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A Pilot Trial of Thymalfasin (Thymosin-α-1) to Treat Hospitalized Patients With Hypoxemia and Lymphocytopenia Due to Coronavirus Disease 2019 Infection

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Background. Thymosin-α-1 (Tα1) may be a treatment option for coronavirus disease 2019 (COVID-19), but efficacy and safety data remain limited.

Methods. Prospective, open-label, randomized trial assessing preliminary efficacy and safety of thymalfasin (synthetic form of Tα1), compared with the standard of care, among hospitalized patients with hypoxemia and lymphocytopenia due to COVID-19.

Results. A total of 49 patients were included in this analysis. Compared with control patients, the incidence of clinical recovery was higher for treated patients with either baseline low-flow oxygen (subdistribution hazard ratio, 1.48 [95% confidence interval, .68–3.25]) or baseline high-flow oxygen (1.28 [.35–4.63]), although neither difference was significant. Among patients with baseline low-flow oxygen, treated patients, compared with control patients, had an average difference of 3.84 times more $CD4^+$ T cells on day 5 than on day 1 ($P = .01$). Nine serious adverse events among treated patients were deemed not related to Tα1.

Conclusions. Tα1 increases $CD4^+$ T-cell count among patients with baseline low-flow oxygen support faster than the standard of care and may have a role in the management of hospitalized patients with hypoxemia and lymphocytopenia due to COVID-19.

Clinical Trials Registration. NCT04487444.

Keywords. COVID-19; efficacy; hypoxemia; lymphocytopenia; lymphopenia; safety; thymalfasin; thymosin-α-1.

In >60% of patients with coronavirus disease 2019 (COVID-19) some degree of lymphocytopenia develops [1–3], a condition caused by pathophysiological mechanisms, such as T-cell apoptosis and exhaustion mediated by both angiotensin-converting enzyme 2–independent infection of activated $CD4^+$ T cells [4–6] and cytokine dysregulation [4, 6–8]. Because lymphocytopenia is associated with severe COVID-19 infection [1, 7, 9] and poor clinical outcomes [10, 11] and is possibly linked with persistent symptoms [12], restoration of lymphocytes may contribute to recovery among patients with COVID-19 and lymphocytopenia.

Thymosin-α-1 (Tα1), produced by the thymus, binds to Toll-like receptors of dendritic cells [13], promotes T-cell maturation into $CD4^+$ and $CD8^+$ T cells [14], modulates signaling of cytokines associated with inflammation, such as interleukin 1β and tumor necrosis factor α [15], and enhances the signaling of interleukin 2 and 10 [15, 16]. Tα1 has yielded encouraging preliminary results in the treatment of cancer [17], infectious diseases such as hepatitis B [18], and sepsis [19]. Notably, Tα1 is also associated with limiting severe acute respiratory syndrome disease progression [20]. Regarding Tα1 as a treatment option for COVID-19, comprehensive efficacy and safety data from randomized clinical trials [21] and observational studies [22–27] are limited.

The objective of the current pilot phase 2 trial was to provide a preliminary assessment of thymalfasin (the synthetic form of Tα1) as a treatment option among hospitalized patients with hypoxemia and lymphocytopenia due to COVID-19. Herein, we discuss interim efficacy and safety findings, as well as trends in total lymphocyte, $CD4^+$ T-cell, $CD8^+$ T-cell, and leukocyte counts, following treatment with either Tα1 or the standard of care alone.

METHODS

Study Setting and Design

We recruited patients from 2 acute care hospitals, Rhode Island Hospital and The Miriam Hospital, located in Providence,

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Rhode Island. The trial protocol was approved by the institutional review board (Lifespan IRB no. 412020) and was monitored by an independent data and safety monitoring board. Starting 10 September 2020, consecutive hospitalized patients with a positive severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) polymerase chain reaction test result were screened for eligibility. Enrolled patients provided informed consent if possible; if the patient could not provide consent, the patient's legally authorized representative provided surrogate consent (NCT04487444; https://clinicaltrials.gov/ct2/show/NCT04487444).

Inclusion and Exclusion Criteria

At screening, eligible participants were ≥18 years old and admitted with (1) polymerase chain reaction–confirmed SARS-CoV-2 infection ≤4 days before enrollment; (2) hypoxemia, defined as either oxygen saturation (Spo_2) ≤93% on room air or a requirement for supplemental oxygen support; and (3) lymphocytopenia, defined as a total lymphocyte count $<1.5 \times 10^9$/L [1].

Key exclusion criteria at screening were (1) use of invasive mechanical ventilation (IMV), (2) multiorgan failure, (3) advanced cancer being treated with cytotoxic chemotherapy, (4) history of solid organ or bone marrow transplantation, (5) use of hydroxychloroquine or other immunomodulatory medications not including standard of care treatments (eg, dexamethasone) for COVID-19, (6) history of allergy or intolerance to Tα1, or (7) current pregnancy or current breastfeeding.

Treatment Assignment

Patients were randomly assigned in a concealed 1:1 allocation ratio using the Research Electronic Data Capture (REDCap) randomization module [28]. Randomization of treatment assignment was ensured by creating the randomization table using the Python random module that implements pseudorandom number generators [29]. Patients in the treatment arm received the standard of care plus thymalfasin subcutaneously, at a daily dose of 1.6 mg in 1 mL of diluent, starting the day of randomization (day 1) for 7 consecutive days or until death, hospital discharge, or withdrawal from the study. Patients who were randomized to the treatment arm and received ≥1 dose of thymalfasin were considered treated with Tα1 in this modified intent-to-treat population.

Assessments

Clinical Assessments

Ascertainment of medical history was conducted at screening. On days 1–7, 10, 14, and 28, the following data were collected: use of concomitant medications (eg, remdesivir, corticosteroids, baricitinib, and tocilizumab) and clinical status data, such as intensive care unit (ICU) admission, supplemental oxygen support (eg, low-flow or high-flow delivery system and IMV), and survival. Telephone interviews were conducted for patients discharged before the end of the follow-up period. Concurrent use of remdesivir and corticosteroids at baseline were defined as receipt of ≥1 dose of the respective medication within 24 hours of randomization.

Laboratory Assessments

While patients remained hospitalized, laboratory assessments were performed, including routine standard chemistry evaluations and complete blood count with white blood cell differential, along with T-cell subsets, either as part of standard clinical care or according to our study schedule of events (days 1, 3, 5, 7, 10, 14, and 28) if not performed as part of standard clinical care. Specifically, we collected data on aspartate transaminase, alanine transaminase, and bilirubin levels and neutrophil and platelet counts. We also performed flow cytometry to determine total lymphocyte counts, with subsets of $CD4^+$ and $CD8^+$ T-cell counts, and leukocyte counts, using the BD FACSCanto System (Becton Dickinson).

End Points

Owing to limited recruitment following the initial Omicron wave, we decided to present interim findings regarding the efficacy and safety of Tα1 as a treatment option for COVID-19, while aiming to enroll 80 participants in this trial. All patients enrolled through 25 May 2022 are included in this analysis. The primary efficacy end point was time to clinical recovery, defined as the length of time required for a patient to either (1) no longer require supplemental oxygen support and sustain Spo_2 on room air or (2) improve Spo_2 to >93% without supplemental oxygen support if Spo_2 was ≤93% at room air at screening, within 28 days.

Secondary efficacy end points included 28-day incidence of both all-cause mortality and use of IMV. We also assessed the 28-day incidence of ICU admission among patients who were not admitted to the ICU on day 1. In addition, we assessed trends from day 1 to day 7 in total lymphocyte, $CD4^+$ T-cell, $CD8^+$ T-cell, and leukocyte counts.

To evaluate the safety of Tα1, we assessed the incidence of serious adverse events (SAEs) and their relation to Tα1. We also assessed the severity of transaminitis, hyperbilirubinemia, neutropenia, and thrombocytopenia, as defined and graded by the Common Terminology Criteria for Adverse Events, version 5.0 [30]. The severity of incident acute kidney injury (AKI) cases was graded based on the Kidney Disease Improving Global Outcomes criteria, which categorizes AKI cases into 1 of 3 severity grades contingent on serum creatinine level increase from baseline [31]. Moreover, to further assess the safety and tolerability of Tα1, we prospectively monitored patients after Tα1 administration to report and manage any adverse reactions, such as irritation, redness, discomfort, or allergic reactions.

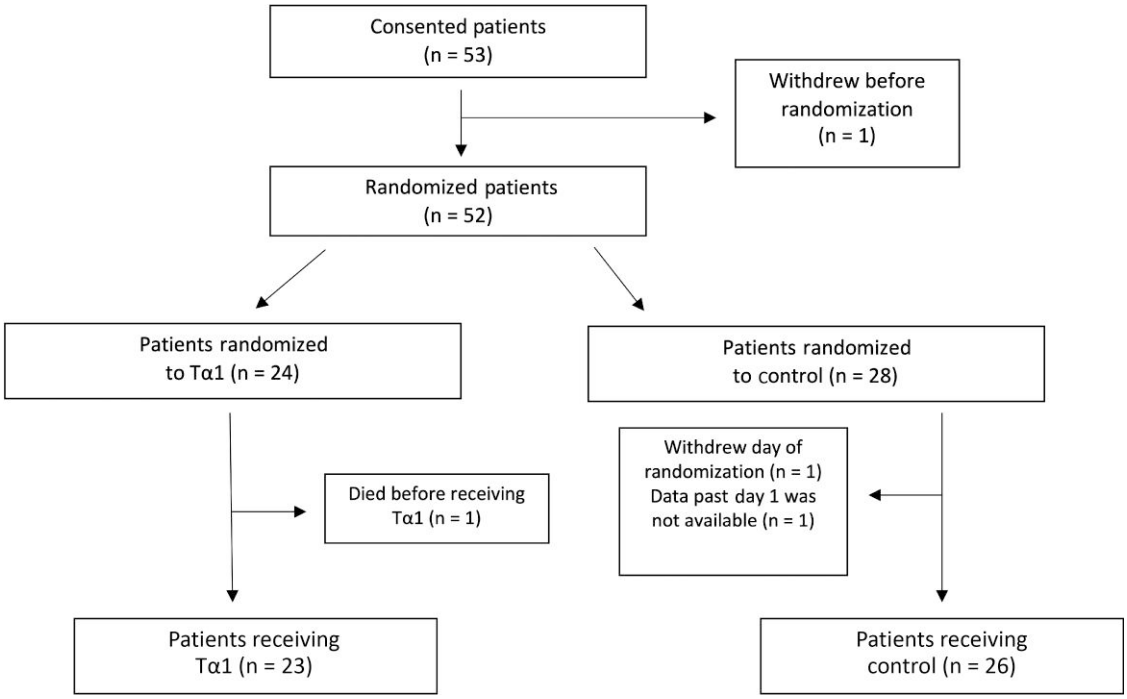

Figure 1. Patient disposition flowchart. Abbreviation: Tα1, thymosin-α-1.

Statistical Analysis

Continuous variables were represented as medians with interquartile ranges. Univariate tests of association between treatment assignment and baseline demographic and health characteristics were performed using Wilcoxon rank sum tests for continuous variables and the Pearson χ^2 test for independence for categorical variables.

Owing to a significant difference in baseline high-flow oxygen support between treatment arms and the clinical merit of stratifying by baseline oxygen support because of relevance in clinical outcomes, as seen in larger trials in this population [32–35], all efficacy end point analyses were stratified by baseline oxygen support. To analyze the time to clinical recovery, we used the Fine and Gray competing risk regression model [36] with death as a competing risk to present subdistribution hazard ratios (SHRs) with 95% confidence intervals (CIs). The SHR allows us to assess the direction of the treatment effect on incidence of clinical recovery in the presence of death as a competing risk [37]. Moreover, the cumulative incidence function of clinical recovery for both treatment arms was estimated using the Aalen-Johansen estimator and compared using Gray's test for equality, with $\rho = 0$ [38].

In addition, the incidence of all-cause mortality, IMV use, and ICU admission were assessed using Pearson χ^2 test for independence. Furthermore, for each specific cell count, we first performed independent Student *t* tests to assess differences between treatment arms in both average absolute cell count (on days 1, 3, 5, and 7) and average rate of change (on days 3, 5, and 7, using day 1 as reference). We then implemented individual mixed-effects models to predict, from day 1 to day 7, the daily average absolute cell count and daily average rate of change for both treatment arms. Day of collection was used as the continuous independent covariate, and predictive cubic growth curves with 95% CIs were plotted.

We also conducted a sensitivity analysis in which we used an inverse probability-weighted competing risk regression analysis [39] to adjust for baseline oxygen support by predicting the propensity of treatment based on a patient's baseline low-flow or high-flow oxygen support status. The incidence and severity of AKI, transaminitis, hyperbilirubinemia, neutropenia, and thrombocytopenia in the treatment arms were evaluated using Pearson χ^2 test for independence. Analyses were performed and plots were produced using either Stata software, version 17.0 (StataCorp) or R language, version 4.1.3 [40]. Significance was set at $\alpha = .05$.

RESULTS

A total of 53 patients consented to enroll, and 4 patients were excluded from analysis, as shown in our patient disposition flowchart in Figure 1. Specifically, 1 patient withdrew consent before randomization, 1 patient in the control arm withdrew

Table 1. Baseline Demographic, Health, and Clinical Characteristics by Treatment Arm

Patient Characteristic	Patients, No. (%)[a] Total (N = 49)	Tα1 Arm (n = 23)	Control Arm (n = 26)	*P* Value
Age, median (IQR), y	58 (49–74)	64 (49–80)	57 (49–68)	.28
Patient sex				
Female	20 (41)	9 (39)	11 (42)	.82
Male	29 (59)	14 (61)	15 (58)	
Race/ethnicity				
Hispanic or Latinx	4 (8)	1 (4)	3 (12)	.11
Non-Hispanic black	4 (8)	4 (17)	0 (0)	
Non-Hispanic white	34 (69)	14 (61)	20 (77)	
Other/unknown	7 (14)	4 (17)	3 (12)	
COVID-19 vaccination[b]				
Not fully vaccinated	39 (80)	20 (87)	19 (73)	.40
Fully vaccinated	10 (20)	3 (13)	7 (27)	
Oxygen support				
Low flow	26 (53)	8 (35)	18 (69)	.02
High flow	23 (47)	15 (65)	8 (31)	
ICU admission status				
Not in ICU	43 (88)	21 (91)	22 (85)	.48
In ICU	6 (12)	2 (9)	4 (15)	
Corticosteroid use[c]	49 (100)	23 (100)	26 (100)	…
Remdesivir use[c]				
No	5 (8)	2 (9)	2 (8)	.90
Yes	45 (92)	21 (91)	24 (92)	
Heart disease	7 (14)	2 (9)	5 (19)	.29
Pulmonary circulation disorders	4 (8)	1 (4)	3 (12)	.36
Peripheral vascular disorders	7 (14)	4 (17)	3 (12)	.56
Hypertension	24 (49)	12 (52)	12 (46)	.67
Chronic pulmonary disease	15 (31)	5 (22)	10 (38)	.20
Diabetes	13 (27)	8 (35)	5 (19)	.22
Hypothyroidism	3 (6)	0 (0)	3 (12)	.09
Renal failure	1 (2)	0 (0)	1 (4)	.34
Liver disease	3 (6)	1 (4)	2 (8)	.63
Solid tumor without metastasis	1 (2)	1 (4)	0 (0)	.28
Coagulopathy	3 (6)	2 (9)	1 (4)	.48
Obesity	21 (43)	12 (52)	9 (35)	.22

Abbreviations: COVID-19, coronavirus disease 2019; ICU, intensive care unit; IQR, interquartile range; Tα1, thymosin-α-1.

[a]Data represent no. (%) of patients unless otherwise specified.

[b]A patient was considered fully vaccinated against COVID-19 if their date of enrollment was ≥14 days after their second messenger RNA vaccine dose or ≥14 days after their Johnson & Johnson vaccine. All patients who did not meet this definition were considered not fully vaccinated.

[c]Concurrent use of remdesivir and corticosteroids at baseline was defined as receipt of ≥1 dose of respective medications within 24 hours of randomization.

consent immediately after randomization, 1 patient in the control arm was lost to follow-up after day 1, and 1 patient died after randomization but before receiving the first dose of Tα1. As a result, 49 patients were included in the analysis, with 23 of 49 (47%) in the treatment arm and 26 of 49 (53%) in the control arm.

Table 2. Efficacy End Points for Patients Overall and by Baseline Oxygen Support

End Point	Tα1, No./ Total (%)	Control, No./ Total (%)	SHR (95% CI)	*P* Value
Overall	n = 23	n = 26		
Clinical recovery[a]	14/23 (61)	17/26 (65)	.80 (.42–1.55)	.52
Death	3/23 (13)	4/26 (15)	…	.82
IMV	1/23 (4)	2/26 (8)	…	.63
ICU admission[b]	7/21 (33)	2/22 (9)	…	.051
Low-flow oxygen	n = 8	n = 18		
Clinical recovery	8/8 (100)	14/18 (78)	1.48 (.68–3.25)	.33
Death	0/8 (0)	2/18 (11)	…	.33
IMV	0/8 (0)	0/18 (0)	…	…
ICU admission[b]	1/8 (13)	0/18 (0)	…	.13
High-flow oxygen	n = 15	n = 8		
Clinical recovery	6/15 (40)	3/8 (38)	1.28 (.35–4.63)	.71
Death	3/15 (20)	2/8 (25)	…	.78
IMV	1/15 (7)	2/8 (25)	…	.20
ICU admission[b]	6/13 (46)	2/4 (50)	…	.89

Abbreviations: CI, confidence interval; ICU, intensive care unit; IMV, invasive mechanical ventilation; SHR, subdistribution hazard ratio; Tα1, thymosin-α-1.

[a]The unadjusted Fine and Gray competing risk analysis for patients overall does not adjust for difference in baseline oxygen support between treatment arms.

[b]The incidence of ICU admission was assessed among patients who were not admitted to the ICU on day 1.

Baseline Characteristics

Most baseline demographic, health, and clinical characteristics were comparable between both treatment arms (Table 1). Patients' median age (interquartile range) was 64 (49–80) years in the Tα1 arm and 57 (49–68) years in the control arm. Women comprised 9 of 23 patients (39%) treated with Tα1 and 11 of 26 (42%) in the control arm. Overall, 34 of 49 enrolled patients (69%) were non-Hispanic white, and 8 of 49 (16%) identified as either non-Hispanic black or Hispanic/Latinx.

All patients required supplemental oxygen support at baseline. Notably, a greater proportion of patients who required higher supplemental oxygenation, suggestive of greater respiratory distress, were treated with Tα1. Specifically, 15 of 23 patients (65%) treated with Tα1 required baseline high-flow oxygen support, while 8 of 26 (31%) in the control arm required baseline high-flow oxygen support.

Primary Efficacy End Point

Primary efficacy end point results for the entire cohort and stratified by baseline oxygen support are presented in Table 2. Overall, 14 of 23 patients (61%) in the Tα1 arm and 17 of 26 (65%) patients in the control arm recovered within 28 days, and 3 of 23 patients (13%) in the Tα1 arm died, compared with 4 of 26 (15%) in the control arm.

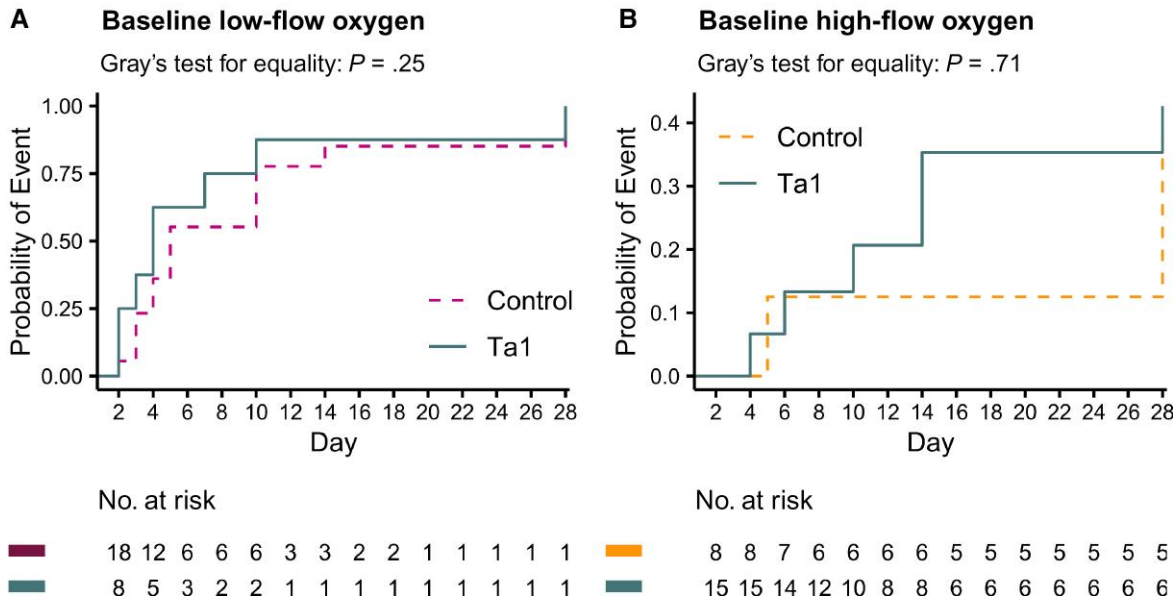

Figure 2. Cumulative incidence function of clinical recovery among patients with baseline low-flow (*A*) and high-flow (*B*) oxygen support. The cumulative incidence function estimates the probability of a patient recovering using the Aalen-Johansen estimator. At each time point, the number at risk represents the number of patients not lost to follow-up, alive, and yet to experience clinical recovery. Gray's test for equality compares cumulative incidence functions to assess the null hypothesis that the cumulative incidence functions are similar. Abbreviation: Tα1, thymosin-α-1.

After accounting for death as a competing risk, the unadjusted competing risk analysis showed that the incidence of clinical recovery was lower among patients in the Tα1 arm (SHR, 0.80 [95% CI, .42–1.55]) compared with those in the control arm, although this different was not statistically significant, and patients treated with Tα1 were more likely to require higher supplemental oxygenation at baseline.

Among patients with baseline low-flow oxygen support, 8 of 8 patients (100%) in the Tα1 arm and 14 of 18 (78%) in the control arm recovered within 28 days. After accounting for death as a competing risk, we found that the incidence of clinical recovery (Figure 2*A*) was higher among patients treated with Tα1 (SHR, 1.48 [95% CI, .68–3.25]) compared with control patients, although this difference was also not significant. Among patients with baseline high-flow oxygen support, 6 of 15 (40%) in the Tα1 arm and 3 of 8 (38%) in the control arm recovered within 28 days. Similarly, we found that the incidence of clinical recovery (Figure 2*B*) was higher among patients treated with Tα1 (SHR, 1.28 [95% CI, .35–4.63]) compared with control patients, although, again, this difference was not significant. After adjusting for baseline oxygen support, we found that the incidence of clinical recovery was higher among patients treated with Tα1 (SHR, 1.40 [95% CI .72–2.72]) compared with control patients, although this difference was not significant.

Secondary Efficacy End Points

Secondary efficacy end points regarding the incidence of all-cause mortality, IMV use, and ICU admission did not differ significantly between treatment arms, irrespective of baseline oxygen support (Table 2). In terms of mortality rates, among patients with baseline low-flow oxygen support, none of 8 patients in the Tα1 arm died, compared with 2 of 18 (11%) in the control arm. Among patients with baseline high-flow oxygen support, 3 of 15 patients (20%) in the Tα1 arm died, compared with 2 of 8 (25%) in the control arm.

In terms of IMV use, among patients with baseline low-flow oxygen support, no patients in either treatment arm required IMV throughout the study period. Among patients with baseline high-flow oxygen support, 1 of 15 patients (7%) in the Tα1 arm required IMV, compared with 2 of 8 (25%) in the control arm. In terms of ICU admission, among patients with baseline low-flow oxygen support, 1 of 8 patients (13%) in the Tα1 arm were admitted to the ICU, compared with none of 18 patients in the control arm. Among patients with baseline high-flow oxygen support, 6 of 13 patients (46%) in the Tα1 arm were admitted to the ICU, compared with 2 of 4 (50%) in the control arm.

Time Trend Analyses

Absolute and relative increases in total lymphocyte (Supplementary Figure 1), $CD4^+$ T-cell (Supplementary Figure 2), $CD8^+$ T-cell (Supplementary Figure 3), and leukocyte (Supplementary Figure 4) counts were generally comparable between treatment arms, irrespective of baseline oxygen support. Notably, we found that among patients with baseline low-flow oxygen, treated patients, compared with control patients,

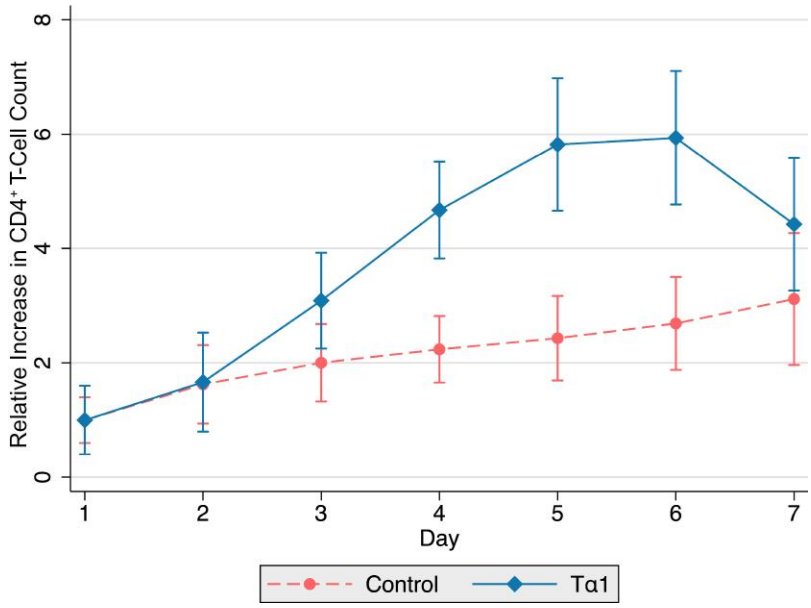

Figure 3. Daily average relative increase in $CD4^+$ T-cell count among patients with baseline low-flow oxygen support. The daily average relative increase was defined as the average ratio between the daily $CD4^+$ T-cell count and the $CD4^+$ T-cell count at baseline (day 1). Abbreviation: Tα1, thymosin-α-1.

had an average difference of 3.84 times more $CD4^+$ T cells on day 5 than on day 1 ($P = .01$; Supplementary Table 1). Moreover, mixed-effects modeling demonstrated that treated patients, compared with control patients, had greater average $CD4^+$ T-cell ratios on days 4, 5, and 6 than on day 1, respectively, as indicated by the nonoverlapping CIs (Figure 3).

Safety End Points

Overall, 10 patients experienced a total of 18 SAEs. For each treatment arm, the SAEs are presented by organ system in Table 3. Among patients in the Tα1 arm, 4 patients experienced a total of 9 SAEs, none of which were deemed related to Tα1 (Supplementary Table 3).

The incidences of AKI, transaminitis, and hyperbilirubinemia classified as either grade 1, grade 2, or grade 3 adverse events, respectively, were similar between treatment arms, while cases of neutropenia and thrombocytopenia classified as either grade 1 or grade 2, respectively, were also similar between treatment arms (Table 4). Notably, there were 4 cases of grade 1 AKI between both treatment arms, with 3 of 4 cases (75%) reported among patients in the Tα1 arm, though this difference was not significant. Importantly, most patients in both treatment arms did not experience AKI, transaminitis, hyperbilirubinemia, neutropenia, or thrombocytopenia. Moreover, no events of irritation, pain, discomfort, or allergic reactions were reported after Tα1 administration.

Table 3. Serious Adverse Events by Organ System and Treatment Arm

Organ System	SAEs in Tα1 Arm	SAEs in Control Arm
Cardiovascular	Thromboembolism (n = 2); deep vein thrombosis (n = 1); pulmonary embolism (n = 1)	Non-ST elevation myocardial infarction (n = 1)
Hematological	Epistaxis (n = 1)	…
Infection/ infestation	Sepsis (n = 2); superimposed bacterial pneumonia (n = 1)	Sepsis (n = 1)
Neurological	…	Brainstem herniation due to left frontal brain lesion (n = 1)
Pulmonary	Respiratory failure (n = 3)	Respiratory failure (n = 5)
Renal	…	Renal failure (n = 1)

Abbreviation: SAEs, serious adverse events; Tα1, thymosin-α-1.

DISCUSSION

In this pilot trial, we assessed the efficacy and safety of Tα1 among hospitalized patients with hypoxemia and lymphocytopenia due to COVID-19. After stratification and adjustment, respectively, for baseline oxygen support, the incidence of clinical recovery was higher among patients in the Tα1 arm than among those in the control arm, although all analyses showed differences that were not statistically significant. In addition, upward trends in total lymphocyte count, $CD4^+$ T-cell, $CD8^+$ T-cell, and leukocyte counts within a week were generally comparable between treatment arms, but Tα1 increased $CD4^+$ T-cell counts in patients with baseline low-flow oxygen support faster than the standard of care alone.

Clinical trial data reported while our manuscript was under review found that Tα1 is associated with a reduced mortality rate, improvement in the World Health Organization 8-point ordinal scale, and increases in both $CD4^+$ and $CD8^+$ T-cell counts [21]. Of note, the study by Shetty et al [21] was not

Table 4. Abnormal Laboratory Values by Treatment Arm

Laboratory Value or Finding[a]	Patients, No. (%)			*P* Value
	Total (N = 49)	Tα1 Arm (n = 23)	Control Arm (n = 26)	
AST				
Grade 1	6 (12)	3 (13)	3 (12)	.91
No increase	42 (88)	20 (87)	22 (88)	
ALT				
Grade 1	10 (21)	4 (17)	6 (24)	.57
No increase	38 (79)	19 (83)	19 (76)	
Bilirubin				
Grade 1	2 (4)	1 (4)	1 (4)	.59
Grade 2	1 (2)	0 (0)	1 (4)	
Grade 3	1 (2)	0 (0)	1 (4)	
No increase	44 (92)	22 (96)	22 (88)	
AKI				
Grade 1	4 (8)	3 (13)	1 (4)	.70
Grade 2	2 (4)	1 (4)	1 (4)	
Grade 3	2 (4)	1 (4)	1 (4)	
No AKI	41 (84)	18 (78)	23 (88)	
Neutrophil count				
Grade 1	6 (12)	2 (9)	4 (16)	.45
Grade 2	1 (2)	1 (4)	0 (0)	
No decrease	41 (85)	20 (87)	21 (84)	
Platelet count				
Grade 1	11 (23)	4 (17)	7 (28)	.68
Grade 2	2 (4)	1 (4)	1 (4)	
No decrease	35 (73)	18 (78)	17 (68)	

Abbreviations: AKI, acute kidney injury; ALT, alanine transaminase; AST, aspartate transaminase; Tα1, thymosin-α-1.

[a]AKI cases were graded using the Kidney Disease Improving Global Outcomes criteria. Abnormal laboratory values were graded using the Common Terminology Criteria for Adverse Events version 5.0.

restricted to patients with lymphocytopenia, and the treatment regimen was defined as a 7-day course of 1.6 mg/mL of Tα1, in which moderately ill patients received Tα1 4 times a day and severely ill patients received it 6 times a day. Overall, our study along with the report by Shetty et al [21] and other observational findings [22, 23] indicate that Tα1 is well tolerated and primed for a larger study in patients with hypoxemia and lymphocytopenia due to COVID-19.

Observational studies have found that Tα1 is associated with both greater [24, 26] and reduced [22, 23] likelihood of death, as well as both greater [26] and reduced [22] likelihood of IMV use among patients with severe COVID-19. Taken in their totality, the observational efficacy findings regarding clinical outcomes following treatment with Tα1 are limited owing to unmeasured confounding and nonstandardized rationale for initiation and duration of Tα1 intervention. Tα1 has also been assessed as a prophylactic agent for COVID-19 among medical staff, but no significant effect was observed [27].

Observational studies [22, 41, 42] have also assessed the effect of Tα1 on restoring both total lymphocyte and T-cell counts in patients with COVID-19. For instance, Yu et al [41] analyzed a small cohort of 25 severely and critically ill patients with COVID-19 and found a larger increase in lymphocyte count for patients treated with Tα1 compared with control patients. In another retrospective study, Liu et al [22] analyzed 34 patients with severe COVID-19 and found that daily Tα1 administration increases $CD4^+$ and $CD8^+$ T-cell counts among patients with counts $<0.650 \times 10^9$/L and $<0.400 \times 10^9$/L, respectively, at admission. Of note, the study by Liu et al is limited by the lack of a comparison group and by an analysis restricted to patients hospitalized for ≥10 days.

$CD4^+$ T cells are critical to establishing protective immunity against SARS-CoV-2 by promoting production and maturation of neutralizing antibodies [43–45], as well as regulating $CD8^+$ T cells to eliminate virally infected cells [45]. Importantly, a coordinated humoral and cellular immune response is associated with mild disease [46, 47] and patient recovery [48] following COVID-19 infection. We found that all patients with baseline low-flow oxygen support who were treated with Tα1 recovered within 28 days. Notably, Tα1 increased $CD4^+$ T-cell counts among patients with baseline low-flow oxygen support faster than the standard of care. Thus, the effect of Tα1 on T-cell restoration may be modified by disease severity and may contribute to patient recovery. Analogous to monoclonal antibodies [49] and oral agents [50] that have demonstrated efficacy in earlier stages of COVID-19 infection, the effect of Tα1 may be limited to patients with hypoxemia and lymphocytopenia before they require high-flow oxygen.

Irritation, redness, and discomfort at the site of injection are the most common reported adverse reactions after Tα1 administration [14]. Liu et al [22] did not observe any adverse reactions among 76 patients with severe COVID-19 treated with Tα1. Similarly, irritation, redness, discomfort, or allergic reactions were not observed among our cohort of patients treated with Tα1, and the incidence and severity of AKI, transaminitis, hyperbilirubinemia, neutropenia, and thrombocytopenia were comparable between treatment arms. Moreover, both treatment arms in our study experienced the same number of SAEs, and none of the SAEs among treated patients were deemed related to Tα1, which is similar to safety data from Shetty et al [21].

Regarding study limitations, the nonblinded study design and patient enrollment limited to a single study center are important considerations. The small sample size of our trial is also a limitation, which resulted in underrepresentation of racial/ethnic groups and contributed to differential baseline oxygen support between treatment arms. In addition, all patients received corticosteroids, so we could not discern the confounding effect of corticosteroid use on cell counts. Another important consideration is that findings from the post hoc analyses should be interpreted with caution because stratification by baseline oxygen support was not planned a priori. Nevertheless, our aim was to offer a preliminary assessment of Tα1 as a treatment

option for COVID-19. Moving forward, larger placebo-controlled clinical trials with standardized Tα1 dosing regimens and more comprehensive follow-up protocols, including consistent collection of blood samples throughout entire study periods, are needed to definitively assess the efficacy and safety of Tα1, as well to appropriately describe trends in total lymphocyte, $CD4^+$ T-cell, $CD8^+$ T-cell, and leukocyte counts in patients with hypoxemia and lymphocytopenia due to COVID-19.

In conclusion, data from our randomized pilot trial offer a first preliminary assessment of the clinical efficacy and safety of Tα1 among hospitalized patients with hypoxemia and lymphocytopenia due to COVID-19. We found that Tα1 is safe and tolerable and increases $CD4^+$ T-cell count among patients. Research from larger studies is encouraged to further assess the clinical benefit of Tα1 in managing COVID-19.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copy-edited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Author contributions. Conceptualization: F. S. and E. M. Methodology: F. S., G. B., and E. M. Formal analysis: F. S., G. B., and E. K. M. Investigation: all authors. Data curation: F. S., G. B., and E. K. M. Writing of original draft: F. S. and G. B. Writing review and editing: all authors. Visualization: F. S. and G. B.

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