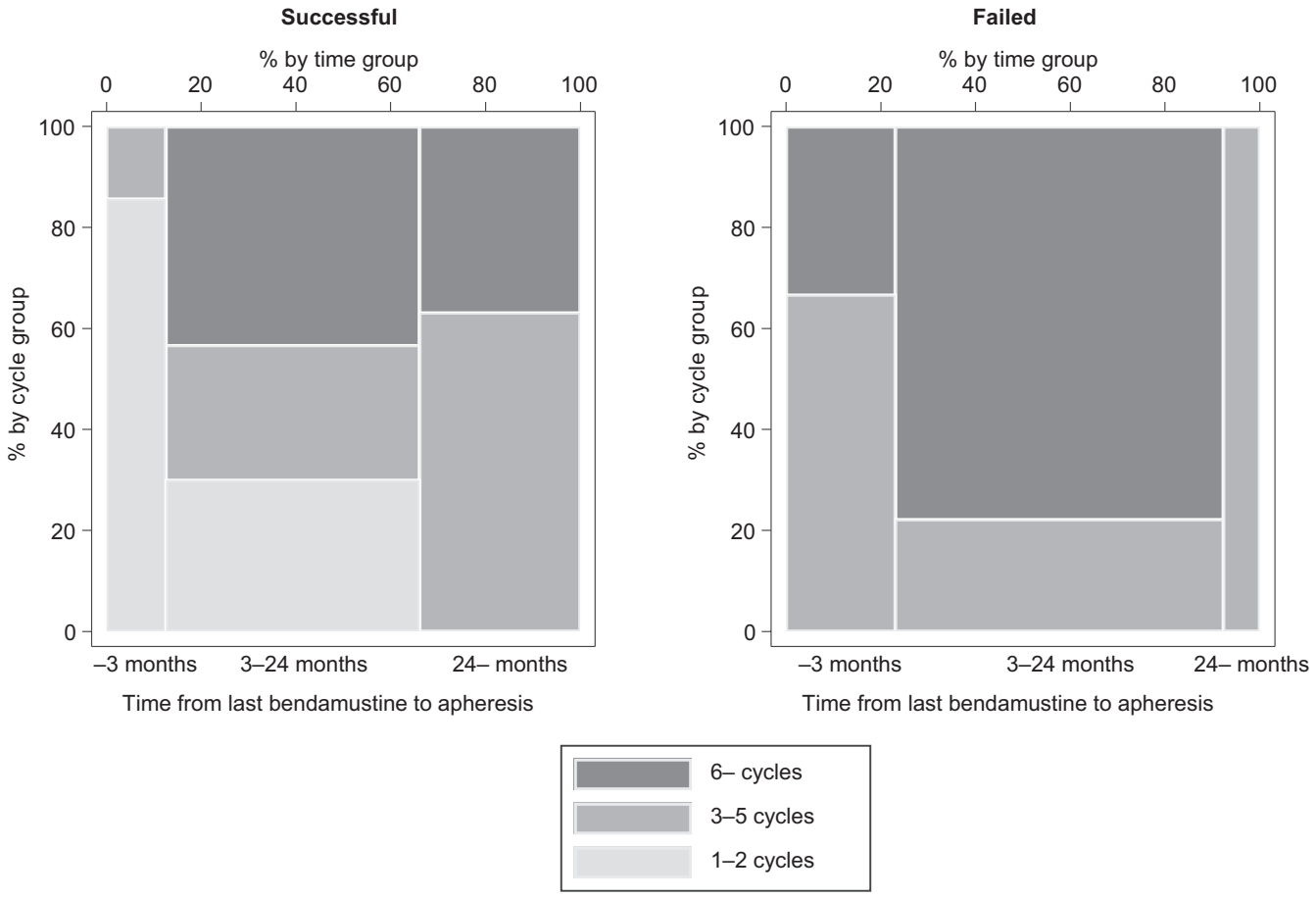

FIGURE 2 Comparison of bendamustine use between the successful and failed groups among patients who had been treated with bendamustine. The vertical axis represents categories for cycles of bendamustine, and the horizontal axis shows categories reflecting the time from last administration of bendamustine to apheresis.

$p = 0.011$) in peripheral blood at apheresis was risk factors for CAR-T manufacturing failure.

Development of a prediction model for manufacturing failure

On the basis of the multiple regression model, estimated probability for manufacturing failure was calculated using the following formula (see also Supplemental Figure S2) Estimated probability for manufacturing failure = odds/(1 + odds), where odds = $0.136 \times 0.494^{(\text{platelet counts [10E5/μl]})} \times$ (3.249, if CD4/CD8 ratios <1/3) × (5.520, if intermediate bendamustine risk) × (57.088, if high bendamustine risk). Using this formula, the estimated probability of manufacturing failure was significantly higher in the failed group than those in the successful group (median 0.175 vs. 0.061, $p < 0.001$; Figure 3).

In order to employ this formula in clinical practice, we created a calculation nomogram based on the formula (Figure 4). For example, for a patient with r/r DLBCL, who has a history of

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TABLE 3 Effects of clinical parameters, including bendamustine use, on manufacturing failure.

		Multivariate		
		Odds ratio	**95% CI**	***p* value**
Bendamustine risk category	Low	Reference		
	Intermediate	5.520	1.436–21.215	0.013*
	High	57.088	3.370–966.996	0.005*
Plt	/10^5/μL	0.495	0.271–0.903	0.022*
CD4/CD8 ratio	>=1/3	Reference		
	<1/3 versus	3.249	1.314–8.036	0.011*

Note: Abbreviations are shown in Tables 1 and 2. Bendamustine risk category was defined as follows: high risk (washout period of <3 months and total cycles of ≥3 cycles), intermediate risk (washout period of 3–24 months and total cycles of ≥6 cycles) and low risk (others including patients with no history of bendamustine).
*indicates $p < 0.05$.

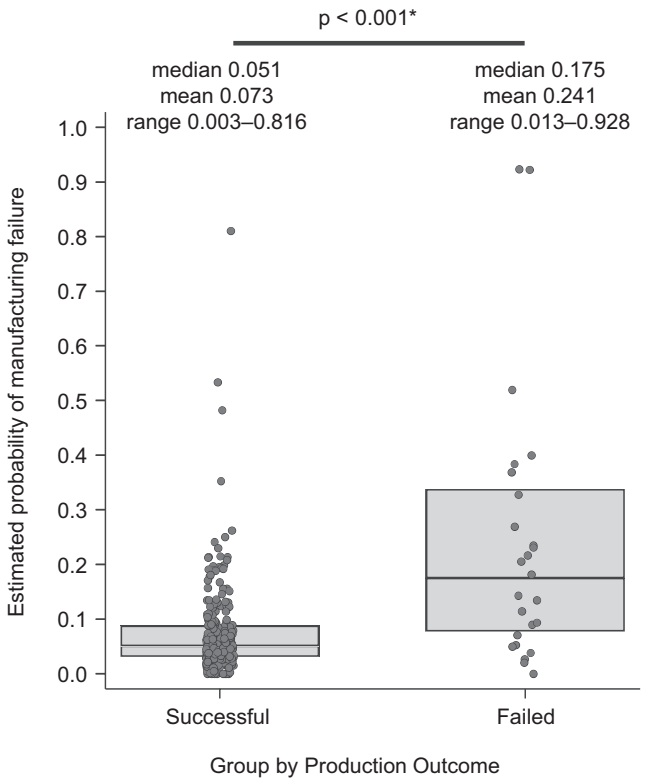

FIGURE 3 Estimated probability of manufacturing failure between the failed and successful groups, based on multivariate logistic analysis.

six cycles of bendamustine with a washout period of 12 months (bendamustine risk category: intermediate), and a platelet count of 20×10^4/μL and a CD4/CD8 ratio of 0.2 (<one third) at apheresis, scores corresponding to the respective factors, namely 4.0 points for bendamustine risk category, 6.7 points for platelet count and 2.8 points for CD4/CD8 ratio, were obtained using the nomogram. A total score of 13.5 points gave an estimated probability of manufacturing failure of 40%.

DISCUSSION

We retrospectively evaluated 408 cases CAR-T cell production for tisa-cel in patients with DLBCL, and found the following: (1) Manufacturing failure occurred in 7.4% of DLBCL patients who underwent apheresis for tisa-cel. (2) Precollection lower platelet counts and CD4/CD8 T-cell ratios < one third in peripheral blood significantly increased risk of CAR-T cell manufacturing failure. (3) For bendamustine use, repeated use and short washout periods increased the risk of manufacturing failure, whereas fewer cycles (≤2) or sufficient washout (≥24 months) did not. (4) Risk of CAR-T cell production failure can be estimated based on patient parameters before apheresis, using the risk calculation formula and the nomogram.

The proportion of patients who experienced manufacturing failure in this study (7.4%), was comparable to the 7% reported in a previous clinical trial.[2] While great effort has been made to optimize the manufacturing process,[7,8] our results indicate that manufacturing failure remains an obstacle to tisa-cel treatment in clinical practice.

Thus, we analysed various clinical factors and risk of tisa-cel manufacturing failure among patients with r/r DLBCL. Low platelet count may reflect residual chemotherapy-induced T-cell toxicity. In this study, low platelet count was significant in the multivariate analysis even after adjusting for the number of chemotherapy cycles and the washout period from chemotherapy to apheresis; therefore, platelet count is a good marker for the risk of manufacturing failure before apheresis.

Next, we considered the impact of harvested T-cell quality on the whole process of manufacturing. As the total number of $CD3^+$ cells shipped for tisa-cel production was higher in the failed group than in the successful group, the absolute number of $CD3^+$ cells themselves may not be associated with production failure. Rather, functional subpopulations of T cells may be responsible for success or failure of CAR-T cell production. Differences in subpopulations may be related to T-cell 'fitness', defined as the ability of T cells collected through apheresis to generate CAR-mediated functional immune responses, which are impaired by various factors, such as ageing, disease and chemotherapy.[9,10] While several groups have tried to evaluate T-cell fitness by thorough analyses of T-cell subsets, that is, naïve/stem cell memory, central memory, effector memory and effector T cells, or by quantifying cytokine production,[11–15] it has been difficult to

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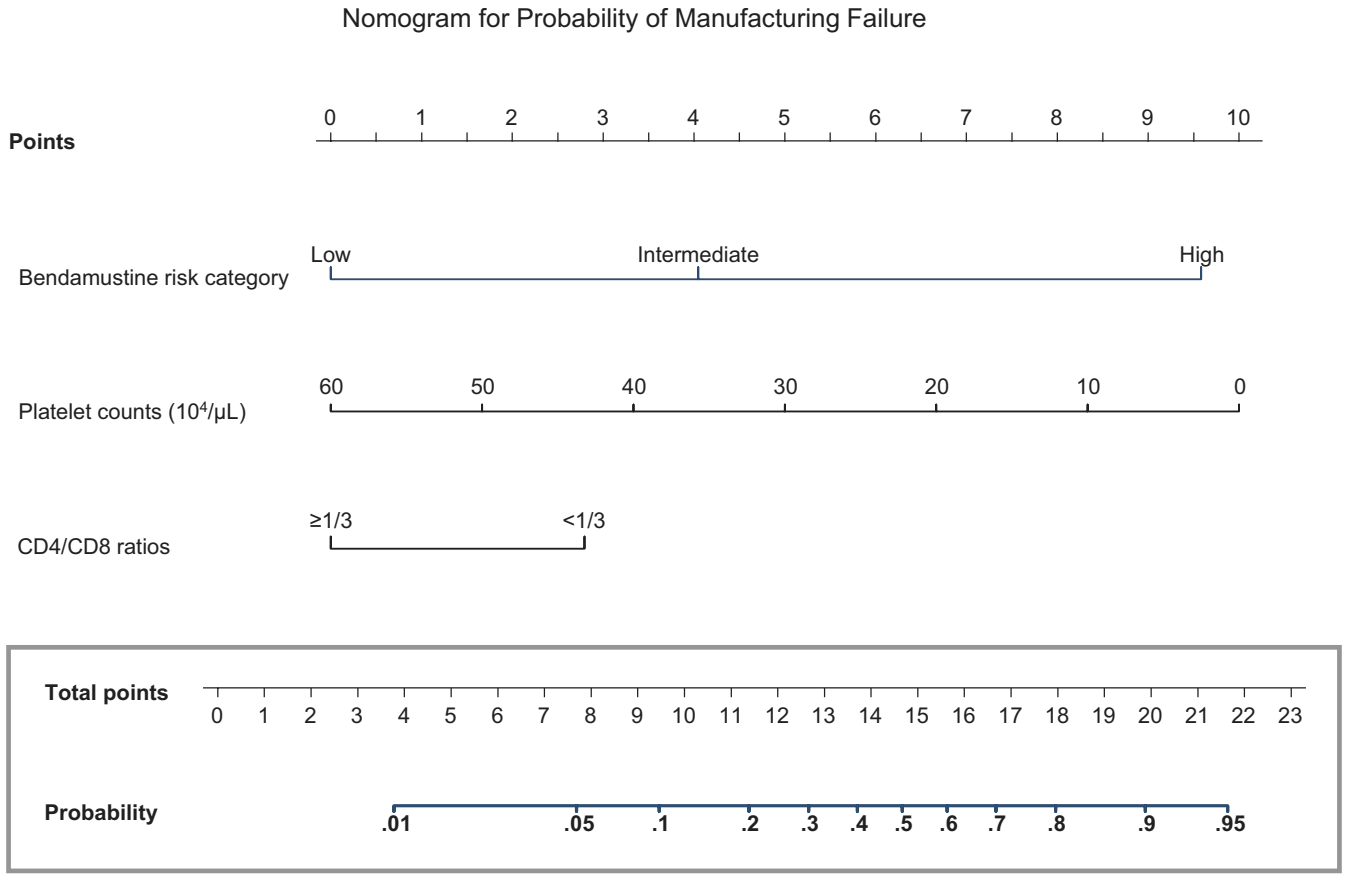

FIGURE 4 Nomogram predicting the probability of CAR-T cell manufacturing failure. Instructions: Locate the patient's bendamustine risk category. Draw a line straight upward to the points axis to determine how many points towards manufacturing failure for bendamustine risk category. Repeat the process for each predictive factor. Sum the points for all predictive factors. Locate the sum for bendamustine risk category, platelet count at apheresis and CD4/CD8 ratio at apheresis on the total points axis. Draw a line straight down to find the estimated probability of manufacturing failure.

evaluate T-cell fitness in clinical practice. Because the CD4/CD8 ratio among T cells in peripheral blood declines with ablative chemotherapies, it is a convenient surrogate marker of T-cell fitness.[16–18] In addition, as it has been reported that proliferation and therapeutic effects of CAR-T cells are improved by a manufacturing process in which collected T cells were separated into $CD4^+$ cells and $CD8^+$ cells, and CAR was introduced with a balanced CD4/CD8 ratio,[19,20] our results suggest that the CD4/CD8 ratio of T cells may also affect efficiency of the subsequent manufacturing process. The adverse effect of an unfavourable CD4/CD8 ratio on CAR-T cell manufacturing may be minimized by optimizing the manufacturing process.

Since the number of $CD3^+$ cells collected or shipped was not lower in the manufacturing failure group than in the successful group, difficulty in harvesting $CD3^+$ cells induced by bendamustine may not be the major reason for impaired CAR-T cell production. Rather, bendamustine may reduce T-cell fitness in CAR-T cell therapy, if it changes T-cell phenotypes over time[21,22] and increases the proportion of T cells expressing exhaustion markers.[23,24] As the clinical efficacy of bendamustine for r/r DLBCL has been demonstrated,[25] bendamustine has already become an essential agent for CAR-T cell therapy in patients with r/r DLBCL. Therefore, clinical evidence that optimizes safe use of bendamustine without interfering with CAR-T cell therapy, is urgently required. Our data, showing that ≤2 cycles of bendamustine, or washout periods ≥24 months do not increase the risk of manufacturing failure and that otherwise it does so significantly, will help clinical decision-making.

We developed a quantitative risk estimation formula and nomogram based on multivariate analysis, which helps to quickly estimate probability of manufacturing failure at the time of apheresis according to three parameters: bendamustine use, platelet count and CD4/CD8 ratio in peripheral (Table 3 and Figures 3 and 4). This estimation formula and nomogram enable rapid estimation of the probability of manufacturing failure before apheresis, and can be used to suggest better treatment strategies before apheresis.

While this study includes detailed analyses of real-world data, some limitations must also be acknowledged. First, because it examined data from multiple facilities, differences in collection procedures and cell count measurements among facilities could potentially affect risk

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of manufacturing failure. For instance, some facilities considered that the lower limit of the cell count requirement of apheresate was sufficient and set a lower target cell number and processing blood volume, while others aimed closer to the upper limit of the cell count requirement allowing more processing blood volume. While, multivariate analysis in this study showed that the number of $CD3^+$ cells harvested or shipped was not associated with risk of manufacturing failure, standard requirements for the number of cells to be harvested or shipped for manufacturing have to be further optimized. While analysis of lymphocyte fraction in peripheral blood at apheresis and $CD3^+$ cells in apheresis products were performed by a standardized method, cell count results by flow cytometry may potentially vary between facilities, as has been reported regarding measurement of $CD34^+$ cells in peripheral blood stem cell collection.[26] In order to further evaluate effects of detailed lymphocyte subsets of peripheral blood and apheresis product on manufacturing outcomes, as well as prognostic outcomes in clinical practice with CAR-T cell therapy, it is necessary to refine standardized methods of lymphocyte subset analysis. Second, as the CAR-T manufacturing process is proprietary to Novartis, aspects of that process that may potentiate manufacturing failure cannot be evaluated. Third, detailed analyses of T-cell subpopulation other than CD4/CD8 ratios, or functional tests were not performed in this study. Fourth, as both the development and validation of the model were performed using the same cohort, the potential for overfitting cannot be excluded. Therefore, our model requires external validation to ensure generalizability. As this study focused on CAR-T manufacturing outcome as well as factors associated with manufacturing failure, the exact impact of manufacturing failure on prognostic outcomes of patients thereafter was not evaluated. Of the 30 cases with manufacturing failure, only 12 cases (40.0%) were eventually infused with CAR-T cells because of reduced chance of remanufacturing due to progressive disease and relatively low probability of remanufacturing success. While majority of cases who once experienced manufacturing failure could not reach infusion of CAR-T cells, which lead to poor prognosis of this group of patients, remanufacturing could rescue a subset of patients among them (data not shown). Further studies are required to clarify prognostic impact of manufacturing failure, as well as therapeutic effects of CAR-T cells remanufactured in patients who once experienced manufacturing failure, thereby determining optimal strategy to counter manufacturing failure.

In conclusion, this study showed that manufacturing failure remains an obstacle to CAR-T cell therapy for some DLBCL patients. Repeated bendamustine administration with inadequate washout, and low platelet counts and low CD4/CD8 ratios in peripheral blood at apheresis significantly increase the risk of manufacturing failure. Strategies to reduce this risk may help to optimize CAR-T cell therapy for patients with r/r DLBCL.

AUTHOR CONTRIBUTIONS

TJ and YA designed the study, reviewed and analysed the data and wrote the paper; SY, YO, KFujii, KK, RY, WT, YU, KFukushima, TA, MY-F, RH, NY, YT, YS, HN, NS, NM, JA, TA and YN contributed to data collection. JI, KI, SF, MR, TN-I and RT interpreted the data. All authors critiqued the manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors declare no competing financial interests.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are not openly available due to reasons of sensitivity and are available from the corresponding author upon reasonable request.

PATIENT CONSENT STATEMENT

Patient information is anonymized, and patients consent was confirmed by the opt-out method during the study.

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SUPPORTING INFORMATION

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