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ORIGINAL RESEARCH

Thymosin α1 Elevates Lymphocyte Counts and Improves Immunoradiotherapy Outcomes in Patients with Advanced Cancer

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Background: Radiotherapy combined with immunotherapy shows increasing efficacy in treating metastatic malignancies; however, positive outcomes may be negatively impacted by lymphocytopenia. Previous studies suggest thymosin α1 (Tα1) may mitigate radiation-induced lymphocytopenia. This study retrospectively evaluated the effects of a Tα1 loading dose on peripheral blood lymphocyte counts and assessed the safety and efficacy of radiotherapy combined with of PD-1 inhibitors in patients with advanced or refractory cancers.

Methods: A total of 48 patients received a 7-day loading dose of Tα1 (1.6 or 3.2 mg, once daily) followed by hypofractionated radiotherapy and PD-1 inhibitors. Peripheral blood T cells, B cells, and natural killer cells were quantified by flow cytometry before and after Tα1 treatment. The primary endpoint was the change from baseline in lymphocyte subset counts. Secondary endpoints included adverse events, objective response rate (ORR), disease control rate (DCR), progression-free survival (PFS), and overall survival (OS).

Results: The median follow-up was 13.7 months. Tα1 treatment for 7 days significantly increased the median counts of peripheral blood total T cells (422.5/μL to 614.0 /μL, P<0.001), $CD4^+$ T cells (244.5/μL to 284.5/μL, P<0.001), and $CD8^+$ T cells (159.0/μL to 222.5/μL, P<0.001). Among the 36 patients with evaluable data, the ORR was 19.4% and DCR was 69.4%. The median PFS and OS were 5.1 months and 9.6 months, respectively. Two patients (4.2%) experienced grade ≥3 treatment-related adverse events.

Conclusion: A 7-day loading dose of Tα1 elevated lymphocyte counts in advanced cancer patients and was accompanied by satisfactory safety and efficacy profiles. It should be noted that the median follow-up of 13.7 months may be insufficient to fully assess long-term survival outcomes and the potential for late-onset toxicities. As this was an exploratory analysis across multiple tumor types, these findings warrant validation in larger, randomized studies with more homogenous cohorts.

Keywords: LYMPHOCYTOPENIA, Radiotherapy, PD-1 inhibitor, thymosin α1, loading dose, advanced cancer

Introduction

Radiotherapy is increasingly recognized as a pivotal additional treatment modality in the evolving landscape of immunotherapy, and it is significantly changing the paradigm in cancer treatment.[1] In particular, the combination of radiotherapy, inhibitors of programmed cell death protein 1 (PD-1), and granulocyte-macrophage colony stimulating factor (GM-CSF) has markedly improved the treatment of advanced cancers, as demonstrated in clinical studies such as the PRaG and SWORD trials.[2,3] Subgroup analysis demonstrated a significant association between lymphocyte subset counts and treatment efficacy in PRaG therapy, where elevated CD3+ T cells, CD3+CD4+ T cells, CD3+CD8+ T cells, and NK cells after one treatment cycle correlated with improved clinical outcomes.[2]

However, lymphopenia is a common challenge for cancer patients at various stages of disease management. A growing body of evidence indicates that patients who develop severe lymphocytopenia after radiotherapy derive substantially less benefit from subsequent immunotherapy than patients who maintain higher lymphocyte counts.[4,5]

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Because advanced cancer patients are frequently immunocompromised due to reduced lymphocyte counts —a condition that can be exacerbated by radiotherapy —the administration of radioimmunotherapy can be challenging. While a universally accepted standard for defining the severity of lymphopenia has yet to be established, the most widely used criterion is the Common Terminology Criteria for Adverse Events (CTCAE) version 5.0, which classifies an absolute lymphocyte count (ALC) $<0.5 \times 10^9$/L as grade 3 or higher lymphopenia. Meta-analyses in various malignancies, including pancreatic and head and neck cancers, have shown that grade 3 or higher lymphopenia is significantly associated with a poorer prognosis.[6]

Therefore, it is crucial to monitor lymphocyte counts and devise strategies to protect or replenish these immune cells, though this remains a significant challenge in clinical practice. Several strategies have been explored to counteract treatment-induced lymphocytopenia, including the administration of cytokines such as interleukin (IL)-2 and IL-7 or the use of adoptive cell transfer. However, these approaches have been hampered by significant adverse effects, restricting their widespread clinical adoption. This underscores the need for a well-tolerated, readily available agent that can effectively preserve lymphocyte counts and function within the context of radioimmunotherapy.

In this context, the immunomodulatory peptide thymosin alpha 1 (Tα1) named thymalfasin, has emerged as a potential solution to this challenge. Tα1 is a highly conserved 28-amino acid peptide produced by the thymus and plays several important roles in the immune-related processes including T cell maturation and differentiation,[7,8] modulation of allogeneic and autologous $CD4^+$ T cell responses, and regulation of sensitivity to radiation. Thymalfasin has been widely applied in both infectious diseases and oncology. Expert consensus recommends its use as an adjunctive therapy for conditions such as sepsis, hepatitis B, and lymphopenic COVID-19 patients. In the oncology setting, it is also suggested as an adjuvant treatment for various malignancies, including hepatocellular carcinoma, pancreatic cancer, and lung cancer, according to expert guidelines. In patients with infection-associated sepsis, Tα1 administered at a loading dose of 1.6 or 3.2 mg daily for 5 to 7 days proved effective in mitigating lymphocytopenia and significantly increased peripheral blood $CD4^+$ and $CD8^+$ T cell counts in patients with severe lymphocytopenia.[9–12]

Based on these findings, we conducted a retrospective study in patients with advanced tumors with the primary goal of evaluating the ability of a loading dose of Tα1 to increase lymphocyte counts, and the secondary goal of evaluating the effects of Tα1 on the safety and efficacy of subsequent radiotherapy and immunotherapy.

Subjects and Methods

Ethics Statement

In this retrospective study, patient consent for reviewing their medical records was waived by the Ethics Committee of the Second Affiliated Hospital of Soochow University because the study involved the secondary use of anonymized medical data for scientific research purposes, and could not reasonably feasible be conducted without it. All patient data were handled with strict confidentiality, and no personal identifying information was included in the dataset. Identifiers were removed to ensure anonymity and protect patient privacy in accordance with institutional policies and national regulations on data protection and medical ethics. The study was conducted in compliance with the principles of the Declaration of Helsinki (World Medical Association, 2013), and received approval from the hospital's Ethics Committee (code: JD-HG-2023-69).

Patient Selection and Data Collection

This study retrospectively analyzed clinical data from patients with advanced solid cancers who were treated at our institution between June 2020 and August 2023 with a PD-1 inhibitor in combination with radiotherapy, after standard treatments were either ineffective or intolerable. The primary aim was to assess the effect of a loading dose of Tα1 on lymphocytopenia, and the secondary aim was to evaluate the safety and treatment outcomes of the combined therapy in this patient population.

The inclusion criteria were as follows: (i) aged ≥18 years; (ii) histologically or radiographically confirmed advanced or metastatic solid cancers; (iii) received Tα1 (ZADAXIN, Sciclone Pharmaceuticals) via subcutaneous (SC) injection at a loading dose of 1.6 or 3.2 mg once daily (QD) for 7 consecutive days; (iv) available flow cytometry data on routine

peripheral blood lymphocyte counts conducted during immunotherapy; (v) treatment regimen consisting of at least two cycles of hypofractionated radiotherapy and PD-1 inhibitor within one week after the completion of radiotherapy; and (vi) follow-up period of at least 6 weeks after the initiation of immunotherapy. The exclusion criteria included: (i) incomplete peripheral blood count data and (ii) severe systemic or hematologic conditions.

Follow-up

The main primary endpoint was the change from baseline to the end of Tα1 treatment in peripheral blood lymphocyte counts. The secondary endpoints were treatment-related adverse events (TRAEs), objective response rate (ORR), disease control rate (DCR), progression-free survival (PFS), and overall survival (OS). The nature, frequency, and severity of adverse events were assessed based on the Common Terminology Criteria for Adverse Events version 5.0 (CTCAE 5.0). Tumor assessments were conducted at baseline, week 6, and every 6 weeks thereafter. Clinical responses were evaluated using the Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1. The ORR was defined as the percentage of patients achieving a complete response (CR) or partial response (PR) to treatment; DCR was defined as the percentage of patients who achieved CR, PR, or stable disease (SD); OS was defined as the time from the initiation of immunotherapy to either the follow-up cutoff date or death; PFS was defined as the time from the start of radiotherapy to the date of progression or death. Patients were followed up every 6 weeks from June 2020 to August 2023 through electronic medical records or telephone calls to monitor their survival status. The final follow-up date was November 30, 2023.

Flow Cytometry

Peripheral blood samples (2 mL) were collected into ethylenediaminetetraacetic acid tubes before and at the end of the 7-day Tα1 treatment. Erythrocytes were lysed using Optilyse (Immunotech, Marseille, France). Leukocytes (50 μL) were then mixed with 20 μL of fluorochrome-conjugated monoclonal antibodies against human CD3, CD4, CD8, CD19, CD16, and CD56 (BD Biosciences, Franklin Lakes, NJ, USA) and incubated for 15–20 min at room temperature in the dark. Data were acquired using a FACSCanto flow cytometer (BD Biosciences) and analyzed with FlowJo 8 software (BD Biosciences). Pretreatment blood samples were also analyzed for levels of cytokines (interleukin [IL]-2, IL-4, IL-6, IL-10, IL-17A, tumor necrosis factor [TNF], and interferon-γ [IFN-γ] using a flow cytometer kit.

Statistical Analysis

Data were analyzed using Python 3.11.2 and the SciPy 1.11.1 package. The normality of continuous variables was evaluated using the Shapiro–Wilk test. Normally distributed continuous data are presented as the mean ± standard deviation, and non-normally distributed data are presented as the median and interquartile range (Q1–Q3). Categorical variables are reported as absolute counts and percentages. T-tests and non-parametric tests were used to compare normally and non-normally distributed data, respectively. Factors influencing T cell counts were analyzed using MLxtend v0.23.1 and statsmodels v0.14.0, with logistic regression employed to identify significant predictors. Survival was analyzed using Lifelines 0.27.7. The Kaplan–Meier method was employed to estimate OS and PFS, and differences were analyzed using the Log rank test. Multivariate Cox proportional hazards regression was used to assess prognostic factors. All reported P values are two-tailed, and a P value of <0.05 was considered statistically significant.

Results

Baseline Characteristics

In total, 48 patients were enrolled in the study and received the loading dose of Tα1. Six patients were excluded due to severe infections that prevented further immunotherapy, and six were excluded for lack of adequate evaluation for tumor assessment or survival outcomes. Ultimately, 36 patients were included in the analysis of radioimmunotherapy treatment outcomes (Figure 1). The baseline characteristics of the 48 patients are summarized in Table 1. The median age was 61 years, and 62.5% of the patients were male. The majority of the patients (68.7%) had an Eastern Cooperative Oncology Group performance status score of 2–3. Colorectal cancer was the most common primary tumor (27.1%). More than half

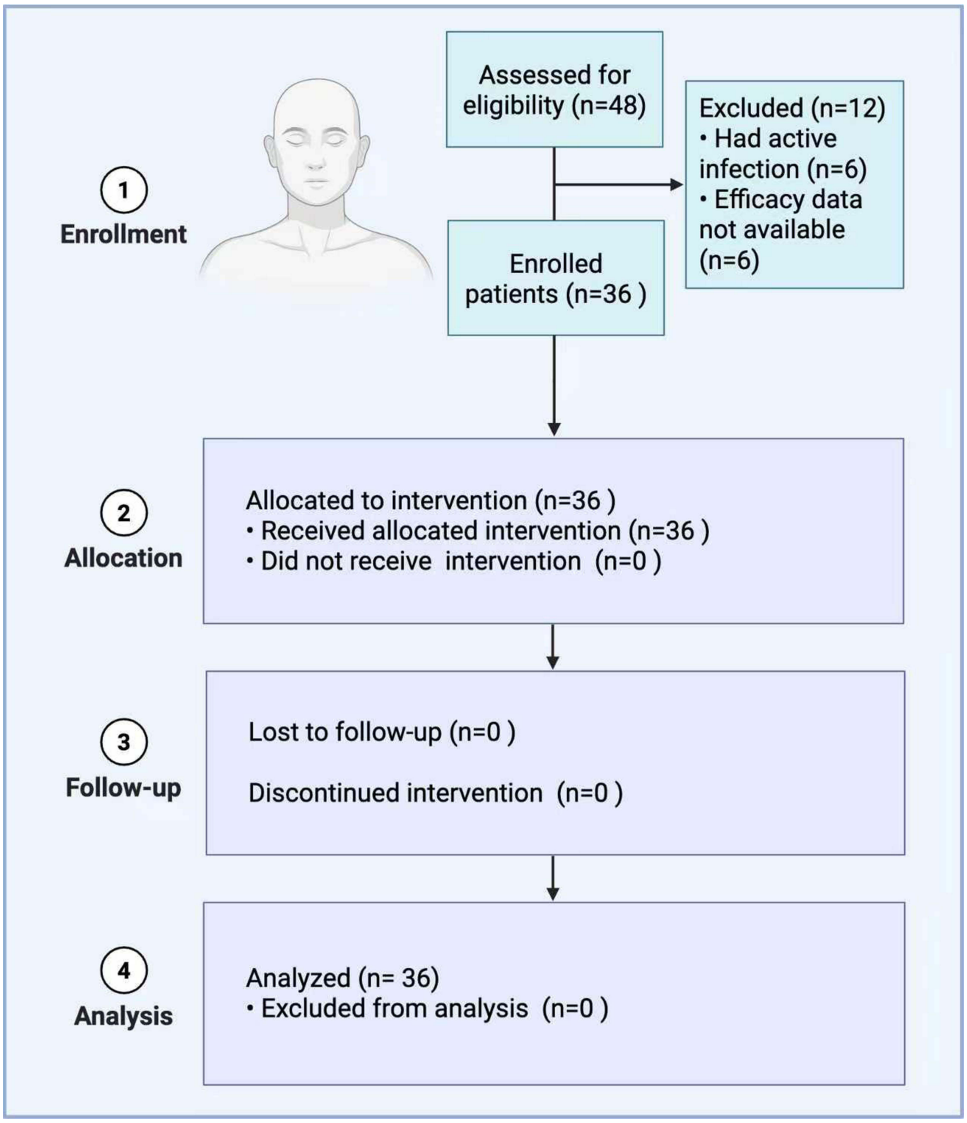

Figure 1 CONSORT diagram.

of the patients (25/48, 52.1%) had received second- or later-line systemic therapy; 18 patients (37.5%) had undergone prior immunotherapy; and 26 patients (54.2%) received radiotherapy concurrently with Tα1 treatment. Tα1 was administered at 3.2 mg QD in 31 patients (64.6%) and at 1.6 mg QD in 17 patients (35.4%).

Table 1 Patient Clinicopathological Characteristics

Characteristic	No. (%)
Age, median, range (years)	61 (36~87)
Gender	
Male	30 (62.5%)
Female	18 (37.5%)
ECOG performance status	
1	15 (31.3%)
2	21 (43.7%)
3	12 (25.0%)

(*Continued*)

Table 1 (Continued).

Characteristic	No. (%)
No. of prior systemic therapies, median, range	
0	9 (18.7%)
1	14 (29.1%)
2	10 (20.8%)
≥3	15 (31.3%)
Primary cancer sites	
Colorectum	13 (27.1%)
Gastric	7 (14.6%)
Pancreatic	6 (12.5%)
Esophagus	5 (10.4%)
Head and neck	4 (8.3%)
Cervix	4 (8.3%)
Others*	9 (18.8%)
*Lung 3 (6.3%), Liver 2 (4.2%), Bile duct 2 (4.2%), Ovary 1 (2.1%), Retroperitoneal 1 (2.1%)	
Primary radiotherapy	
Yes	20 (41.7%)
No	28 (58.3%)
Primary immunotherapy	
Yes	18 (37.5%)
No	30 (62.5%)
Dose of thymalfasin	
1.6mg QD	17 (35.4%)
3.2mg QD	31 (64.6%)
Radiotherapy during thymalfasin	
Yes	26 (54.2%)
No	22 (45.8%)
Dose and fractions	
5Gy*3f	12 (25.0%)
8Gy*2f	13 (27.1%)
8Gy*3f	15 (31.2%)
Others	8 (16.7%)
Irradiation site	
Chest	12 (25.0%)
Abdomen	21 (43.7%)
Pelvic	8 (16.7%)
None	5 (10.4%)
Others	2 (4.2%)
Peripheral lymphocyte counts	
$<0.5\times10^9$/L	14 (29.2%)
$\geq0.5\times10^9$/L	34 (70.8%)

Abbreviation: ECOG, Eastern Cooperative Oncology Group.

Changes in Lymphocyte Counts

Tα1 treatment resulted in rapid changes in several peripheral blood lymphocyte subsets (Table 2 and Figure 2). At baseline, the median total ($CD3^+$) T cell count was 422.5 cells/μL, $CD4^+$ T cell count was 244.5 cells/μL, and $CD8^+$ T cell count 159.0 cells/μL. After one week of Tα1 treatment, the total T cell count increased to 614.0 cells/μL ($P<0.001$), the $CD4^+$ T cell count to 284.5 cells/μL ($P<0.001$), and the $CD8^+$ T cell count to 222.5 cells/μL ($P<0.001$). These findings indicate that a loading dose of Tα1 rapidly increased the abundance of total $CD3^+$, $CD4^+$, and $CD8^+$ T lymphocytes in peripheral blood.

Table 2 Treatment-Related Changes in Peripheral Blood Lymphocytes

	Before Treatment	After Treatment	*P* value
$CD3^+$ T cell	422.5 (285.0–547.5)	614.0 (379.3–726.5)	<0.001
$CD3^+CD4^+$ T cell	244.5 (151.5–287.3)	284.5(197.0–402.8)	<0.001
$CD3^+CD8^+$ T cell	159.0 (117.0–232.8)	222.5 (156.0–343.8)	<0.001
B cell	44.0 (21.8–73.5)	35.5(20.8–69.3)	0.419
NK cell	159.8±106.2	224.1±164.8	<0.001
$CD4^+/CD8^+$ ratio	1.4(0.99–2.0)	1.3 (0.9–2.2)	0.703

Factors Influencing Tα1-Induced Changes in T Lymphocyte Counts

Stepwise regression analysis was conducted on baseline characteristics, pretreatment lymphocyte subsets, and cytokines, which resulted in the inclusion of eight factors in the multivariate analysis of parameters associated with the change in T cell counts: radiation dose, age, neutrophil-to-lymphocyte ratio (NLR), neutrophil count, and pretreatment levels of IL-2, IL-4, IL-10, and TNF. The multiple linear regression model demonstrated strong predictive power, with an R-squared value of 0.916, an adjusted R-squared value of 0.892, and an F value of 38.21 (P<0.001). The results of the multivariate analysis indicated that NLR, neutrophil count, and IL-2 level were significant factors associated with the increase in T lymphocyte counts (Table 3).

Safety Profile

The observed TRAEs are listed in Table 4. A total of 18 patients experienced TRAEs, the most common being fatigue (14.6%), followed by appetite loss (10.4%), fever (6.3%), thyroid dysfunction (2.1%), rash (2.1%), alopecia (2.1%), myocarditis (2.1%), and encephalitis (2.1%). Two patients experienced grade 3 TRAEs, one each of myocarditis and encephalitis; both were attributed to immunotherapy and improved with appropriate symptomatic treatment. No grade 4 TRAEs or deaths occurred in this study.

Tumor Responses and Patient Survival

Tumor responses were evaluated using RECIST v1.1 criteria. CR was observed in 2 patients, PR in 5 patients, and SD in 18 patients. The ORR was 19.4% and the DCR was 69.4%. The median follow-up time was 13.7 months. The median PFS was 5.13 months (95% confidence interval [CI] = 2.97–8.0 months) and the median OS was 9.6 months (95% CI = 6.33–19.67 months) (Figure 3).

Univariate analysis identified several factors significantly associated with OS, including number of cycles of PD-1 inhibitor combined with radiotherapy, tumor response, pretreatment γ-IFN level, and NLR (Supplementary Table 1). In multivariate analysis, NLR (hazard ratio [HR] = 1.08, 95% CI = 1.009–1.167; P = 0.029) and the number of therapy cycles (HR = 0.844, 95% CI = 0.749–0.951; P = 0.005) remained significant independent factors associated with OS (Figure 4). Further analysis revealed no significant correlation between severe lymphocytopenia at baseline and OS (Supplementary Figure 1). Univariate analysis also identified factors significantly associated with PFS, including number of prior lines of systemic therapy, prior immunotherapy, number of therapy cycles, radiotherapy dose, tumor response, and percentage of $CD3^+$ T cells (Supplementary Table 2). Multivariate analysis confirmed that the number of therapy cycles (HR = 0.869, 95% CI = 0.781–0.968; P = 0.011) and tumor response (HR = 0.041, 95% CI = 0.009–0.192; P<0.001) were significant independent factors associated with PFS (Figure 5).

Discussion

In this study, we retrospectively evaluated the immunomodulatory effects of a loading dose of Tα1 (3.2 mg or 1.6 mg QD for 7 days) in patients with advanced tumors who were treated with a PD-1 inhibitor in combination with radiotherapy. Our findings indicate that Tα1 significantly increased total $CD3^+$ T cells, as well as $CD4^+$ and $CD8^+$ T cell subsets, in the peripheral blood. To the best of our knowledge, this is the first study to document that a loading dose of Tα1 increases the

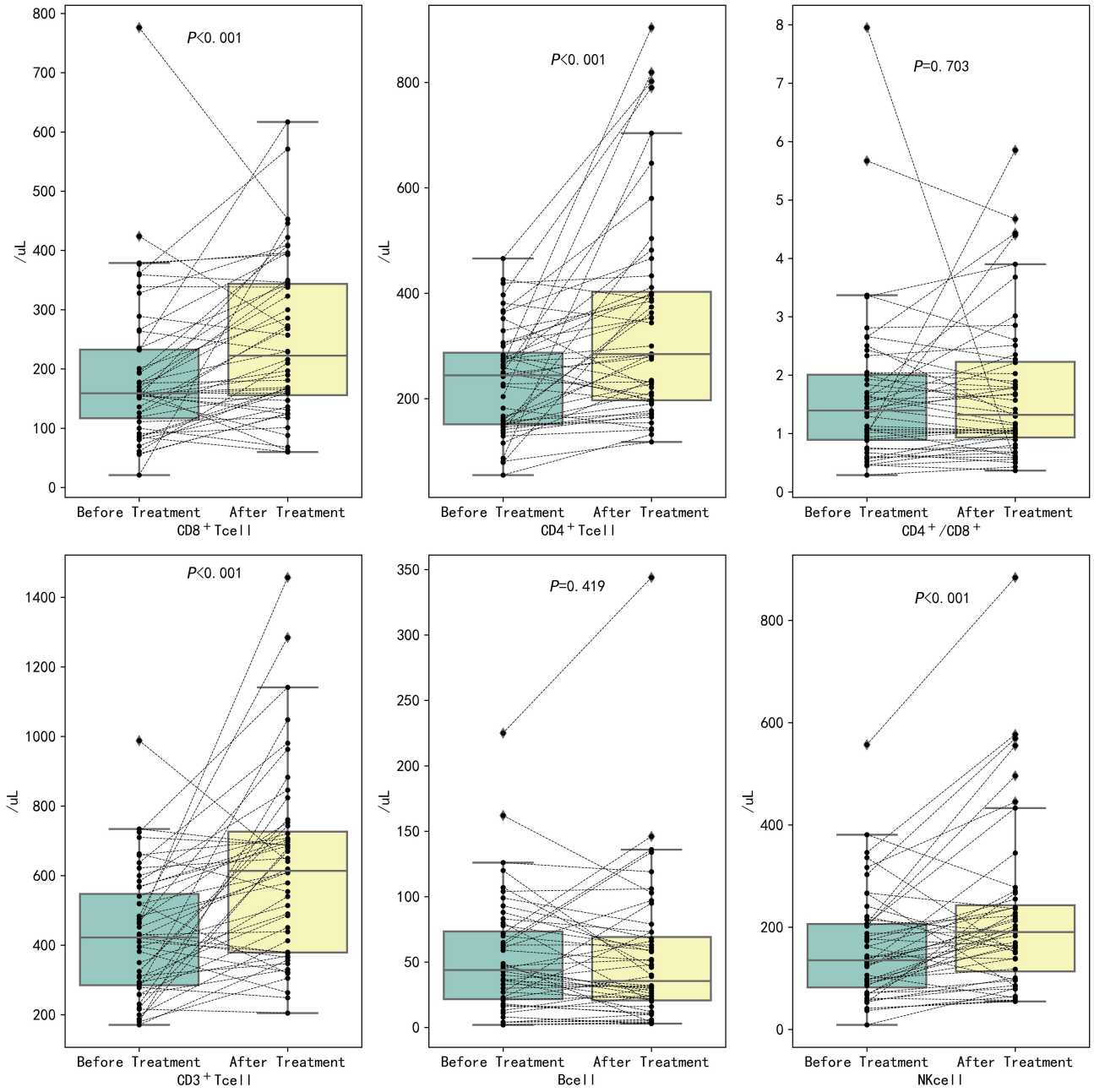

Figure 2 Treatment-related changes in peripheral blood lymphocytes. Each panel shows the paired comparison of specific immune cell subsets before and after treatment. Data are presented as paired individual values (black dots and connecting lines) and boxplots summarizing group distributions (25th to 75th percentiles with whiskers extending to 1.5× the interquartile range). Statistical significance was evaluated using a paired test, and p-values are indicated above each plot.

abundance of T cells and T cell subsets in patients with advanced tumors. The findings in this study preliminarily elucidated the "signal" of safety and efficacy of Thymosin α1 in the context of radiotherapy combined with immunotherapy, and provided preliminary rationale and clues for future research directions and patient selection.

We previously published a retrospective analysis demonstrating that exposure of the spleen to an irradiation dose as low as 5 Gy can significantly reduce lymphocyte counts and adversely affect the survival of patients with advanced solid tumors.[13] We also showed that a new regimen of PD-1 inhibitors combined with GM-CSF and radiotherapy was efficacious in patients with advanced malignancies (REF: PRaG1.0). In that study, subgroup analysis indicated a positive correlation between better outcome and increases in lymphocyte subsets, particularly total $CD3^+$ T cells, $CD4^+$ and $CD8^+$ T cell subsets, and natural killer (NK) cells.[2] It has been widely recognized that low lymphocyte counts

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Table 3 Multiple Linear Regression Analysis of Factors Associated with Increases in T Lymphocyte Counts

	β value	Lower 95%	Upper 95%	*P* value
Radiation doses	−0.0163	−0.045	0.012	0.251
Age	0.0038	−0.006	0.014	0.452
NLR	0.0546	0.014	0.096	0.011
N	0.2006	0.048	0.353	0.012
IL-2	0.1214	0.023	0.22	0.017
IL-10	−0.0069	−0.02	0.006	0.301
IL-4	−0.0103	−0.038	0.017	0.443
TNF	−0.0017	−0.042	0.039	0.931

Abbreviations: NLR, neutrophil-to-lymphocyte ratio; N, neutrophil count; IL-2, interleukin-2; IL-4, interleukin-4; IL-10, interleukin-10; TNF, tumor necrosis factor.

Table 4 Treatment-Related Adverse Events

Adverse Events	Any Grade	Grade 3
Fatigue	7 (14.6%)	0 (0%)
Anorexia	5 (10.4%)	0 (0%)
Fever	3 (6.3%)	0 (0%)
Hypothyroidism	1 (2.1%)	0 (0%)
Rash	1 (2.1%)	0 (0%)
Alopeciaxi	1 (2.1%)	0 (0%)
Carditis	1 (2.1%)	1 (2.1%)
Encephalitis	1 (2.1%)	1 (2.1%)

are associated with poor prognosis in patients with various malignancies, especially after chemoradiotherapy. For instance, decreases from baseline in NK cells and $CD8^+$ T cells are linked to an increased risk of early mortality following chemotherapy in patients with metastatic breast cancer;[14–16] a reduced $CD4^+$ T cell count is an independent prognostic factor for poor outcomes in patients with advanced breast cancer and other solid tumors; and poor responses of patients with head and neck squamous carcinoma to immune checkpoint inhibitors are associated with lymphocytopenia, particularly grade 3 or higher, both before and 2–3 months after treatment.[17] Thus, our current findings that a Tα1 loading dose improves lymphocyte counts could have significant implications for the management of lymphocytopenia in patients with advanced malignancies.

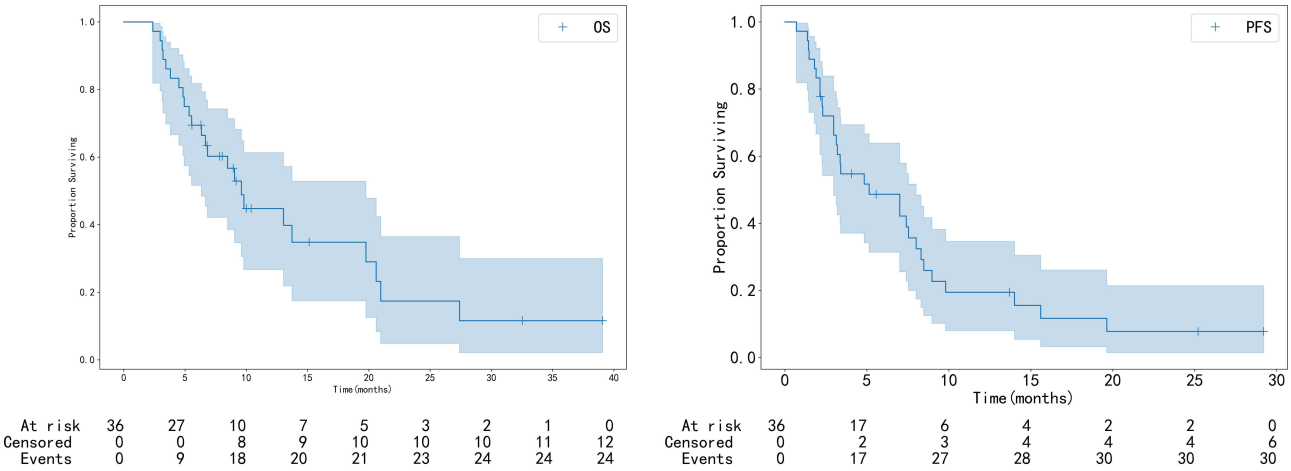

Figure 3 Kaplan–Meier curves of overall survival (left) and progression-free survival (right).

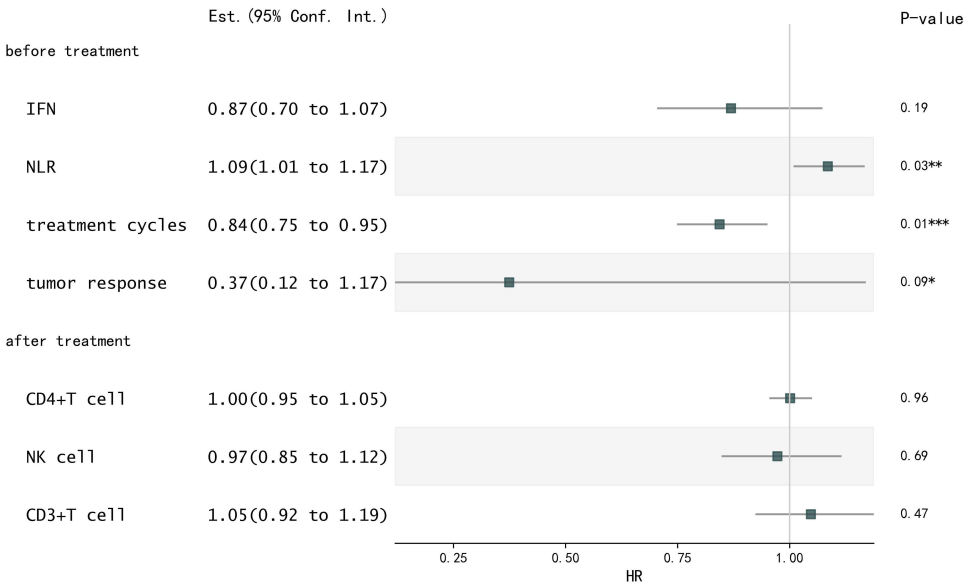

Figure 4 Forest plot showing multivariable hazard ratios for factors associated with death. Hazard ratios (and confidence intervals) were computed using a Cox proportional hazards model.

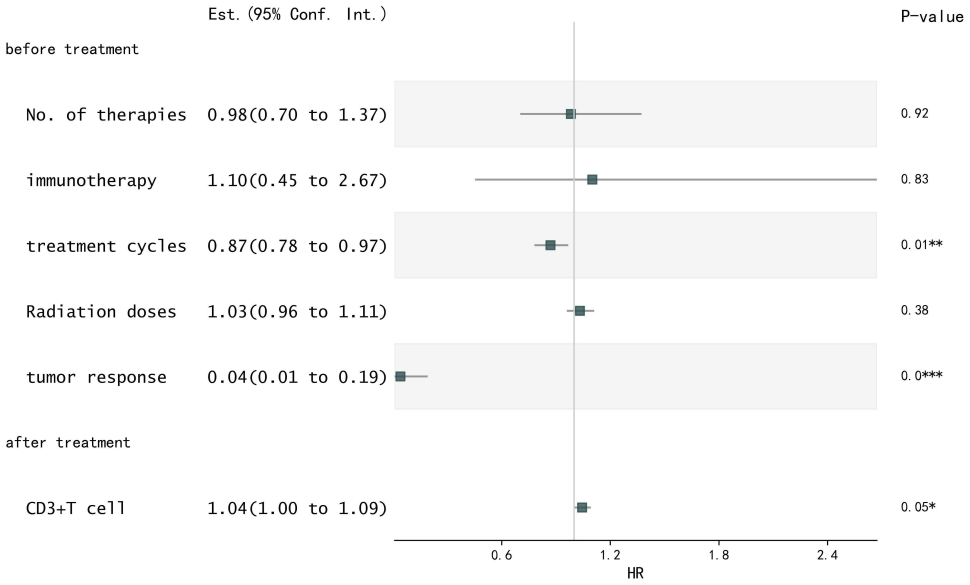

Figure 5 Forest plot displaying multivariable hazard ratios (HR) for factors associated with disease progression. Each factor's HR and corresponding 95% confidence interval (CI) are derived using a Cox proportional hazards model.

Currently, there are few effective treatments for lymphocytopenia in patients with advanced solid tumors. IL-2, IL-7, and IL-15 each play important roles in T cell development, proliferation, and survival; however, some clinical trials of these compounds have failed due to unacceptable side effects, while others are still in the developmental stage. In contrast, Tα1 has been widely used in immunocompromised patients, including those with various advanced tumors and sepsis. For example, GASTO-1043, a prospective cohort study in patients with inoperable locally advanced non-small cell lung cancer (NSCLC), showed that combination Tα1 therapy (1.6 mg weekly) and chemoradiotherapy significantly reduced the incidence of grade 3 or higher lymphocytopenia compared with chemoradiotherapy alone (19.1% vs 62.1%, P<0.001), suggesting that Tα1 may help mitigate chemoradiotherapy-induced lymphocyte depletion.[18] An earlier randomized controlled trial showed that loading doses of Tα1 improved immune function and prolonged the OS

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compared with placebo in patients with advanced NSCLC treated with curative radiotherapy.[19] A prospective randomized controlled trial of patients with sepsis found that twice-daily subcutaneous injections of Tα1 significantly reduced mortality in critically ill patients without increasing drug-related adverse events.[10] Zeng et al[20] conducted a meta-analysis of 27 randomized controlled trials investigating combined treatment with Tα1 and chemotherapy for patients with NSCLC, and they found that Tα1 enhanced peripheral blood lymphocyte counts in these patients. Our findings are consistent with this observation, even though the general condition of our patient cohort was poorer than that of the patients included in the meta-analysis by Zeng et al.[20]

In the present study, baseline NLR showed a significant positive association with Tα1-induced increases in $CD8^+$ T cell counts. NLR is a measure of systemic inflammation, and the inflammatory response itself is considered an important factor leading to T cell lymphocytopenia.[21] Evidence also suggests that NLR might predict the response to PD-1 inhibitors in patients with NSCLC.[22,23] Tα1 has been associated with improvements in systemic inflammatory responses, and a study of patients undergoing liver cancer resection showed that NLR was significantly reduced in the patient cohort treated with Tα1.[24] Liu et al found that Tα1 alleviated inflammation in patients with sepsis who were experiencing both hyperinflammation and immunosuppression.[25] Given that an elevated NLR is linked to a stronger inflammatory response, which can result in reduced T lymphocyte counts, Tα1 might enhance lymphocyte abundance by attenuating the hyperinflammatory state in patients with advanced solid tumors, thereby reducing inflammation-induced reductions in T lymphocyte counts. However, studies with larger sample sizes will be needed to validate this hypothesis. Future research should focus on the dynamics of specific lymphocyte subsets, such as $CD4^+$ T cells, $CD8^+$ effector memory T cells, T regulatory cells, as well as changes in related cytokines.[26–28]

In this present study, we focused on the patients with recurrent metastatic or advanced solid tumors and usually had severe lymphocytopenia, which had been demonstrated a poor prognosis indicator for immunotherapy. Qin's study of patients with advanced esophageal cancer treated with radiotherapy receiving immunotherapy found that the median OS was 6 months for patients with lymphocytopenia (absolute lymphocyte count [ALC]) ≤625 cells/μL) compared with 12 months for those in non-lymphopenia group (ALC >625 cells/μL).[29] Another retrospective study of the efficacy of immunotherapy in patients with advanced NSCLC reported median PFS and OS of 2.2 and 5.7 months, respectively, for the patients with lymphocytopenia (ALC <1000 cells/μL) compared with 5.9 and 12.1 months, respectively, for patients with normal lymphocyte counts.[30] When compared with these studies and our previous preliminary report on the study of patients with heavily treated advanced solid tumors treated with radiotherapy and immunotherapy,[6] patients enrolled in this study had lower baseline $CD3^+$ T cell counts (422.5 cells/μL) and more than half of them had G3~4 lymphocytopenia, while better ORR, DCR, PFS, and OS were obtained after addition of a loading dose of Tα1, suggested that mitigating lymphocytopenia by loading-dose Tα1 to a regimen of PD-1 inhibitors and radiotherapy may further improve the tumor response and survival outcomes in patients with advanced cancers, and warrants further exploration.

Theelen et al[31] demonstrated that the number of radiotherapy fractions and the total dose correlated with treatment efficacy in patients with metastatic NSCLC. They found that the incidence of the abscopal effect differed significantly in patients receiving three different fractionation regimens, and was 20% with 45 Gy/15 fractions, 47% with 24 Gy/3 fractions, and 56% with 50 Gy/4 fractions. The authors suggested that these findings might be related to the significant T cell depletion specifically associated with the 45 Gy /15 fractions regimen. In our study, hypofractionated radiotherapy was administered in only 2 or 3 fractions, resulting in no substantial differences in lymphocyte counts before and after treatment. Furthermore, we did observe no significant differences in T lymphocyte changes among different irradiation sites (Supplementary Figure 2). However, the optimal Tα1 dose and frequency of administration in combination with PD-1 inhibitors and radiotherapy require further investigation.

Our study has several limitations. It is important to emphasize that this study is a retrospective analysis, and its inherent design limitations, including potential selection bias and uncontrolled confounding factors, restrict its ability to draw causal conclusions. Furthermore, the inclusion of patients with diverse tumor types, most of whom were heavily pretreated, makes it difficult to draw definitive conclusions for any specific cancer type. This also suggests that our findings need to be validated in more homogeneous prospective cohorts. Despite the limitations mentioned above, the observed effectiveness of Thymosin α1 in increasing lymphocyte counts, its combination therapy demonstrating a favorable safety profile, and the encouraging survival trends seen in some patients with refractory disease—these

data provide preliminary support for a promising treatment strategy. This strongly suggests the necessity of further validating the role of Thymosin α1 in prospective clinical trials. Future studies should include a larger sample size and aim to optimize the study design and adjust the Tα1 dosage and treatment cycles based on patient T lymphocyte counts, and include serial lymphocyte monitoring throughout the entire treatment course to determine the sustainability of the effect and its relationship with long-term outcomes. An ongoing prospective clinical study "anonymized for review" (NCT: anonymized for review)[32] is expected to provide support for the therapeutic benefit of radio-immunotherapy via the improvement of lymphocyte counts.

Conclusions

Administration of a loading dose of Tα1 rapidly and safely elevated peripheral blood counts of T lymphocytes and subsets in patients with advanced cancer, suggesting that this strategy may have utility for enhancing the anti-tumor efficacy of PD-1 inhibitors combined with radiotherapy. Additional well-designed prospective clinical trials will be necessary to validate these findings.

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Disclosure

The authors report no conflicts of interest in this work.

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