Impact of thymosin $\alpha 1$ as an immunomodulatory therapy on long-term survival of non-small cell lung cancer patients after R0 resection: a propensity score-matched analysis

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Abstract

Background: There is limited information about thymosin $\alpha 1$ (Tα1) as adjuvant immunomodulatory therapy, either used alone or combined with other treatments, in patients with non-small cell lung cancer (NSCLC). This study aimed to evaluate the effect of adjuvant Tα1 treatment on long-term survival in margin-free (R0)-resected stage IA–IIB NSCLC patients.

Methods: A total of 5746 patients with pathologic stage IA–IIB NSCLC who underwent R0 resection were included. The patients were divided into the Tα1 group and the control group according to whether they received Tα1 or not. A propensity score matching (PSM) analysis was performed to reduce bias, resulting in 1027 pairs of patients.

Results: After PSM, the baseline clinicopathological characteristics were similar between the two groups. The 5-year disease-free survival (DFS) and overall survival (OS) rates were significantly higher in the Tα1 group compared with the control group. The multivariable analysis showed that Tα1 treatment was independently associated with an improved prognosis. A longer duration of Tα1 treatment was associated with improved OS and DFS. The subgroup analyses showed that Tα1 therapy could improve the DFS and/or OS in all subgroups of age, sex, Charlson Comorbidity Index (CCI), smoking status, and pathological tumor-node-metastasis (TNM) stage, especially for patients with non-squamous cell NSCLC and without targeted therapy.

Conclusion: Tα1 as an adjuvant immunomodulatory therapy can significantly improve DFS and OS in patients with NSCLC after R0 resection, except for patients with squamous cell carcinoma and those receiving targeted therapy. The duration of Tα1 treatment is recommended to be $>24$ months.

Keywords: Non-small cell lung cancer; Resection; Adjuvant therapy; Thymosin $\alpha 1$

Introduction

Lung cancer is the leading cause of cancer-related deaths for men and women globally. Non-small cell lung cancer (NSCLC) is the most common type of lung cancer, accounting for approximately 85% of the cases. The selection of the therapeutic options for NSCLC is based on the tumor-node-metastasis (TNM) classification system. Complete surgical resection is the most effective treatment for patients with stage I and II disease and resectable stage IIIA disease. Postoperative recurrence is the most important issue affecting patient survival. Although adjuvant therapy (such as chemotherapy, radiotherapy, and targeted therapy) has been improved in the last decades, overall survival (OS) remains poor. The frequency of postoperative recurrence increases with tumor stage, ranging from 15% in stage IA to 60% in stage IIIA, resulting in a decrease in the expected 5-year survival rate after surgery from 90% to 41%. Therefore, exploring effective adjuvant treatments is important to reduce the recurrence risk and improve prognosis.

The treatment of cancer is entering the era of immunotherapy. This therapy can assist the immune system in attacking and eradicating the cancer cells by enhancing the antitumor immune response and reversing the immune tolerance toward the tumor. Cancer immunotherapy can be broadly classified into two general categories: active and passive. The active approach includes the induction of a tumor-directed immune response through vaccination.

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Immunomodulatory therapy, as a type of non-specific immune stimulation, has been widely used as adjuvant therapy and can improve the long-term outcomes of cancer patients. Thymosin α1 (Tα1) is one of the commonly used immunomodulators and consists of an N-terminal acetylated acidic peptide containing 28 amino acids with a molecular weight of 3108 Da. Tα1 treatment combined with chemotherapy and/or radiotherapy for non-surgical NSCLC patients is associated with improved immune parameters and prolonged progression-free survival (PFS) and OS. Still, whether Tα1 can improve the long-term prognosis of NSCLC patients who underwent complete surgical resection has not been confirmed. The present study aimed to evaluate the impact of Tα1 as an adjuvant immunomodulatory therapy on the long-term survival of patients with NSCLC who underwent complete surgical resection.

Methods

Ethical approval
This retrospective study was approved by the Institutional Review Board of the West China Hospital, Sichuan University (No. 2020-344). Informed consent was waived by the Institutional Review Board because of the retrospective nature of the study.

Patients
Consecutive patients who underwent surgery for primary NSCLC between May 2005 and December 2018 were identified from the Western China Lung Cancer Database (WCLCD), West China Hospital, Sichuan University. The data did not contain any identifiable patient information. The patients were staged according to the seventh edition of the American Joint Committee on Cancer (AJCC) TNM staging system for lung cancer.

The exclusion criteria were pathological stage 0 (ie, carcinoma in situ), IIIIB, or IV disease, positive surgical margin (R1 or R2), lack of staging data, previous history of malignancy, lack of data on postoperative immunomodulator use, adjuvant therapy using other synthetic thymic peptides (eg, thymosin or thymopentin), or death within 90 days after surgery regardless of causes.

The patients were grouped into the Tα1 group or the control group according to whether Tα1 was used or not after surgery.

Data collection
The durations of all prescriptions were calculated to determine the duration of Tα1 administration. Clinicopathological data were collected, including the year of surgery, age at surgery (dichotomized according to the median age of 59 years), sex, body mass index (BMI), history of comorbidity according to the Charlson Comorbidity Index (CCI), smoking status, surgical approach, type of surgery, histological subtypes, pathological TNM stage, neoadjuvant therapy, adjuvant therapy, and follow-up information.

Follow-up
All patients were followed according to the established institutional standards, that is, every 3 to 6 months during the first 5 years after surgery and annually after that. Chest and upper abdominal computed tomography (CT) and brain magnetic resonance imaging (MRI) or CT were performed at every follow-up. Whole-body bone scintigraphy was performed annually. A telephone follow-up was conducted for patients from distant geographical locations and followed at a local hospital. OS was calculated as the time from surgery until death from any cause or last follow-up. Disease-free survival (DFS) was defined as the period from the surgery date until any local or distant recurrence or death or last follow-up. Survival and postoperative therapy data were recorded in the WCLCD at West China Hospital, Sichuan University.

Statistics analysis
The baseline characteristics are presented as counts and proportions. Pearson’s chi-squared test or Fisher’s exact test was used to compare the frequencies of the categorical measures. In order to minimize the selection bias between the two groups, propensity score matching (PSM) was performed using R (version 3.5.2, R Core Team, 2018) and the MatchIt package (Daniel Ho, 2018). The logistic regression model was used as the link model. For regression adjustment to be trustworthy, the standardized mean differences of all the included confounding variables were requested within the recommended limits of −0.25 and 0.25. The following statistically different confounding variables were included: year of surgery, sex, CCI, smoking status, surgical approach, type of surgery, histologic subtypes, pathological TNM stage, neoadjuvant therapy, and adjuvant therapy. At last, the patients were matched 1:1, without replacement, using a nearest neighbor approach without a preset caliper width. The Kaplan–Meier method was used to generate the OS and DFS curves before and after PSM. The differences between the curves were analyzed using the log-rank test. Univariable and multivariable analyses for OS and DFS were carried out using the Cox proportional hazard regression model before and after PSM. Subgroup analyses of OS and DFS were also performed by the Cox proportional hazard regression model after PSM. The level of statistical significance was set at 0.05. All comparisons were two tailed. Statistical tests were performed using SAS for Windows (version 9.4, SAS Institute Inc., Cary, NC, USA) and R (version 3.5.2, R Core Team, 2018).

Results

Characteristics of the patients
A total of 5746 patients were included (1027 in the Tα1 group and 4719 in the control group) [Figure 1]. Among all
Patients with primary NSCLC who underwent surgery between May 2005 and December 2018 (n=10,550) were 5746 selected patients. After PSM, 1027 pairs of patients were obtained, and there were no significant differences in all variables mentioned above. The clinical characteristics of the two groups, both before and after PSM, are shown in Table 1.

Survival outcomes

By December 2019, the median follow-up was 25 (range, 4–160) months for the unmatched patients and 26 (range, 4–159) months for the matched patients. Before PSM, there were 10 (1.0%) and 67 patients (1.4%) lost to follow-up in the T1 group and control groups, respectively. After PSM, 10 (1.0%) and 9 patients (0.9%) in the T1 and control groups were lost to follow-up, respectively. Both before and after PSM, the 5-year DFS was higher in the T1 group than in the control group (before matching: 77.3% vs. 72.7%, P < 0.0001; after matching: 83.3% vs. 72.7%, P < 0.0001) [Figure 2].

The univariable and multivariable analyses for DFS and OS before PSM are presented in Supplementary Tables 1 and 2, http://links.lww.com/CM9/A797. The results after PSM are shown in Tables 2 and 3. In the univariable analyses, adjuvant treatment with T1, the later surgery period (2012–2018), age < 59 years, female sex, CCI scores of 0, no smoking history, adenocarcinoma, and early pathologic stage were associated with better DFS and OS. In the multivariable analyses, adjuvant treatment with T1 (DFS: hazard ratio [HR], 0.655; 95% confidence interval [CI], 0.533–0.805; P < 0.0001; OS: HR, 0.548; 95% CI, 0.426–0.703; P < 0.0001) and early pathologic stage (all stages, P < 0.0001 vs. IA for DFS and OS) were independently associated with better DFS and OS, while non-adenocarcinoma and non-squamous cell carcinoma subtypes (DFS: HR, 1.706; 95% CI, 1.188–2.449; P = 0.0038; OS: HR, 2.019; 95% CI, 1.333–3.058;
Table 1: Baseline characteristics in the overall study population.

| Variables                  | Overall cohort (N = 5746) | Control group (N = 4719) | T1 group (N = 1027) | χ²   | P value | Matched cohort (N = 2504) | Control group (N = 2017) | T1 group (N = 1027) | χ²   | P value |
|---------------------------|---------------------------|--------------------------|---------------------|------|---------|---------------------------|--------------------------|---------------------|------|---------|
| Year of surgery           |                           |                          |                     |      |         |                           |                          |                     |      |         |
| 2005-2011                 | 1135 (19.8)               | 1013 (21.5)              | 122 (11.9)          | 48.97| <0.0001 |                           |                          |                     |      |         |
| 2012-2018                 | 4611 (80.2)               | 3706 (78.5)              | 905 (88.1)          |      |         |                           |                          |                     |      |         |
| Age (years)               |                           |                          |                     |      |         |                           |                          |                     |      |         |
| <50                       | 2868 (49.9)               | 2345 (49.4)              | 514 (50.0)          | 0.009| 0.9235  |                           |                          |                     |      |         |
| ≥59                       | 2878 (50.1)               | 2365 (50.6)              | 513 (50.0)          |      |         |                           |                          |                     |      |         |
| BMI (kg/m²)               |                           |                          |                     |      |         |                           |                          |                     |      |         |
| <24                       | 3762 (65.5)               | 3072 (65.1)              | 690 (67.2)          | 2.566| 0.2772  |                           |                          |                     |      |         |
| 24 to <28                 | 1678 (29.2)               | 1387 (29.4)              | 291 (28.3)          |      |         |                           |                          |                     |      |         |
| ≥28                       | 306 (5.3)                 | 260 (5.5)                | 46 (4.5)            |      |         |                           |                          |                     |      |         |
| Sex                       |                           |                          |                     |      |         |                           |                          |                     |      |         |
| Female                    | 2595 (45.2)               | 2024 (42.9)              | 571 (55.6)          | 55.023| <0.0001 |                           |                          |                     |      |         |
| Male                      | 3151 (54.8)               | 2695 (57.1)              | 464 (44.4)          |      |         |                           |                          |                     |      |         |
| CCI                        |                           |                          |                     |      |         |                           |                          |                     |      |         |
| 0                         | 1575 (27.4)               | 1336 (28.3)              | 239 (23.3)          |      |         |                           |                          |                     |      |         |
| 1                         | 1439 (25.0)               | 1188 (25.2)              | 251 (24.4)          |      |         |                           |                          |                     |      |         |
| 2                         | 1413 (24.6)               | 1166 (24.7)              | 247 (24.1)          |      |         |                           |                          |                     |      |         |
| 3                         | 808 (14.1)                | 620 (13.8)               | 158 (15.4)          |      |         |                           |                          |                     |      |         |
| 4–8                       | 511 (8.9)                 | 379 (8.0)                | 132 (12.8)          |      |         |                           |                          |                     |      |         |
| Smoking status            |                           |                          |                     |      |         |                           |                          |                     |      |         |
| Current/former            | 2469 (43.0)               | 2193 (45.3)              | 330 (32.1)          | 59.925| <0.0001 |                           |                          |                     |      |         |
| Never                     | 3277 (57.0)               | 2580 (54.7)              | 697 (67.9)          |      |         |                           |                          |                     |      |         |
| Surgical approach         |                           |                          |                     |      |         |                           |                          |                     |      |         |
| VATS                      | 4165 (72.5)               | 3308 (70.1)              | 857 (83.5)          | 75.341| <0.0001 |                           |                          |                     |      |         |
| Thoracotomy               | 1581 (27.5)               | 1411 (29.9)              | 170 (16.5)          |      |         |                           |                          |                     |      |         |
| Type of surgery           |                           |                          |                     |      |         |                           |                          |                     |      |         |
| Sublobectomy              | 1018 (18.7)               | 794 (16.8)               | 224 (21.8)          | 18.921| <0.0001 |                           |                          |                     |      |         |
| Lobectomy                 | 93 (1.6)                  | 85 (1.8)                 | 8 (0.8)             |      |         |                           |                          |                     |      |         |
| Pneumonectomy             | 516 (9.1)                 | 410 (8.7)                | 45 (4.4)            |      |         |                           |                          |                     |      |         |
| Pathological TNM stage    |                           |                          |                     |      |         |                           |                          |                     |      |         |
| IA                        | 1846 (32.1)               | 1443 (30.6)              | 403 (39.2)          | 104.796| <0.0001 |                           |                          |                     |      |         |
| IB                        | 2028 (35.3)               | 1601 (33.9)              | 427 (41.6)          |      |         |                           |                          |                     |      |         |
| II                        | 589 (10.3)                | 516 (10.9)               | 73 (7.1)            |      |         |                           |                          |                     |      |         |
| III                       | 236 (4.1)                 | 211 (4.5)                | 25 (2.4)            |      |         |                           |                          |                     |      |         |
| IIA                       | 1047 (18.2)               | 948 (20.1)               | 99 (9.6)            |      |         |                           |                          |                     |      |         |
| Neadsauvant therapy       |                           |                          |                     |      |         |                           |                          |                     |      |         |
| Yes                       | 63 (1.1)                  | 58 (1.2)                 | 5 (0.5)             | 4.285| 0.0385  |                           |                          |                     |      |         |
| No                        | 5683 (98.9)               | 4661 (98.8)              | 1022 (99.5)         |      |         |                           |                          |                     |      |         |
| Postoperative therapy     |                           |                          |                     |      |         |                           |                          |                     |      |         |
| None                      | 3297 (57.4)               | 2605 (55.2)              | 692 (67.3)          | 65.654| <0.0001 |                           |                          |                     |      |         |
| Chemotherapy              | 1207 (21.0)               | 1043 (22.1)              | 164 (16.0)          |      |         |                           |                          |                     |      |         |
| Targeted therapy          | 290 (5.1)                 | 232 (4.9)                | 58 (5.7)            |      |         |                           |                          |                     |      |         |
| Chemoradiotherapy         | 534 (9.5)                 | 483 (10.2)               | 51 (5.0)            |      |         |                           |                          |                     |      |         |
| Chemotherapy plus         | 186 (3.2)                 | 159 (3.4)                | 27 (2.6)            |      |         |                           |                          |                     |      |         |
| targeted therapy          | 232 (4.0)                 | 197 (4.2)                | 35 (3.4)            |      |         |                           |                          |                     |      |         |

Data are shown as n (%). BMI: Body mass index; CCI: Charlson Comorbidity Index; T1: Thyminos α1; TNM: Tumor-Node-Metastasis; VATS: Video-assisted thoracic surgery.

P = 0.0009), were independently associated with worse DFS and OS [Tables 2 and 3].

**Medication duration**

To investigate the effect of the duration of medication on the long-term outcomes, the patients in the T1 group were further divided into three groups: < 12 months (n = 375), 12 to 24 months (n = 282), and > 24 months (n = 370). The median duration of medication was 4.18, and 36 months, respectively. The median follow-up was 20 (range, 4–159) months. There were significant differences in DFS and OS among the three subgroups. The 5-year DFS rates for the three groups were 66.1%, 81.0%, and 84.7%, respectively. The 5-year OS rates were 64.5%, 83.7%, and 92.2%, respectively [Figure 3].

**Subgroup analyses for OS and DFS**

In order to identify which specific subgroups were more likely to benefit from adjuvant T1 treatment, subgroup analyses for OS and DFS were performed after PSM. The patients were divided into subgroups by median age, sex, BMI, CCI, smoking status, histological subtypes, pathological TNM stage, and adjuvant therapy.

The patients in the different subgroups of age, sex, smoking status, and pathological TNM stage benefited from adjuvant T1 treatment when considering DFS and OS [Figure 4]. As for comorbidities, although only those patients with CCI 0 and 1 had improved DFS in the T1 group, all subgroups showed better OS. The patients with BMI > 28 kg/m² and those with squamous cell carcinoma...
might not benefit from postoperative Ta1 injection. Moreover, as for the combination with other adjuvant drugs, patients without postoperative adjuvant therapy and those who received chemotherapy alone had better DFS and OS in the Ta1 group, while those who received chemoradiotherapy had a better OS. The patients who received targeted therapy had neither DFS nor OS benefits in the Ta1 group.

Discussion

This study showed a significant survival advantage of Ta1 therapy in patients with NSCLC and R0 resection. It yielded a 5-year DFS rate of 77.3% and a 5-year OS rate of 83.3%, whereas patients without Ta1 therapy had DFS and OS rates of 64.7% and 72.7%, respectively. Combining the results from univariable and multivariable Cox regression analyses, it reasonably suggests that Ta1 therapy is an independent predictor of DFS and OS. Besides, subgroup analyses showed that Ta1 as an immunomodulatory therapy improved the DFS and/or OS in all subgroups of age, sex, CCI, smoking status, and pathological TNM stage, especially for patients with non-squamous cell NSCLC and no targeted therapy.

As an immunomodulator for cancer therapy, Ta1 has been used in liver cancer, melanoma, and lung cancer for decades,[12] but its efficacy and safety in NSCLC have not been well characterized. It has been confirmed that the combination of cyclophosphamide, murine interferon α/β, and Ta1 has a certain effect on the treatment of advanced lung cancer in mouse models,[17] and it has also been demonstrated that Ta1 could inhibit the growth of lung cancer cells and prolong the survival in mouse models.[18] Still, there is a lack of solid clinical evidence on the effects of Ta1 in lung cancer patients. The first clinical study for NSCLC treatment with Ta1 was reported by Schulof et al[14] in 1985. That study showed that Ta1 treatment after radiotherapy was associated with significant improvements in recurrence-free survival (RFS) and OS for NSCLC patients. Still, that study only enrolled 42 patients with a short follow-up (8–108 weeks). Several mechanisms might be related to the efficacy of Ta1 in improving the outcomes. Ta1 can trigger the differentiation of human CD34+ hematopoietic stem cells into CD3+CD4+ T cells,[19] which play a crucial role in tumor immune surveillance and pathogen clearance. Ta1 has immunomodulating effects by primarily increasing the ability of T cells to produce a variety of cytokines, such as interleukin (IL)-2, IL-7, IL-10, IL-12, IL-15, interferon-α, and interferon-γ and further increasing the efficiency of T-cell maturation.[20] Ta1 can also promote dendritic cells, natural killer (NK) cells, and macrophage activity.[21-23] Moreover, Ta1 increases the expression of the major
Table 2: Univariable and multivariable Cox regression for DFS after PSM.

| Variables                                      | Univariable analysis | Multivariable model |
|------------------------------------------------|----------------------|---------------------|
|                                                 | HR 95% CI | P value | HR 95% CI | P value |
| Adjuvant Ta1 treatment (yes vs. no)             | 0.618     | 0.505–0.758 | <0.0001   | 0.655     | 0.533–0.805 | <0.0001   |
| Year of surgery (2012–2018 vs. 2005–2011)      | 0.606     | 0.476–0.772 | <0.0001   | 0.884     | 0.692–1.131 | 0.3277    |
| Age (years, ≥59 vs. <59)                       | 1.420     | 1.155–1.745 | 0.0009    | 1.232     | 0.880–1.781 | 0.2108    |
| Sex (male vs. female)                          | 1.828     | 1.489–2.245 | <0.0001   | 1.166     | 0.856–1.589 | 0.3297    |
| CCI (vs. 0)                                    |           |           |           |           |
| 1                                              | 1.145     | 0.814–1.610 | 0.4367    | 0.940     | 0.663–1.333 | 0.7292    |
| 2                                              | 1.341     | 0.968–1.859 | 0.0778    | 0.924     | 0.598–1.426 | 0.7204    |
| 3                                              | 1.327     | 0.935–1.884 | 0.1128    | 0.805     | 0.497–1.305 | 0.3794    |
| ≥8                                             | 1.668     | 1.173–2.371 | 0.0044    | 1.244     | 0.758–2.042 | 0.3883    |
| BMI (kg/m², vs. ≤24)                           |           |           |           |           |
| 24 to <28                                      | 0.941     | 0.750–1.182 | 0.6039    | 0.922     | 0.729–1.165 | 0.4961    |
| ≥28                                            | 1.259     | 0.780–2.031 | 0.3452    | 1.102     | 0.674–1.800 | 0.6955    |
| Smoking status (never vs. current/former)      | 0.535     | 0.438–0.635 | <0.0001   | 0.886     | 0.646–1.213 | 0.4496    |
| Histologic subtypes (vs. adenocarcinoma)        |           |           |           |           |
| Squamous cell carcinoma                         | 2.015     | 1.586–2.561 | <0.0001   | 0.892     | 0.673–1.183 | 0.4276    |
| Others                                         | 2.862     | 2.029–4.038 | <0.0001   | 1.706     | 1.188–2.449 | 0.0038    |
| Pathological TNM stage (vs. IA)                 |           |           |           |           |
| IB                                             | 3.999     | 2.666–5.999 | <0.0001   | 3.649     | 2.427–5.484 | <0.0001   |
| IIA                                            | 11.661    | 7.470–18.204 | <0.0001  | 9.944     | 6.250–15.819 | <0.0001 |
| IIB                                            | 10.845    | 5.993–19.625 | <0.0001  | 10.072    | 5.432–18.676 | <0.0001 |
| IIIA                                           | 19.642    | 13.055–29.550 | <0.0001  | 17.510    | 11.546–26.555 | <0.0001 |

The total number of patients in the Cox regression before adjustment was 2054 (1027 each group); BMI: Body mass index; CCI: Charlson Comorbidity Index; CI: Confidence interval; DFS: Disease-free survival; HR: Hazard ratio; PSM: Propensity score matching; Ta1: Thymosin α1; TNM: Tumor-Node-Metastasis.

Table 3: Univariable and multivariable Cox regression for OS after PSM.

| Variables                                      | Univariable analysis | Multivariable model |
|------------------------------------------------|----------------------|---------------------|
|                                                 | HR 95% CI | P value | HR 95% CI | P value |
| Adjuvant Ta1 treatment (yes vs. no)             | 0.505     | 0.394–0.646 | <0.0001   | 0.548     | 0.426–0.705 | <0.0001   |
| Year of surgery (2012–2018 vs. 2005–2011)      | 0.700     | 0.525–0.933 | 0.0149    | 0.911     | 0.681–1.218 | 0.5286    |
| Age (years, ≥59 vs. <59)                       | 1.769     | 1.366–2.289 | <0.0001   | 1.554     | 0.981–2.462 | 0.0604    |
| Sex (male vs. female)                          | 2.031     | 1.576–2.617 | <0.0001   | 1.089     | 0.740–1.603 | 0.6641    |
| CCI (vs. 0)                                    |           |           |           |           |
| 1                                              | 0.861     | 0.552–1.341 | 0.5068    | 0.686     | 0.435–1.082 | 0.1052    |
| 2                                              | 1.249     | 0.836–1.867 | 0.2781    | 0.808     | 0.462–1.414 | 0.4551    |
| 3                                              | 1.499     | 0.987–2.277 | 0.0577    | 0.838     | 0.467–1.578 | 0.6226    |
| ≥8                                             | 1.853     | 1.216–2.821 | 0.0041    | 1.299     | 0.695–2.429 | 0.4123    |
| BMI (kg/m², vs. ≤24)                           |           |           |           |           |
| 24 to <28                                      | 0.945     | 0.720–1.239 | 0.6807    | 0.886     | 0.669–1.173 | 0.3964    |
| ≥28                                            | 0.676     | 0.317–1.440 | 0.3102    | 0.511     | 0.236–1.060 | 0.0881    |
| Smoking status (never vs. current/former)      | 0.475     | 0.372–0.606 | <0.0001   | 0.772     | 0.526–1.132 | 0.1846    |
| Histologic subtypes (vs. adenocarcinoma)        |           |           |           |           |
| Squamous cell carcinoma                         | 2.248     | 1.703–2.968 | <0.0001   | 1.136     | 0.819–1.574 | 0.4454    |
| Others                                         | 2.972     | 2.006–4.405 | <0.0001   | 2.019     | 1.333–3.058 | 0.0009    |
| Pathological TNM stage (vs. IA)                 |           |           |           |           |
| IB                                             | 4.369     | 2.533–7.537 | <0.0001   | 3.819     | 2.210–6.598 | <0.0001   |
| IIA                                            | 12.771    | 7.126–22.888 | <0.0001  | 9.966     | 5.451–18.219 | <0.0001 |
| IIB                                            | 10.058    | 4.778–21.172 | <0.0001  | 7.808     | 3.627–16.809 | <0.0001 |
| IIIA                                           | 19.327    | 11.201–33.348 | <0.0001  | 17.221    | 9.926–29.878 | <0.0001 |

The total number of patients in the Cox regression before adjustment was 2054 (1027 each group); BMI: Body mass index; CCI: Charlson Comorbidity Index; CI: Confidence interval; HR: Hazard ratio; OS: Overall survival; PSM: Propensity score matching; Ta1: Thymosin α1; TNM: Tumor-Node-Metastasis.

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The immunomodulatory effect and apoptotic effects of T cells. Immune system dysregulation plays a significant role in cancer progression. Besides these immunomodulatory effects, To1 can exert antitumor effects by acting directly on tumor cells. Moody et al[25] found that biologically active To1 receptors were present on NCI-H1299 NSCLC cells, and To1 could inhibit lung cancer growth in vivo and in vitro by stimulating arachidonic acid release. Giuliani et al[24] showed that the treatment of murine and human tumor cell lines with To1 could increase the expression of MHC-I. Studies also revealed the antiproliferative and apoptotic effects of To1 on lung cancer, breast cancer, and leukemia cells.[18,26,27] The immunomodulatory effect and direct action on tumor cells of To1 might be beneficial in increasing antitumor immunity of the tumor-bearing host, which improves the survival outcomes.

Nevertheless, which specific subgroups of patients are more likely to benefit from To1 therapy remains an issue. This study suggests prognostic benefits of To1 therapy in all subgroups of age, sex, smoking history, CCI, and stage I–III lung cancer. Patients who were not eligible for adjuvant therapy (eg, stage I disease not requiring postoperative adjuvant therapy) and those who received adjuvant chemotherapy or chemoradiotherapy also had benefits in OS. On the other hand, there was no survival benefit from To1 therapy in patients with a BMI >28 kg/m². It might be because a dose of only 1.6 mg might be insufficient in obese patients. The results also showed that there was no survival benefit for squamous cell carcinoma. Patients who received targeted therapy (alone or plus chemotherapy and chemoradiotherapy) had neither DFS nor OS benefits in the To1 group. These results might help clinicians target the patients who are likely to benefit from To1 therapy.

More importantly, To1 treatment led to a survival benefit in early (stage I) and locally advanced (stage II and IIIA) stages. For patients with stage I NSCLC, surgery with curative intent is the standard treatment, but approximately, 30% to 40% of the postoperative patients die of recurrent disease.[28] The indication of adjuvant treatment remains a matter of debate,[29] and there is still no standard adjuvant treatment regimen for stage I patients. The results of our study have a guiding value for the adjuvant treatment of stage I NSCLC and might provide an adjuvant treatment option for these patients. Adjuvant cisplatin-based chemotherapy is recommended for R0-resected stage II and IIIA NSCLC to eradicate any remaining cancer cells and prolong survival.[30] Several clinical trials evaluating To1 with chemotherapy have been reported. Garaci et al[31] demonstrated that sequential chemoimmunotherapy based on cisplatin, etoposide, To1, and interferon-α2a could improve the response rate of chemotherapy. Similarly, combined treatment with To1 and low-dose interferon-α after ifosfamide enhanced the response rates compared with chemotherapy alone (33% vs. 10%).[13] A recent meta-analysis of 27 randomized controlled trials, including 1925 late-stage NSCLC patients from China, evaluated To1 and chemotherapy combination therapy compared with chemotherapy alone and showed that the addition of To1 could improve antitumor immunity, tumor response, quality of life, and the 1-year OS rate.[10] The results of the present study are consistent with the previous studies, and the OS was significantly longer in the patients with chemotherapy combined with To1. It might benefit from the increased response rate of chemotherapy. In addition to chemotherapy, postoperative adjuvant targeted therapy is one of the alternative treatment modalities for patients with sensitive gene mutations.[32] Nevertheless, there are no previous clinical studies about the efficacy of To1 combined with targeted therapy for lung cancer. This study’s findings suggest no DFS benefits in patients treated with targeted therapy and targeted therapy plus other therapies combined with To1. It indicates that To1 might not be synergistic with targeted therapy or that the effect of targeted therapy in sensitive tumors is stronger and masks the effect of To1.

Another issue that bothers clinicians is how long To1 should be administered. This study suggests significant differences in DFS and OS among <12 months, 12 to 24 months, and >24 months of To1 therapy. Therefore, we recommend that the duration of medication should preferably be >24 months. Still, it should be confirmed by prospective trials.
Figure 4: Subgroup analysis of (A) DFS and (B) OS between the Tα1 group and control group in the matched cohort. CI: Confidence interval; DFS: Disease-free survival; HR: Hazard ratio; OS: Overall survival; Tα1: Thymosin α1.
In this study, there were no drug-related serious adverse events that affected the survival of the patients nor adverse events that led to To1 discontinuation. No new safety signals were identified. To1 was well tolerated by all patients.

There are several limitations to this study. First, although we attempted to balance the variables between the two groups using PSM, selection bias and unobserved confounding associated with the retrospective nature of the study cannot be eliminated. Second, the generalization of the observed outcomes in the subgroup analyses to clinical practice must be cautiously scrutinized because the sample size for some subsets in this series was small. Third, the Eastern Cooperative Oncology Group’s performance status was missing in most patients. Finally, the data were derived from a single institution. Thus, more studies from other institutions, preferably multicenter studies, are encouraged to validate our results.

In conclusion, the present study suggests that To1 as adjuvant therapy could delay recurrence and prolong OS in stage I-III NSCLC patients following margin-free resection, except for patients with squamous carcinoma and those who received targeted therapy. The duration of To1 treatment is recommended to be >24 months.

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**Conflicts of interest**

None.

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