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Prospective study of adoptive activated αβT lymphocyte immunotherapy for refractory cancers: development and validation of a response scoring system

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ABSTRACT

Background aims: This prospective clinical study aimed to determine the efficacy and prognostic factors of adoptive activated αβT lymphocyte immunotherapy for various refractory cancers. The primary endpoint was overall survival (OS), and the secondary endpoint was radiological response.
Methods: The authors treated 96 patients. Activated αβT lymphocytes were infused every 2 weeks for a total of six times. Prognostic factors were identified by analyzing clinical and laboratory data obtained before therapy.
Results: Median survival time (MST) was 150 days (95% confidence interval, 105–191), and approximately 20% of patients achieved disease control (complete response + partial response + stable disease). According to the multivariate Cox proportional hazards model with Akaike information criterion–best subset selection, sex, concurrent therapy, neutrophil/lymphocyte ratio, albumin, lactate dehydrogenase, CD4:CD8 ratio and T helper (Th)1:Th2 ratio were strong prognostic factors. Using parameter estimates of the Cox analysis, the authors developed a response scoring system. The authors then determined the threshold of the response score between responders and non-responders. This threshold was able to significantly differentiate OS of responders from that of non-responders. MST of responders was longer than that of non-responders (317.5 days versus 74 days). The validity of this response scoring system was then confirmed by internal validation.
Conclusions: Adoptive activated αβT lymphocyte immunotherapy has clinical efficacy in certain patients. The authors' scoring system is the first prognostic model reported for this therapy, and it is useful for selecting patients who might obtain a better prognosis through this modality.

Introduction

Recently, anti-cancer immunotherapy has emerged as the fourth leading cancer treatment after surgery, radiotherapy and chemotherapy, and it is expected to greatly improve the prognosis of patients. One of the various immunotherapies currently in use is adoptive activated αβT lymphocyte immunotherapy, which was developed in the 1990s. This form of immunotherapy uses T cells activated by IL-2 and anti-CD3 antibody. Mechanistically, Noguchi *et al.* [1] reported that adoptive activated αβT lymphocyte immunotherapy can restore an impaired and imbalanced T-cell immune status. Takayama *et al.* [2] also concluded that it is a safe and feasible treatment that can improve recurrence-free outcomes after surgery for hepatocellular carcinoma (HCC). Others have reported its clinical effectiveness in a particular subtype of lung cancer [3] and stage IV colorectal cancer [4]. In addition, this therapy is less toxic than conventional chemotherapy and novel therapies such as immune checkpoint inhibitor therapy and chimeric antigen receptor T-cell therapy [5]. However, most reports are retrospective studies or have a small sample size;

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reports of prospective studies with a significant number of patients are also very limited. The reports that are available suggest that this immunotherapy might be effective for particular groups. Unfortunately, the prognostic factors associated with this therapy remain unknown.

Hence, the authors conducted a prospective observational clinical study of adoptive activated $\alpha\beta$T lymphocyte immunotherapy (UMIN000019367). This study aimed to assess the efficacy and safety of this therapy and to develop and validate a responder prediction model. This study is the first to report a prognostic model for this immunotherapy. This model can be used for selecting patients who are suitable for this treatment.

Methods

Patients, study design and assessments

The authors treated 96 patients aged greater than 20 years who were diagnosed with cancers resistant to current standard treatments or who were ineligible for standard treatments (see supplementary Figure 1). The authors excluded those who had an autoimmune disease with immunosuppressive therapy or had previously received programmed cell death protein 1/programmed death ligand 1 blockade therapy or organ transplantation. This study was a prospective, single-arm, observational, single-center study of adoptive activated $\alpha\beta$T lymphocyte immunotherapy in patients with refractory cancers. Registration began September 1, 2011, and ended March 31, 2016. The observation period ended November 30, 2018. The primary endpoint was overall survival (OS), and the secondary endpoints were radiological response.

Tumors were evaluated by contrast-enhanced computed tomography before and after therapy. Two radiologists who did not have a conflict of interest with the study evaluated radiological changes as complete response (CR), partial response (PR), stable disease (SD) or progressive disease according to Response Evaluation Criteria in Solid Tumors version 1.1. One patient was withdrawn from the study because of proliferation failure of the $\alpha\beta$T lymphocytes. All recorded adverse events were graded using Common Terminology Criteria for Adverse Events version 4.0. Deaths from primary cancer were excluded from the adverse events. This study followed the principles of the Declaration of Helsinki and the Japanese Ethical Guidelines for Clinical Research. The institutional review board of the Kyushu university hospital approved the study and written informed consent was obtained from all patients.

Adoptive activated $\alpha\beta$T lymphocyte immunotherapy

Peripheral blood mononuclear cells were obtained by continuous peripheral apheresis and then cultured with immobilized anti-CD3 antibody and IL-2 for 14 days as described previously [3,5]. These activated T lymphocytes were administered intravenously every 2 weeks for a total of six times. In some patients, therapy was discontinued because of exacerbation of the general condition.

Flow cytometry analysis

The peripheral blood of patients underwent immunophenotyping before the start of therapy and before the fourth therapy. For flow cytometry analysis, the authors used the following monoclonal antibodies: anti-CD3-FITC, anti-CD3-PC5, anti-CD4-PE, anti-CD8-PC5, anti CD25-FITC, anti-CD45-ECD, anti-CD56-PE, anti-TCRpan$\delta\gamma$-FITC, anti-TCRpan$\alpha\beta$-PE, anti-TCRVγ9-FITC, anti-IFNγ-FITC, anti-IL4-PE (Beckman Coulter, Brea, CA, USA) and anti-Foxp3-PE (Becton Dickinson, Franklin Lakes, NJ, USA). Data were analyzed using Cytomics FC 500 (Beckman Coulter).

Statistical analysis and development and validation of scoring system

To predict immunotherapy responders, the authors developed a response scoring system. The authors first randomly divided patients into two groups, with 68 (70%) patients in a development group and 28 (30%) patients in a validation group. The authors also categorized consecutive variables into tertiles.

Furthermore, the authors used a combination of step-wise selection and best subset selection using the Akaike information criterion developed by Shtatland *et al.* [6]. First, the optimal number of explanatory variables for a Cox proportional hazards model was determined by step-wise selection. Next, the authors assessed a broad spectrum of candidate variables using best subset selection. This analysis identified seven explanatory variables (sex, concurrent therapy, neutrophil/lymphocyte ratio [NLR], albumin, lactate dehydrogenase [LDH], CD4:CD8 ratio and T helper (Th)1:Th2 ratio) out of 23 potential candidate variables from demographic, laboratory and flow cytometry variables.

Table 3 lists the parameter estimate of each explanatory variable, which was considered a coefficient of the linear prediction model. To simplify the prognostic score model, the authors assigned an integer parameter for each parameter estimate to approximate the estimates. Basically, the authors set the first tertile as a reference value. However, the authors reversed the order of categories (Tables 3, 4) when a parameter estimate was negative to make the response score the natural number (Table 3). In this way, clinicians can easily sum up each score (Table 4).

Next, the authors divided the hazard ratios (HRs) of the 10 subgroups by the decile of the response scores. These HRs were then used to decide the cutoff value of the response scores to predict the responders. This threshold was applied in the development group (n = 68) (Figure 2A). Subsequently, the performance of this responder prediction model was evaluated in the validation group (n = 28) using a Kaplan–Meier plot and generalized Wilcoxon test (Figure 2B). All analyses were conducted using JMP 14 software (JMP Statistical Discovery LLC, Cary, NC, USA) and SAS 9.4 software (SAS Institute Inc, Cary, NC, USA).

Results

Patient characteristics and laboratory data

The authors treated 96 patients (median age, 64 years), approximately 40% of whom were male. Performance status was $\leq$2 in approximately 90% of patients, and more than 50% received concurrent treatment during $\alpha\beta$T lymphocyte immunotherapy. Patient characteristics as well as peripheral blood data (NLR, albumin and LDH) of all patients before the first therapy are shown in Table 1. A total of 15, 11 and 10 patients had gastric, colon and non-small cell lung cancer, respectively (see supplementary Table 1).

Flow cytometry analysis

Table 1 shows data with regard to peripheral blood immunophenotyping in all patients before the first therapy, and supplementary Table 2 presents the average number of various cell populations in the first three batches of infused cells. The median number of CD45+ mononuclear cells infused per treatment was 6.39×10^9, and the median number of CD3+$\alpha\beta$+ cells was 5.78×10^9; thus, approximately 84% of infused cells were CD3+$\alpha\beta$+ cells. Furthermore, the median numbers of CD3+CD4+CD8− and CD3+CD4−CD8+ cells infused per therapy were 3.13×10^9 and 2.12×10^9, respectively.

In addition, the authors analyzed the immune cells of 66 patients before the first therapy as well as before the fourth therapy to evaluate the effect of infusing cultured activated $\alpha\beta$T lymphocytes. After therapy, the average numbers of CD3+$\alpha\beta$+, CD3+CD4+ and CD3+CD8

Table 1
Characteristics of all patients treated with adoptive activated αβT lymphocyte immunotherapy.

Variable	All patients			Development group			Validation group		
Nominal variables	n	%		n	%		n	%	
Sex									
Male	40	41.7		28	41.2		12	42.9	
Female	56	58.3		40	58.8		16	57.1	
Concurrent therapy									
Negative	44	45.8		30	44.1		14	50	
Positive	52	54.2		38	55.9		14	50	
Performance status									
0–2	85	88.5		61	89.7		24	85.7	
3–4	11	11.5		7	10.3		4	14.3	
Continuous variables	n	Median	IQR	n	Median	IQR	n	Median	IQR
Age	96	64	56–70	68	63	56–70	28	65	59–68
NLR	95	3.5	2.2–6.8	67	3.5	2.2–6.2	28	3.2	2.2–7.2
Albumin	96	4	3.5–4.3	68	4	3.5–4.3	28	4.1	3.6–4.3
LDH	96	220	172–298	68	232	178–334	28	204	158–232
CD45+	96	4669	3613–6040	68	4656	3216–5867	28	4940	3886–6318
(MNC)CD3+αβ+	96	635	446–825	68	567	373–793	28	698	518–899
(MNC)CD3+γδ+	96	18	10–35	68	17	9–34	28	22	14–43
(MNC)CD3+CD4+CD8-	96	420	274-568	68	391	263–561	28	457	345–591
(MNC)SCD3+CD4−CD8+	96	198	134–275	68	179	123–260	28	228	168–338
(MNC)CD3−CD56+	96	134	81–255	68	141	82–255	28	125	77–247
(MNC)CD3+CD56+	82	26	15–45	59	26	13–45	23	26	16–47
(CD3+CD4+)IFN-γ+IL4−	96	49	32–84	68	49	31–82	28	56	34–95
(CD3+CD4+)IFN-γ−IL4+	96	14	9.0–25	68	13	9–26	28	16	10–23
(CD3+CD4+)Foxp3+	96	1	0.4–2.0	68	1	0.4–2.0	28	1	1.0–2.5
(CD3+CD4+)Foxp3+CD	96	4.1	2.2–7.0	68	4.1	2.1–7.0	28	4	2.4–7.1
CD4:CD8	96	2	1.3–2.7	68	2	1.4–2.8	28	2.1	1.0–2.7
Th1:Th2	95	3.1	2.0–5.7	67	3.2	1.9–5.4	28	3.1	2.1–6.3

Results of laboratory tests and flow cytometry analysis of peripheral blood of patients before therapy are shown.
IFN-γ, interferon gamma; n; number, IQR, interquartile range; MNC, mononuclear cell.

+ cells were significantly increased ($P < 0.05$) (see supplementary Table 3). Conversely, some cell populations, including CD3+CD56+ and CD3+CD4+Foxp3+ cells, did not significantly change.

OS and results of radiological evaluation by Response Evaluation Criteria in Solid Tumors version 1.1

Figure 1 illustrates the Kaplan–Meier curve of the patients. The median survival time (MST) was 150 days (95% confidence interval, 105–191). The disease control rate (CR+PR+SD) was 21.2%, and the overall response rate (CR+PR) was 7.6% (see supplementary Table 1),

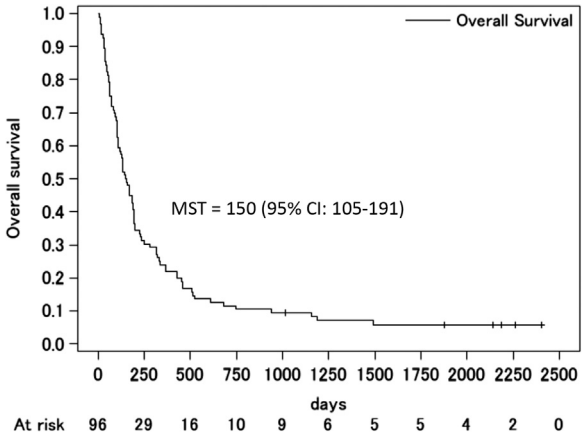

Fig. 1. Kaplan–Meier curve of the 96 patients treated with adoptive activated αβT lymphocyte immunotherapy. MST was 150 days (95% CI: 105–191). CI, confidence interval.

Factors associated with OS

The authors used the clinical, peripheral blood and flow cytometry data obtained before the therapy to identify the factors associated with OS. As shown in Table 2, the univariate Cox proportional hazards model revealed that age, NLR, albumin, LDH and number of CD45+ and CD3+CD4+CD8− cells were significantly associated with OS ($P < 0.05$).

Development of a prognostic model

The authors' next goal was to develop a responder scoring model. Since it is suggested that approximately 70% of the total population (in the authors' case, the enrollment number) be used for model development and 30% for model validation [7], the authors first randomly divided the data of patients into two groups: a development group (n = 68) and a validation group (n = 28). Table 1 shows the characteristics and other data of these two groups. Patient characteristics were basically comparable between the two groups. Second, continuous variable data were categorized into tertile groups. The multivariate Cox proportional hazards model using a combination of step-wise selection and best subset selection according to Akaike information criterion revealed that of the 23 candidate variables, seven (sex, concurrent therapy, NLR, albumin, LDH, CD4:CD8 ratio and Th1:Th2 ratio) were strong predictors of immunotherapy response. Table 3 lists the parameter estimates and HRs. To simplify the model, the authors approximated and modified the parameter estimates into natural numbers, as described earlier. By using these modified parameters, the authors developed a response scoring model (Table 4).

Next, patients from the development group were divided into 10 groups by the decile of the response scores, and the HRs of these subgroups are shown in supplementary Figure 2. HRs seemingly showed

Table 2
Results of univariate Cox proportional hazards regression analysis.

Clinical data/flow cytometry population	Unit increase *	HR	95% CI	*P* value
Age	1	1.585	1.041–2.437	0.032
Sex, male	Female	0.989	0.969–1.009	0.272
Positive concurrent therapy	Negative concurrent therapy	0.691	0.454–1.056	0.087
PS 3–4	PS 0–2	0.5	0.270–1.012	0.054
NLR	1	1.204	1.090–1.309	0.001
Albumin	1	0.393	0.255–0.609	≤ 0.0001
LDH	100	1.12	1.069–1.167	≤ 0.0001
CD45+	100	1.014	1.004–1.021	0.002
(MNC)CD3+$\alpha\beta$+	100	0.969	0.904–1.032	0.348
(MNC)CD3+$\gamma\delta$+	100	0.803	0.358–1.603	0.555
(MNC)CD3+CD4+CD8−	100	0.884	0.793–0.984	0.024
(MNC)CD3+CD4−CD8+	100	0.963	0.829–1.100	0.597
(MNC)CD3−CD56+	100	0.959	0.861–1.044	0.362
(MNC)CD3+CD56+	10	1	0.948–1.046	0.993
(MNC)CD3+&CD4+IFN-γ+IL4−	10	0.974	0.927–1.018	0.253
(MNC)CD3+CD4+IFN-γ−IL4+	10	0.908	0.757–1.076	0.273
(MNC)CD3+CD4+Foxp3+	1	0.905	0.770–1.041	0.172
(MNC)CD3+CD4+Foxp3+CD25+	0.1	0.998	0.992–1.003	0.408
CD4:CD8	1	0.964	0.803–1.138	0.67
Th1:Th2	1	0.992	0.943–1.035	0.728

Results of univariate Cox proportional hazards regression analysis of OS using clinical, laboratory and flow cytometry data are shown. Parameters with a correlation efficiency of more than 0.8 were excluded.
IFN-γ, interferon gamma; MNC, mononuclear cell; PS, performance status. *Hazard ratio is calculated per unit increase.

a clear difference between scores 12 and 13, and the cutoff value of the responders was thus defined as 13. The Kaplan–Meier plot of these groups divided by the threshold showed a significant difference ($P < 0.0001$) (Figure 2A). MST of the responders was 317.5 days, whereas MST of the non-responders was 74 days.

Validation of the prediction model

Next, the authors evaluated the performance of the prediction model by internal validation. The scores in the validation group (n = 28) were divided into two groups according to the same threshold described earlier. The Kaplan–Meier plot was significantly different between the two groups ($P = 0.027$) (Figure 2B), suggesting that the novel model was valid.

Adverse events

Two patients experienced grade 1 fever (Common Terminology Criteria for Adverse Events version 4.0). Thus, the adverse event rate was 2.1%.

Table 4
Response scores of seven parameters for the predictor model.

Categorized parameter	Response score
Sex	
Female	4
Male	0
Concurrent therapy	
Yes	1
No	0
NLR	
<2.65, first tertile	10
2.65–5.10, second tertile	5
≥5.10, third tertile	0
Albumin	
≥4.2, third tertile	10
3.8–4.2, second tertile	5
<3.8, first tertile	0
LDH	
<187, first tertile	2
188–270, second tertile	1
≥270, third tertile	0
CD4:CD8 ratio	
≥2.445, third tertile	4
1.485–2.445, second tertile	2
<1.485, first tertile	0
Th1:Th2 ratio	
≥4.88, third tertile	4
2.3–4.88, second tertile	2
<2.3, first tertile	0

Discussion

This prospective clinical study evaluated the efficacy and prognostic factors of adoptive activated $\alpha\beta$T lymphocyte immunotherapy for various refractory cancers. MST was 150 days, and in terms of radiological evaluation, the disease control rate (CR+PR+SD) was 21.2%.

The authors' aim was not only to evaluate the therapeutic efficacy of adoptive activated $\alpha\beta$T lymphocyte immunotherapy but also to develop a scoring system using OS data. This scoring system could be used in the daily clinical setting. The authors hypothesized that prognosis should depend on multiple factors, including patient characteristics (e.g., age, sex and performance status), immunological status (e.g., number of CD4+ and CD8+ T and natural killer cells) and cancer status (LDH). Thus, all of these variables were analyzed simultaneously.

The authors' multivariate Cox proportional hazards regression with Akaike information criterion revealed that seven variables (sex, concurrent therapy, NLR, albumin, LDH, CD4:CD8 ratio and Th1:Th2 ratio) were strong predictors of immunotherapy response (Table 3). It is recommended that the number of variables used in multivariate Cox regression analysis be approximately one tenth the number of events (in our case, the number of the event (death) is 90) while avoiding overfitting [8]. Thus, using seven variables was thought to be reasonable for developing a model using the entire population.

Table 3
Results of multivariate Cox proportional hazards regression analysis.

Clinical data/flow cytometry population	Reference	Parameter estimate	HR	Integerized parameter	Reference for modified parameter	Modified parameter
Sex, male	Sex, female	0.567	1.763	−4	Sex, male	4
Positive concurrent therapy	Negative concurrent therapy	−0.107	0.898	1	Negative concurrent therapy	1
NLR	First tertile	0.761	2.14	−5	Third tertile	5
Albumin	First tertile	−0.682	0.506	5	First tertile	5
LDH	First tertile	0.141	1.152	−1	Third tertile	1
CD4:CD8	First tertile	−0.341	0.711	2	First tertile	2
Th1:Th2	First tertile	−0.287	0.751	2	First tertile	2

Results of multivariate Cox proportional hazards regression analysis performed by applying Akaike information criterion using the data of 68 patients randomly selected from the development group are shown.

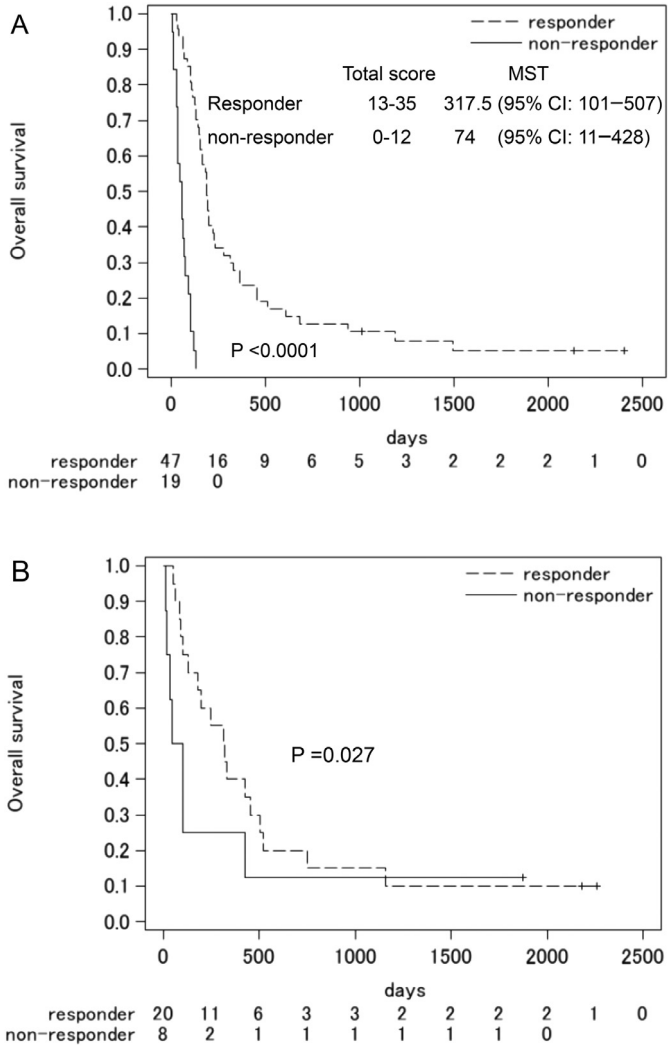

Fig. 2. (A) Kaplan–Meier curves of responder group and non-responder group of development group (n = 68) showed a statistically significant difference, with MST of 317.5 days and 74 days, respectively. (B) Kaplan–Meier curves of responder group and non-responder group of validation group (n = 28) showed a statistically significant difference.

The authors conducted multivariate Cox proportional hazards regression "without" Akaike information criterion using all variables as "consecutive variables" (except for nominal variables such as sex and age) as a sensitivity analysis to show the robustness of the model, although the results are not shown in this article. According to the results shown in this article, the authors categorized the data as described and then performed multivariate Cox proportional hazards regression "with" Akaike information criterion–best subset selection using the "nominal variables." This revealed that NLR, LDH and CD3+CD4+CD8– cells were the statistically significant prognostic factors. These two analyses revealed basically comparable results, suggesting that both methods are reliable.

The scoring system could be used for patient selection whenever $\alpha\beta$T lymphocyte immunotherapy is considered. The authors' data demonstrated that patients with a score of 13 or more would have a better prognosis and would be good candidates for this immunotherapy. This study is the first to report the prognostic model of $\alpha\beta$T lymphocyte immunotherapy.

The authors discussed some of the key variables included in this model. NLR has been reported as a prognostic marker for many cancer types [8,9], including colorectal cancer [10,11] non-small cell lung cancer [12], HCC [13], nasopharyngeal carcinoma [14] melanoma [15] and breast cancer [16]. NLR in the peripheral blood is generally elevated in patients with more advanced or aggressive disease [17]. Fujisawa *et al.* [18] reported that NLR is associated with the outcome of nivolumab immunotherapy in advanced melanoma, and Bagley *et al.* [19] demonstrated that it is a marker of outcome in nivolumab-treated patients with advanced non-small cell lung cancer. The authors' study suggests that NLR could be a prognostic variable of adoptive $\alpha\beta$T lymphocyte immunotherapy. Further study with a larger sample size is warranted to validate NLR's prognostic ability.

According to the authors' study, LDH could also be a prognostic factor of this therapy. Baseline LDH level is reportedly associated with the outcome of nivolumab or ipilimumab immunotherapy in advanced melanoma [20]. In addition, Lobefaro *et al.* [21] showed that a higher LDH is associated with a worse outcome in non-small cell lung cancer treated with immunotherapy. LDH is a tumor marker of some cancers, including colon cancer and HCC [22]. The prognostic ability of LDH might depend on the cancer type, and additional investigation of the results for each cancer type is necessary.

Moreover, flow cytometry analysis revealed that CD4:CD8 ratio and Th1:Th2 ratio in the peripheral blood of patients before therapy were prognostic factors. Peripheral blood CD4:CD8 ratio was also previously considered a prognostic factor in pancreatic cancer [23]. In addition, CD3+CD4+CD8– cells have multifaceted roles in cancer immunity [24,25], among which are helping to target anti-tumor cytotoxic lymphocytes through both direct and indirect mechanisms, to perform direct anti-tumor activity through effector cytokine (interferon gamma and tumor necrosis factor alpha) secretion and to assist B cells in producing antibodies. Thus, patients with more CD3+CD4+CD8– cells could have more robust cancer immunity, making the association between these cells and prognosis reasonable. CD4:CD8 ratio before therapy might also be associated with prognosis regardless of whether adoptive activated $\alpha\beta$T lymphocyte immunotherapy is performed. However, considering that CD3+CD4+CD8– cells were most abundant among cultured cells infused during therapy (see supplementary Table 2) and that CD4:CD8 ratio was significantly increased after therapy (see supplementary Table 3), cancer immunity might further improve by performing immunotherapy to help achieve a better prognosis. A prospective randomized study is necessary to confirm the presence of prognostic factors. In addition, CD4 and Foxp3-expressing regulatory T cells suppress effective tumor immunity [26], thereby affecting cancer prognosis. However, the number of CD3+CD4+Foxp3+ and CD3+CD4+Foxp3+CD25+ cells before therapy was not significantly associated with prognosis in the authors' analysis (Tables 2, 3).

The authors' flow cytometry analysis also revealed Th1:Th2 ratio as another prognostic factor. Hao *et al.* [27] reported that serum tumor necrosis factor:IL-4 ratio, which mainly reflects Th1:Th2 ratio, positively correlates with survival in patients with ovarian cancer. Additionally, Lee *et al.* [28] showed that a shift toward increased Th1 response exerts favorable immunological effects on HCC prognosis. In advanced cancer, the Th cell balance tends to shift from Th1 to Th2 predominance, and immune function, including the cell-mediated immunity mainly exerted by Th1-positive cells, is impaired by cytokines produced by Th2 cells [29]. Thus, an increased Th1:Th2 ratio before therapy is possibly related to a better prognosis.

This study has some limitations. Regarding external generalizability, this study involved single-center, single-arm research. Nevertheless, it is prospective in design, and the outcome used in the prediction model is all-cause mortality, suggesting that the study's external generalizability remains preserved. However, model validation using external data is required to truly generalize the model, although it was not possible this time because this was a single-center clinical study, and there was no other dataset from the same study with a large number of patients with the same analysis, including the flow cytometry analysis, available. With respect to internal generalizability, the extent of differentiation between the responder and non-

responder groups is smaller in the validation group than in the development group (Figure 2A,B), possibly reflecting overfitting during the process of model development. However, the authors were able to significantly differentiate the two groups statistically in the validation group with only 26 patients, and the selected predictive variables were reasonably scientifically associated with cancer immunity. Hence, the internal generalizability is maintained.

Conclusions

By using OS data in this prospective clinical study, the authors developed and validated a prognostic model for selecting candidates for adoptive activated $\alpha\beta$T lymphocyte immunotherapy. This model is the first prognostic model reported for this immunotherapy and could be used to select patients who are suitable for therapy at an outpatient level. Given that all variables are available in the outpatient setting and this immunotherapy is costly, this system could be applied in daily clinical practice and would be useful in terms of medical economy. In addition, the method used to develop the model could be employed when establishing other prognostic models for novel immunotherapies such as immune checkpoint inhibitor therapy and chimeric antigen receptor T-cell therapy.

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Declaration of Competing Interest

The authors have no commercial, proprietary or financial interest in the products or companies described in this article.

Author Contributions

ST and KA designed this study. AN, TI, ST and SM conducted the immunotherapy. AN, TI, ST, SM, TN and SG collected and analyzed the data. AN, RM and KF performed statistical analysis and interpretation. AN, KF and ME wrote the manuscript. All the authors read and approved the final version.

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Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.jcyt.2022.09.007.

References

[1] Noguchi A, Kaneko T, Naitoh K, Saito M, Iwai K, Maekawa R, et al. Impaired and imbalanced cellular immunological status assessed in advanced cancer patients and restoration of the T cell immune status by adoptive T-cell immunotherapy. Int Immunopharmacol 2014;18:90–7.
[2] Takayama T, Sekine T, Makuuchi M, Yamasaki S, Kosuge T, Yamamoto J. et al Adoptive immunotherapy to lower postsurgical recurrence rates of hepatocellular carcinoma: a randomized trial. Lancet 2000;356:802–7.
[3] Iwai K, Soejima K, Kudoh S, Umezato Y, Kaneko T, Yoshimori K, et al. Extended survival observed in adoptive activated T lymphocyte immunotherapy for advanced lung cancer: results of a multicenter historical cohort study. Cancer Immunol Immunother 2012;61:1781–90.
[4] Yoshida Y, Naito M, Yamada T, Aisu N, Kojima D, Mera T, et al. Clinical study on the medical value of combination therapy involving adoptive immunotherapy and chemotherapy for stage IV colorectal cancer (COMVI Study). Anticancer Res 2017;37:3941–6.
[5] Kamigaki T, Matsuda E, Okada S, Naitoh K, Kondo T, Ibe H, et al. Prospective evaluation of safety of immune-cell therapy for patients with various types of advanced cancer. Anticancer Res 2014;34:4601–7.
[6] Shtatland ES, Kleinman K. Cain EM Model building in PROC PHREG with automatic variable selection and information criteria. Stat Data Anal 2005;30:206–30.
[7] Collins GS, Reitsma JB, Altman DG, Karel G M, Moons KGM. Transparent Reporting of a Multivariable Prediction Model for Individual Prognosis or Diagnosis (TRIPOD). Circulation 2015;131:211–9.
[8] Harrell Jr. FE, Lee KL, Califf RM, Pryor DB, Rosati RA. Regression modelling strategies for improved prognostic prediction. Stat Med 1984;3:143–52.
[9] Uribe-Querol E, Rosales CJ. Neutrophils in cancer: two sides of the same coin. J Immunol Res 2015;2015:983698.
[10] Walsh SR, Cook EJ, Goulder F, Justin TA, Keeling NJ. Neutrophil-lymphocyte ratio as a prognostic factor in colorectal cancer. J Surg Oncol 2005;91:181–4.
[11] Malietzis G, Giacometti M, Kennedy RH, Athanasiou T, Aziz O, Jenkins JT, et al. The emerging role of neutrophil to lymphocyte ratio in determining colorectal cancer treatment outcomes: a systematic review and meta-analysis. Ann Surg Oncol 2014;21:3938–46.
[12] Zhang N, Jiang J, Tang S, Sun G. Predictive value of neutrophil-lymphocyte ratio and platelet-lymphocyte ratio in non-small cell lung cancer patients treated with immune checkpoint inhibitors: a meta-analysis. Int Immunopharmacol 2020;85:106677.
[13] Halazun KJ, Hardy MA, Rana AA, Woodland DC, Luyten EJ, Mahadev S, et al. Negative impact of neutrophil-lymphocyte ratio on outcome after liver transplantation for hepatocellular carcinoma. Ann Surg 2009;250:141–51.
[14] He JR, Shen GP, Ren ZF, Qin H, Cui C, Zhang Y, et al. Pretreatment levels of peripheral neutrophils and lymphocytes as independent prognostic factors in patients with nasopharyngeal carcinoma. Head and Neck 2012;34:1769–76.
[15] Schmidt H, Bastholt L, Geertsen P, Christensen IJ, Larsen S, Gehl J, et al. Elevated neutrophil and monocyte counts in peripheral blood are associated with poor survival in patients with metastatic melanoma: a prognostic model. Br J Cancer 2005;93:273–8.
[16] Krenn-Pilko S, Langsenlehner U, Turner EM, Stojakovic T, Pichler M, Gerger A, et al. The elevated preoperative derived neutrophil-to-lymphocyte ratio predicts poor clinical outcome in breast cancer patients. Br J Cancer 2014;110:2524–30.
[17] Cupp MA, Cariolou M, Tzoulaki I, Aune D, Evangelou E. Berlanga-Taylor AJ et al Neutrophil to lymphocyte ratio and cancer prognosis: an umbrella review of systematic reviews and meta-analyses of observational studies. BMC Med 2020;18:360.
[18] Fujisawa Y, Yoshino K, Otsuka A, Funakoshi T, Fujimura T, Yamamoto Y, et al. Baseline neutrophil to lymphocyte ratio combined with serum lactate dehydrogenase level associated with outcome of nivolumab immunotherapy in a Japanese advanced melanoma population. Br J Dermatol 2018;179:213–5.
[19] Bagley SJ, Kothari S, Aggarwal C, Bauml JM, Alley EW. Evans TL et al Pretreatment neutrophil-to-lymphocyte ratio as a marker of outcomes in nivolumab-treated patients with advanced non-small-cell. Lung cancer 2017;106:1–7.
[20] Martens A, Wistuba-Hamprecht K, Geukes Foppen M, Yuan J, Postow MA, Wong P, et al. Baseline peripheral blood biomarkers associated with clinical outcome of advanced melanoma patients treated with ipilimumab. Clin Cancer Res 2016;22:2908–18.
[21] Lobefaro R, Viscardi G, Di Liello R, Massa G, Iacovino ML, Sparano F, et al. Immunotherapy in advanced non-small cell lung cancer patients with poor performance status: the role of clinical-pathological variables and inflammatory biomarkers. Lung Cancer 2021;152:165–73.
[22] Schwartz MK. Enzymes as prognostic markers and therapeutic indicators in patients with cancer. Clin Chim Acta 1992;206:77–82.
[23] Yang C, Cheng H, Luo G, Lu Y, Guo M, Jin K, et al. The metastasis status and tumor burden-associated CA125 level combined with the CD4/CD8 ratio predicts the prognosis of patients with advanced pancreatic cancer: A new scoring system. Eur J Surg Oncol 2017;43:2112–8.
[24] Tay RE, Richardson EK, Toh HC. Revisiting the role of CD4+ T cells in cancer immunotherapy—new insights into old paradigms. Cancer Gene Ther 2021;28:5–17.
[25] Cachot A, Bilous M, Liu YC, Li X, Saillard M, Cenerenti M, et al. Tumor-specific cytolytic CD4 T cells mediate immunity against human cancer. Sci Adv 2021;7:eabe3348.
[26] Tanaka A, Sakaguchi S. Targeting Treg cells in cancer immunotherapy. Eur J Immunol 2019;49:1140–6.
[27] Hao CJ, Li J, Liu P, Li XL, Hu YQ, Sun JC, et al. Effects of the balance between type 1 and type 2 T helper cells on ovarian cancer. Genet Mol Res 2016. https://doi.org/10.4238/gmr.15027936.
[28] Lee HL, Jang JW, Lee SW, Yoo SH, Kwon JH, Nam SW, et al. Inflammatory cytokines and change of Th1/Th2 balance as prognostic indicators for hepatocellular carcinoma in patients treated with transarterial chemoembolization. Sci Rep 2019;9:3260.
[29] Gonda K, Shibata M, Ohtake T, Matsumoto Y, Tachibana K, Abe N, et al. Myeloid-derived suppressor cells are increased and correlated with type 2 immune responses, malnutrition, inflammation, and poor prognosis in patients with breast cancer. Oncol Lett 2017;14:1766–74.