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ORIGINAL ARTICLE

Prognostic Impact of Sarcopenia and Blood Biomarkers in Advanced Non-Small Cell Lung Cancer on First-Line Immune Checkpoint Inhibitors

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ABSTRACT

Although immune checkpoint inhibitors (ICIs) have improved outcomes in advanced non-small cell lung cancer (NSCLC), the prognostic tools for programmed death-ligand 1 (PD-L1), the only approved biomarker, remain limited. We comprehensively evaluated the prognostic impact of sarcopenia and various blood biomarkers in 74 NSCLC patients receiving first-line ICIs. Sarcopenia was diagnosed using dual-energy X-ray absorptiometry. Cytokines and myokines in peripheral blood samples were assessed using Enzyme-linked immunosorbent assay (ELISA) and cytometric bead array, while lymphocyte subsets were characterized using flow cytometry. Sarcopenia group had significantly shorter progression-free survival (PFS) (P = 0.018). Sarcopenia was revealed as an independent poor prognostic factor for PFS (HR 2.63, 95% CI 1.19−5.8; P = 0.017). Patients with sarcopenia showed elevated levels of inflammatory cytokines (interleukin (IL)-6, IL-8, IL-10, IL-15, and tumor necrosis factor). Furthermore, sarcopenia index and muscle mass were negatively correlated with exhausted CD8+ T cells (CD8+ TIGIT+). The sarcopenia with high TIGIT expression group exhibited the worst prognosis (HR 3.5, P=0.0087). Sarcopenia, immune-related blood biomarkers and high TIGIT expression were identified as independent poor prognostic factors in advanced NSCLC patients receiving first-line ICI therapy.

Keywords: non-small-cell lung cancer, T lymphocytes, immunotherapy, systemic inflammatory status, sarcopenia, prognostic biomarker

1. INTRODUCTION

Immune checkpoint inhibitors (ICIs) have significantly improved patient survival outcomes with advanced non-small cell lung cancer (NSCLC), and extensive research has been aimed at identifying prognostic and predictive biomarkers of ICIs. However, programmed cell death ligand 1 (PD-L1) expression, assessed using immunohistochemistry, remains the only fully implemented Food and Drug Administration-approved biomarker for predicting ICI responses in clinical practice [1,2]. Although ICI treatment outcomes vary based on a patient's health history, the absence of standardized quantifiable measures to evaluate the overall condition presents a significant challenge for accurate prediction.

Sarcopenia, a condition characterized by reduced skeletal muscle mass, strength, and function, is an indicator of a patient's overall physical status. This is associated with various factors that may adversely impact the efficacy of ICIs [3]. Particularly, sarcopenia has been linked to a reduction in cytotoxic lymphocytes, an increase in senescent lymphocytes [4], and abnormal inflammatory responses with elevated cytokine levels, and a decrease in normal myokine levels [5]. Furthermore, it is associated with an increased risk of adverse side-effects from cytotoxic chemotherapeutic drugs [6]. While many studies have analyzed the relationship between sarcopenia and NSCLC from the cytotoxic chemotherapy perspective, data on the impact of sarcopenia on ICI therapy remain limited. Numerous studies have reported the associations between individual blood biomarkers and clinical outcomes;

however, comprehensive analyses evaluating their independent prognostic values remain scarce.

We aimed to evaluate two key aspects of patients with advanced NSCLC receiving first-line ICIs. The primary objective was to analyze ICI treatment outcomes in patients with sarcopenia using various blood biomarkers, including T lymphocytes subsets, cytokines, and myokines. The secondary objective was to investigate how sarcopenia influences the systemic inflammatory status and modulates ICI response by associating it with secretory factors and immune cell populations.

2. MATERIALS AND METHODS

2.1 Study design

This prospective study enrolled patients with advanced NSCLC who received first-line ICIs at Korea University Guro Hospital between July 2021 and December 2023. The exclusion criteria included prior cancer treatment (surgery, cytotoxic chemotherapy, ICIs, or targeted therapy), history of other cancers, and age < 18 years. Disease progression was evaluated radiographically using the Response Evaluation Criteria in Solid Tumors (RECIST; version 1.1) guidelines [7]. Progression-free survival (PFS) was defined as the time from treatment initiation to radiological disease progression or death from any cause. The data cutoff date was July 2024. **Supplementary figure 1** illustrates the study design.

This study was approved by the Ethics Committee for Clinical Research of Korea University Guro Hospital (IRB No. 2021GR0409, IRB Approval: August 26, 2021) and was conducted in accordance with the 1964 Declaration of Helsinki and its later amendments. The privacy rights of the patients were ensured and informed consent was obtained for this study.

2.2 Diagnosis of sarcopenia

Sarcopenia was diagnosed using dual-energy X-ray absorptiometry (DXA) to assess the appendicular skeletal muscle mass (ASM). ASM was normalized using height squared (ASM/height^2). Sarcopenia was defined as ASM/height^2 values <6.43 kg/m^2 in men and <5.34 kg/m^2 in women, following the Sarcopenia Study Group of the Korean Geriatrics Society [8].

2.3 Sample preparation

Single blood samples were collected in 10-mL ethylenediaminetetraacetic acid (EDTA)-anticoagulated tubes before ICI administration. Within 2 h of collection, the samples were centrifuged at 120 × g for 20 min. Following this, the supernatant was transferred to a separate tube and further centrifuged at 2500 × g for 15 min. The final supernatant, designated as platelet-poor plasma (PPP), was stored at −80 °C.

The lower layer from the first centrifugation was mixed (1:1) with phosphate-buffered saline (PBS). Peripheral blood mononuclear cells

(PBMCs) were isolated from this mixture using Lymphosep (Biowest, Bradenton, FL, USA; cat# L0560) following the manufacturer's protocol. Afterward, isolated PBMCs were resuspended in freezing medium (90% fetal bovine serum and 10% dimethyl sulfoxide). One mL aliquots were transferred to cryogenic cell vials and stored in liquid nitrogen tanks.

2.4 Cytometric bead array

The concentrations of interleukin (IL)-2, IL-4, IL-6, IL-10, tumor necrosis factor (TNF), interferon (IFN)-γ, and IL-17A in the PPP were quantified using the BD Cytometric Bead Array Human Th1/Th2/Th17 Cytokine Kit (BD Biosciences, San Jose, CA, USA; cat# 560484) in accordance with the manufacturer's protocol. The concentrations of granzyme A (cat# 560299), granzyme B (cat# 560304), IL-8 (cat# 558277), and IL-1β (cat# 558279) in the PPP were analyzed using the BD CBA Human Soluble Protein Master Buffer Kit (BD Biosciences; cat# 558265) in conjunction with the corresponding CBA Flex Set for each analyte. Data acquisition was performed using a BD FACSLyric instrument (BD Biosciences), and the collected data were analyzed using the FCAP Array™ Software (BD Biosciences).

2.5 Enzyme-linked immunosorbent assay (ELISA)

IL-15 and decorin concentrations in the PPP were measured using the Human Decorin DuoSet ELISA (R&D Systems, Minneapolis, MN, USA; cat# DY143) and Human IL-15 DuoSet ELISA (R&D Systems; cat# DY247-05), respectively, following the manufacturer's instructions. The

optical density was measured at 450 nm using a microplate colorimeter (TECAN Sunrise, Tecan, Switzerland).

2.6 Flow cytometry

Flow cytometry was employed to analyze T-regulatory lymphocytes (Tregs), exhausted T lymphocytes, and memory T lymphocytes. PBMCs stored in liquid nitrogen were rapidly thawed at 37 °C, resuspended in flow cytometry buffer (PBS containing 1% bovine serum albumin), and then centrifuged (1,000 rpm for 5 min). Afterward, the cells were washed once with flow cytometry buffer. Live/dead discrimination was carried out using the Zombie Aqua™ Fixable Viability Kit (BioLegend, San Diego, CA, USA; cat# 423102); and Fc receptor blocking was performed with Human TruStain FcX™ (BioLegend; cat# 422302), both as per manufacturer's instructions. Antibody cocktails were prepared and incubated in the dark at 4 °C for 30 min. Subsequently, the antibodies used included anti-human CD3 (BioLegend; cat# 300324), anti-human CD4 (BioLegend; cat# 300514), anti-human CD8 (BioLegend; cat# 344710), anti-human CD45RO (BioLegend; cat# 304229), anti-human CD45RA (BioLegend; cat# 304106), anti-human CD25 (BioLegend, cat# 302606), anti-human CD127 (BioLegend, cat#351310), anti-human TIGIT (BioLegend; cat# 372709), and anti-human PD-1 (BioLegend; cat# 329918). After incubation, 1 mL fluorescence-activated cell sorting (FACS) buffer was added to each tube, followed by centrifugation at 500 × g for 5 min. The supernatant was discarded, and 0.3 mL of FACS buffer was added.

Samples were then analyzed using the BD LSRFortessa or BD FACSLyric flow cytometer (BD Biosciences). Batch correction was performed by extracting T-cells and applying batch alignment using Spectre [9]. Subset analyses were performed following the alignment. A representative gating strategy is shown in **Fig. 1**. Tregs were defined as CD4+/CD25+/CD127dim; exhausted T cells as PD-1+ or TIGIT+; T_{CM} and T_{EM} as CD45RA–/CD45RO+; and terminally differentiated effector T cells (T_{TE}) as CD45RA+/CD45RO– [10].

2.7 Statistical analysis

Categorical variables were analyzed using Fisher's exact or chi-square test and a two-tailed significance test. Normality was assessed using the Shapiro–Wilk test, with Welch's t-test adopted for normally distributed data, and the Mann–Whitney U test for non-normally distributed data. Furthermore, Kaplan–Meier survival curves were compared using the log-rank test. Cutoff values were determined using the Maxstat R package. Univariate and multivariate analyses were performed using Cox proportional hazard regression models. Statistical significance was set at $P<0.05$. All analyses and graphical outputs were generated using R Studio (version 4.2.3), GraphPad Prism (GraphPad Software Inc., San Diego, CA, USA), and FlowJo (version 10.0.7; FlowJo LLC, Ashland, OR, USA).

3. RESULTS

3.1 Clinicopathological characteristics of the enrolled patients

Seventy-four patients were included in this analysis. Of these, 58 (78.4%) were diagnosed with sarcopenia, 46 (62.2%) were aged ≥70 years, and 67 (90.5%) were male. Histologically, adenocarcinomas and squamous cell carcinomas were observed in 39 (52.7%) and 27 (36.5%) patients, respectively. All the patients received pembrolizumab as an ICIs, 21 (28.4%) received ICI monotherapy, and 53 (71.6%) received a combination of ICIs and cytotoxic chemotherapy. PD-L1 SP263 expression was <50% in 49 (66.2%) patients. Treatment responses included: 1 patient (1.4%) with a complete response, 33 (44.6%) with partial responses, 13 (17.6%) with stable disease, and 27 (36.5%) with progressive disease. ICI-induced pneumonitis occurred in 18 patients (24.3%), and 5 (6.8%) discontinued ICI owing to adverse events. Although not statistically significant, the probability of treatment discontinuation among patients who experienced adverse events was higher in the sarcopenia group (5/13, 38.5%) than in the nonsarcopenia group (0/5, 0.0%) ($P = 0.150$). No significant differences in the baseline characteristics were observed between the sarcopenia and nonsarcopenia groups. Patient characteristics are summarized in **Table 1**.

3.2 Progression-free survival

As for the data cutoff, 62 patients experienced PFS events and 31 (41.9%) died within 1 year. Sarcopenia group reported significantly

shorter median PFS (N=16, 74 days, 95% CI; 41-129 days) than nonsarcopenia group (N=58, 172 days, 95% CI; 126-NA days, $P = 0.018$; **Fig. 2**).

Within the sarcopenia group, PFS was further analyzed across the various clinical factors (**Additional File 2**). No statistical differences in median PFS were observed based on (1) PD-L1 SP263 expression (≥50%: N=44, 127 days, 95% CI; 37-262 vs. <50%: N=29, 82 days, 95% CI; 44-155 days, $P = 0.975$), (2) body mass index (BMI) (<20: N=11, 48 days, 95% CI; 34-NA days vs. ≥20: N=63, 119 days, 95% CI; 51-172 days, $P = 0.742$), (3) ICI treatment type (monotherapy: N=21, 97 days, 95% CI; 36-406 days vs. combination: N=53, 82 days, 95% CI; 48-155 days, $P = 0.824$), (4) Disease stage at diagnosis (IVA: N=33, 71 days, 95% CI; 36-171 days vs. IVB: N=41, 126 days, 95% CI; 51-200 days, $P = 0.246$) [11]. In addition, a PFS analysis (Cox regression analysis) adjusted for histological subtype and treatment regimen (ICI monotherapy vs. combination therapy) to assess PD-L1 expression as a prognostic marker didn`t show statistically significant differences in sarcopenia group (hazard ratio [HR] 0.997, 95% confidence interval [CI], 0.988–1.007; $P = 0.601$).

3.3 Lymphocyte subset and cytokine profile analysis

Thirteen cytokines and myokines were evaluated in this study (**Table 2**). The levels of key cytokines associated with poor prognosis, including IL-6, IL-8, IL-10, IL-15, and tumor necrosis factor (TNF) were

significantly elevated in the sarcopenia group (**Fig. 3**). Although IL-1β, IL-2, IL-4, IL-17A, decorin, IFN-γ, and granzyme A were also elevated in the sarcopenia group, these differences were not statistically significant.

Flow cytometric analysis included CD4+ T cells, CD8+ T cells, Tregs (CD4+/CD25+/CD127dim), exhausted T cells (PD-1+ or TIGIT+), T_{CM} and T_{EM} (CD45RA–/CD45RO+), and T_{TE} (CD45RA+/CD45RO–). The frequency of CD4+ T-cells expressing PD-1 was significantly higher in the nonsarcopenia group ($P = 0.022$), whereas the number of CD8+ T_{TE} cells was significantly higher in the sarcopenia group ($P = 0.004$). No significant differences were observed between other T cell subsets (**Table 2**).

3.4 Univariate and multivariate analysis of PFS

Univariate analyses were performed to evaluate the association between PFS and all factors listed in Tables 1–2 (**Table 3**). Sarcopenia was the only clinicopathological factor significantly associated with poor prognosis (HR 2.4, 95% CI 1.1−5.0; $P = 0.022$). From the cytokine profile, only granzyme A was identified as a poor prognostic factor (HR 2.3, 95% CI 1.1−4.9; $P = 0.031$). In the lymphocyte subset analysis, CD4+ T cells (HR 2.13, 95% CI 1.2−3.7; $P = 0.011$) and CD8+ T_{CM} and T_{EM} (HR 1.9, 95% CI 1.1−3.1; $P = 0.02$) were unfavorable prognostic factors. Conversely, Tregs (HR 0.58, 95% CI 0.35−0.97; $P = 0.037$), Tregs expressing PD-1 (HR 0.47, 95% CI 0.26−0.86; $P = 0.031$), CD4+ T cells expressing PD-1 (HR 0.43, 95% CI 0.2−0.93; $P = 0.032$), CD8+ T

cells (HR 0.41, 95% CI 0.18−0.92, P = 0.03), and CD8+ T cells expressing PD-1 (HR 0.37, 95% CI 0.19−0.74; P = 0.0047) were associated with favorable prognosis.

We performed the multivariate analysis including seven factors selected from the 11 variables with P < 0.1 in the univariate analysis. This was done to mitigate the risk of model overfitting that may arise when too many factors are included relative to the sample size. Among the 11 candidates, we selected seven variables that we considered to be clinically important prognostic factors for outcomes with ICIs. (**Fig. 4**). Six independent poor prognostic factors were identified: sarcopenia (HR 2.63, 95% CI 1.19-5.8; P = 0.017), IL-8 (HR 2.31, 95% CI 1.19-4.5; P = 0.014), granzyme A (HR 2.73, 95% CI 1.20−6.2; P = 0.016), CD4+ T cells (HR 2.73, 95% CI 1.38−5.4; P = 0.004), CD8+ T cells expressing TIGIT (HR 2.79, 95% CI 1.30−6.0; P = 0.008), and CD8+ T_{CM} + T_{EM} (HR 2.40, 95% CI 1.34−4.3; P = 0.004).

3.5 Further analysis of the association between sarcopenia and TIGIT

Pearson's correlation analysis was performed to evaluate the relationship between the sarcopenia index (ASM/height^2), muscle mass (ASM), and the six significant poor prognostic factors identified in the multivariate analysis (IL-8, granzyme A, CD4+ T cells, CD8+ T cells expressing TIGIT, and CD8+ T_{CM} + T_{EM}). Only CD8+ T cells expressing TIGIT showed significant negative correlation with the sarcopenia index

($P = 0.006$; r = −0.316) and ASM ($P = 0.002$; r = −0.359) (**Additional File 3**).

We analyzed PFS by stratifying the patients into three groups according to TIGIT expression: nonsarcopenia with low TIGIT expression, sarcopenia with low TIGIT expression, and sarcopenia with high TIGIT expression. (In the nonsarcopenia group, none of the patients exhibited high TIGIT expression.) In the three-group comparison, relative to the nonsarcopenia, the sarcopenia with low TIGIT expression group had a significantly worse PFS (HR 2.2, P=0.038), and the sarcopenia with high TIGIT expression group also exhibited significantly worse PFS (HR 3.5, P=0.0087) (**Fig. 5**).

4. DISCUSSION

We aimed to assess independent associations between sarcopenia and various blood-based biomarkers with ICI treatment outcomes and identify key factors associated with poor prognosis. Given the limitations of obtaining tumor tissues from patients with advanced NSCLC, we focused on blood-based biomarkers, including T lymphocytes subsets and cytokines, as complementary indicators of the systemic inflammatory status. Numerous studies have analyzed immune cells in the blood of patients using flow cytometry and, more recently, single-cell RNA sequencing. However, its clinical application remains limited owing to inaccessibility, the need for specialized equipment, and challenges regarding standardization and reproducibility [12].

Cytometric studies often involve multiple experimental groups and are susceptible to batch effects, which compromise the reproducibility. Although standardized protocols minimize these effects [13,14], they may not fully eliminate them. Manual batch-to-batch gating adjustment for cell subsets remains the simplest approach to multibatch integration [15,16]. We applied Spectre, an R package for high-dimensional cytometry data analysis, to rectify batch effects in the flow cytometry data [9]. Computational approaches improve data consistency, and enhance the reliability and clinical relevance of immune cell analyses. It has been well established by numerous studies that sarcopenia is associated with poor outcomes in patients receiving ICIs. The unique value of our study lies in the accurate diagnosis of sarcopenia based on DXA and in demonstrating its prognostic relevance through high-dimensional immune profiling using the Spectre package to rectify batch effects.

We confirmed that the PFS was significantly shorter in the sarcopenia group than in the nonsarcopenia group among patients with advanced NSCLC undergoing first-line immunotherapy. Multivariate analysis identified sarcopenia as an independent adverse prognostic factor of PFS. According to the 9th revision of the International Classification of Diseases (ICD), sarcopenia is characterized by low muscle mass, decreased muscle strength, and impaired physical performance. While factors such as Eastern Cooperative Oncology Group performance scale, activities of daily living, and BMI can provide insights into a patient’s general condition, sarcopenia is recognized as

the most objective and quantifiable indicator of overall health [17]. Diagnostic modalities for sarcopenia include DXA, bioelectrical impedance analysis, computed tomography, and magnetic resonance imaging [18]. Among these, DXA used in this study was the most reliable. Sarcopenia was evaluated based on ASM, as measured by DXA and normalized to height squared, yielding an absolute value. As these values vary by ethnicity and sex, we used diagnostic thresholds validated for the Korean population [8].

Sarcopenia remains under-diagnosed and poorly understood in clinical practice. This stems from the limited research and the lack of effective pharmacological interventions. Although sarcopenia is widely acknowledged to be associated with numerous chronic diseases and the development and treatment of cancer, comprehensive research, especially in the context of ICIs for NSCLC, is still lacking [19,20]. To address this gap, we investigated the relationship between sarcopenia and lung cancer by performing a detailed analysis of lymphocyte subsets, cytokines, and myokines in patients with NSCLC receiving first-line ICI therapy. Ongoing clinical trials are exploring the potential treatments for sarcopenia, including agents targeting muscle-specific proteins, myostatin inhibitors, and hormone-based therapies [21-23]. If these interventions are effective, they may offer valuable combination strategies to enhance the treatment efficacy for cancer and other diseases.

Sarcopenia is an independent prognostic factor in NSCLC patients receiving first-line ICI therapy. This negative effect likely stems from two

factors. First, patients with sarcopenia may not be able to continue treatment after experiencing adverse events. Although not statistically significant owing to the limited sample size, the pneumonitis-related ICI discontinuation rate was higher in the sarcopenia group than in the nonsarcopenia group. We hypothesized that although the incidence of adverse events may be comparable, prolonged recovery or an inability to restore the general condition for ICI reinitiation contributes to poorer outcomes in the sarcopenia group. Second, although not statistically significant in our study, the progressive disease rate in the first-response assessment was higher in the sarcopenia group than in the nonsarcopenia group, suggesting that patients with sarcopenia may have a reduced early response to ICIs [24].

A challenge faced in treating patients with sarcopenia using ICIs is the lack of clear predictive biomarkers. Although PD-L1 expression is a well-established predictive biomarker in well-designed randomized controlled trials (RCTs), these trials often exclude patients with poor general health, unlike in real-world clinical practice. Given the prevalence of sarcopenia in advanced cancer [25] and its high incidence in patients with advanced NSCLC, PD-L1 expression demonstrated an HR of 0.997 in our study, making it challenging to predict outcomes in the sarcopenia group. Additionally, PD-L1 expression showed no significant difference in PFS outcomes when stratified by sarcopenia index. Thus, more practical and effective prognostic markers are needed for patients with sarcopenia in real-world clinical settings, despite PD-L1’s overall robustness.

Sarcopenia primarily impairs ICI treatment outcomes by exacerbating abnormal inflammatory responses and is marked by elevated levels of inflammatory cytokines such as IL-1β, IL-6, and IL-8 [26-28]. Our analysis of 13 cytokines and myokines revealed elevated IL-6, IL-8, IL-10, IL-15, and TNF levels in the sarcopenia group. First, the deleterious effect of IL-8 on ICI efficacy likely stemmed from its paracrine activity, which facilitates the recruitment of myeloid-derived suppressor cells and promotes angiogenesis, and its autocrine function, which induces and maintains epithelial-mesenchymal transition (EMT) [29]. Notably, IL-8-mediated EMT appears to be closely associated with the activation of key signaling pathways, including AKT, MAPK/ERK, and JAK2/STAT3 [30]. Furthermore, *in vitro* assays demonstrated that IL-8 promotes C2C12 myotube atrophy [31], a mechanism commonly researched in sarcopenia. This is indicative of a potential feedback loop where sarcopenia enhances IL-8 secretion, which, in turn, exacerbates muscle wasting. Thus, IL-8 appears to be central to the pathophysiology of sarcopenia, and anti-IL-8 antibodies represent a promising therapeutic strategy for patients with NSCLC and sarcopenia [32]. Second, IL-6, a key regulator of energy metabolism, enhances energy absorption during energy deficits and transiently suppresses immune function [33]. Although IL-6 is classified as a myokine, it promotes muscle atrophy and wasting [34]. It can also impair ICI efficacy by directly inhibiting cytotoxic T lymphocyte function, reducing PD-L1 sensitivity, or disrupting memory cell function via the FOXO1 pathway [35]. Elevated inflammatory cytokines and an increased systemic

inflammatory tone are associated with muscle wasting and atrophy, which ultimately diminish the efficacy of ICIs.

The skeletal muscle plays a critical role in preventing T cell exhaustion, and physical exercise has been shown to mitigate T cell senescence and systemic inflammation. We focused on PD-1 and TIGIT, common markers of exhausted T cells [36]. Our analysis revealed that CD8+ T cells expressing TIGIT were associated with a poorer prognosis. Additionally, TIGIT expression was negatively correlated with both sarcopenia and muscle indices. Although TIGIT is a promising ICI target [37], its association with sarcopenia remains unclear. Given that TIGIT+ T cells increase with age [38], and TIGIT marks senescent T cells [39], our findings suggest that sarcopenia may be associated with elevated TIGIT expression, positioning TIGIT as a potential key prognostic marker in patients with sarcopenia undergoing ICI therapy.

T lymphocytes undergo continuous differentiation from naïve T cells to memory T cells (T_{CM}, T_{EM}) and ultimately to T_{TE} [10]. In the present study, the sarcopenia group showed a significant increase in the CD8+ T_{TE} cells. Furthermore, multivariate analysis identified CD8+ T_{CM}+T_{EM} as a poor prognostic factor for PFS. This contradicts the previous reports of favorable prognoses in cancer patients with higher memory T-cell levels [40], and the cause of this discrepancy remains unclear. Future longitudinal studies with serial sampling are needed to confirm our findings and explore the underlying mechanisms.

Our study has certain limitations. First, the analysis was based on a single blood sample, and muscle mass measurements were obtained

before ICI administration. This limits the evaluation of treatment-related changes and impact of longitudinal variations in muscle mass on ICI treatment outcomes. Future studies should include serial blood sampling and DXA-based measurements. Second, we were unable to perform tissue-based analyses because of limited tumor tissue availability and the infeasibility of repeated tissue biopsies. Consequently, the extent to which our blood-based findings reflect the systemic inflammatory status remains unclear. Third, although various cytokines and lymphocyte subsets were analyzed, direct investigation of immune cell-cytokine interactions was not conducted. Future studies should aim to establish this relationship using mouse models of sarcopenia and cell lines *in vitro*. Finally, overall survival analysis was not performed because of the variability in second-line therapies, as some patients were enrolled in ICI retreatment trials or RCTs for rare mutations.

In conclusion, sarcopenia is an independent poor prognostic factor in NSCLC patients receiving first-line ICI. And, sarcopenia appears to be associated with dysregulated inflammatory responses and high TIGIT expression. Other independent indicators of poor prognosis included IL-8, granzyme A, CD4+ T cells, and CD8+ T cells expressing TIGIT, CD8+ T_{CM} and T_{EM}. Notably, TIGIT-expressing CD8 + T cells were associated with worse outcomes, highlighting TIGIT's potential as both a prognostic marker and therapeutic target in sarcopenia patients.

ABBREVIATIONS

ASM, appendicular skeletal muscle mass; BMI, body mass index; CI, confidence interval; DXA, dual-energy X-ray absorptiometry; EDTA, ethylenediaminetetraacetic acid; ELISA, enzyme-linked immunosorbent assay; EMT, epithelial-mesenchymal transition; HR, hazard ratio; ICI, immune checkpoint inhibitor; ICD; International Classification of Diseases; IFN, interferon; IL, interleukin; NSCLC, non-small cell lung cancer; PBMC, peripheral blood mononuclear cell; PBS, phosphate-buffered saline; PD-L1, programmed cell death ligand 1; PFS, progression-free survival; PPP, platelet-poor plasma; RCT, randomized controlled trial; TNF, tumor necrosis factor; T_{reg}, T-regulatory lymphocyte; T_{TE}, terminally differentiated effector T cell.

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AUTHOR CONTRIBUTIONS

Jieun Park: Conceptualization (equal), Methodology (equal), Formal analysis (equal), Investigation (equal), Visualization (equal), Writing - Review and editing (equal). **Juwhan Choi:** Conceptualization (equal), Methodology (equal), Formal analysis (equal), Investigation (equal), Visualization (equal), Writing - Original draft preparation (lead). **Seunghun Lee:** Methodology (equal), Formal analysis (equal). **Jihyun Park:** Methodology (equal), Formal analysis (equal**). Chae Rin Kim:** Methodology (equal), Formal analysis (equal). **Yulim Lee:** Methodology

(equal), Formal analysis (equal). **Young Kee Shin:** Conceptualization (equal), Writing - Review and editing (equal), Supervision (equal). **Sung Yong Lee:** Conceptualization (equal), Writing - Review and editing (equal), Supervision (equal). All the authors have read and agreed to the published version of this manuscript.

DATA STATEMENT

The datasets used and analyzed in the current study are available from the corresponding authors upon reasonable request.

DECLARATION OF COMPETING INTERESTS

The authors declare that they have no competing financial interests or personal relationships that may have influenced the work reported in this study.

DECLARATION OF GENERATIVE AI AND AI-ASSISTED TECHNOLOGIES IN THE WRITING PROCESS

During the preparation of this work, the authors used ChatGPT (OpenAI) in order to assist with English language editing. After using this tool, the authors reviewed and edited the content as needed, and take full responsibility for the content of the published article.

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TABLES

Table 1. Baseline characteristics of patients.

	Total patients (N = 74)	Nonsarcopenia (N = 16)	Sarcopenia (N = 58)	*P-value*
Age (years)				
Median (min–max)		70 (50–88)	72 (50–95)	
<70	28	7 (43.8)	21 (36.2)	0.80
≥70	46	9 (56.3)	37 (63.8)	
Sex				
Male	67	15 (93.8)	52 (89.7)	1.00
Female	7	1 (6.3)	6 (10.3)	
Histology				
Adenocarcinoma	39	9 (56.3)	30 (51.7)	0.33
Squamous cell carcinoma	27	7 (43.8)	20 (34.5)	
Others	8	0 (0.0)	8 (13.8)	
Treatment regimen				
ICI monotherapy	21	5 (31.3)	16 (27.6)	0.76
ICI + ChT combination	53	11 (68.8)	42 (72.4)	
PD-L1 IHC (clone: SP263)				
<50	49	10 (62.5)	39 (67.2)	0.96
≥50	25	6 (37.5)	19 (32.8)	

Response evaluation				
CR	1	0 (0.0)	1 (1.7)	0.39
PR	33	7 (43.8)	26 (44.8)	
SD	13	5 (31.3)	8 (13.8)	
PD	27	4 (25.0)	23 (39.7)	
DCR	47	12 (75.0)	35 (60.3)	0.28
ORR	34	7 (43.8)	27 (46.6)	0.84
ICI-induced pneumonitis				
No	56	11 (68.8)	45 (77.6)	0.51
Yes	18	5 (31.3)	13 (22.4)	
ICI hold due to irAE				
No	69	16 (100.0)	53 (91.4)	0.58
Yes	5	0 (0.0)	5 (8.6)	

Abbreviations: ICI, immune checkpoint inhibitor; ChT, chemotherapy; PD-L1, programmed cell death ligand 1; IHC, immunohistochemistry; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; DCR, disease control rate; ORR, objective response rate; irAE, immune-related adverse events

Table 2. Differences in cytokine levels, myokine levels, and lymphocyte subsets between the sarcopenia and nonsarcopenia groups.

	Nonsarcopenia (N = 16)	Sarcopenia (N = 58)	*P-value*
Cytokines and myokines (Unit = pg/mL)			
IL-1β	0.0 (0.0–0.0)	6.9 (0.0–133.7)	0.10
IL-2	1.3 (0.0–5.9)	2.1 (0.0–13.9)	0.59
IL-4	2.1 (0.0–8.9)	3.0 (0.0–27.9)	0.59
IL-6	12.5 (1.6–48.4)	33.9 (0.0–553.2)	0.006
IL-8	0.4 (0.0–2.2)	7.4 (0.12–32.5)	0.038
IL-10	1.4 (0.0–8.2)	7.3 (0.0–250.5)	0.0055
IL-15	0.2 (0.0–2.8)	7.6 (0.0–254.8)	0.027
IL-17A	12.4 (0.0–148.9)	12.8 (0.0–138.3)	0.34
TNF	0.4 (0.0–2.2)	1.2 (0.0–10.1)	0.038
Decorin	6.5 (3.4–15.4)	6.9 (4.2–14.4)	0.29
IFN	1.3 (0.0–5.2)	1.8 (0.0–8.8)	0.46
Granzyme A	1.2 (0.0–10.3)	28.2 (0.0–447.4)	0.16
Granzyme B	136.6 (0.0–1178.5)	35.6 (0.0–579.2)	0.56
Lymphocyte subsets (Unit=% of total T lymphocytes)			
CD4+ T cells	54.1 (22.4–79.5)	48.8 (13.5–78.4)	0.21
Treg cells*	4.0 (1.1–11.8)	3.4 (0.2-–11.0)	0.52
Treg cells, PD-1+	2.7 (0.7–7.2)	2.1 (0.05–8.8)	0.14
CD4+ T cells, PD-1+	31.0 (15.2–46.5)	24.7 (2.0–50.1)	0.022

CD4+ T cells, TIGIT+	21.7 (8.8-40.5)	18.8 (5.2-33.0)	0.26
CD8+ T cells	25.0 (12.9–44.5)	29.4 (9.0–67.2)	0.092
CD8+ T cells, PD-1+	16.5 (3.4–43.3)	16.1 (0.1–60.3)	0.53
CD8+ T cells, TIGIT+	15.0 (6.4–26.0)	17.7 (3.5–50.5)	0.39
CD8+ T_{CM} and T_{EM}‡	6.9 (2.2–21.6)	8.9 (0.02–38.2)	0.17
CD8+ T_{TE} §	3.8 (0.2–9.0)	7.5 (1.2–22.6)	0.004

Abbreviations: IL, interleukin; TNF, tumor necrosis factor; IFN, interferon; Treg, regulatory T cell; TIGIT, T cell immunoreceptor with immunoglobulin and ITIM domain; T_{CM}, central memory T cell; T_{EM}, effector memory T cell; T_{EMRA}, effector memory cell re-expressing CD45RA; T_{TE}, terminally differentiated effector T cell

* Tregs are defined as CD4+, CD25+, or CD127dim.

‡ T_{CM} and T_{EM} are defined as CD45RA and CD45RO+, respectively.

§ T_{TE} is defined as CD45RA+ and CD45RO–.

Table 3. Univariate analysis of baseline characteristics, cytokine and myokine levels, and lymphocyte subsets associated with progression-free survival

	Hazard Ratio (95% CI)	*P-value*
Baseline characteristics		
Sarcopenia	2.4 (1.1–5)	0.022
Age (<70 vs. ≥70 years)	1.4 (0.81–2.3)	0.23
Sex (Male vs. Female)	1.2 (0.49–3.1)	0.65
Histology (Adenocarcinoma vs. Nonadenocarcinoma)	0.89 (0.62–1.3)	0.55
Treatment regimen (ICI mono vs. ICI + ChT)	1.3 (0.73–2.3)	0.37
PD-L1 IHC (clone: SP263) (<50 vs. ≥50)	0.98 (0.57–1.7)	0.94
ICI-induced pneumonitis	1 (0.58–1.9)	0.88
ICI hold due to irAE	2.1 (0.82–5.2)	0.12
Cytokines, myokines		
IL-1β	0.88 (0.4–1.9)	0.75
IL-2	1.4 (0.83–2.3)	0.22
IL-4	1.5 (0.88–2.5)	0.14
IL-6	2.1 (0.83–5.4)	0.12
IL-8	1.8 (0.95–3.2)	0.073

IL-10	1.6 (0.77–3.2)	0.21
IL-15	1.5 (0.87–2.5)	0.15
IL-17A	0.66 (0.31–1.4)	0.28
TNF	0.63 (0.27–1.5)	0.29
Decorin	0.7 (0.33–1.5)	0.35
IFN	1.3 (0.79–2.3)	0.27
Granzyme A	2.3 (1.1–4.9)	0.031
Granzyme B	1.2 (0.62–2.4)	0.55
Lymphocyte subsets		
CD4+ T cells	2.1 (1.2–3.7)	0.011
Treg cells*	0.58 (0.35–0.97)	0.037
Treg cells, PD-1+	0.47 (0.26–0.86)	0.013
CD4+ T cells, PD-1+	0.43 (0.2–0.93)	0.032
CD4+ T cells, TIGIT+	2 (0.79-–5)	0.14
CD8+ T cells	0.41 (0.18–0.92)	0.03
CD8+ T cells, PD-1+	0.37 (0.19–0.74)	0.0047
CD8+ T cells, TIGIT+	1.9 (0.94–3.7)	0.075
CD8+ T_{CM} and T_{EM} ‡	1.9 (1.1–3.1)	0.02
CD8+ T_{TE}§	2.2 (0.81–6.2)	0.12

Abbreviations: IL, interleukin; TNF, tumor necrosis factor; IFN, interferon; Treg, regulatory T cell; TIGIT, T-cell immunoreceptor with immunoglobulin and ITIM domain; T_{CM}, central memory T cells; T_{EM}, effector memory T cells; T_{EMRA}, effector memory cells expressing CD45RA; T_{TE}, terminally differentiated effector T cells; ICI, immune

checkpoint inhibitor; ChT, chemotherapy; PD-L1, programmed cell death ligand 1; IHC, immunohistochemistry; irAE, immune-related adverse events.

* Tregs are defined as CD4+, CD25+, or CD127dim.

T_{CM} and T_{EM} are defined as CD45RA and CD45RO+, respectively.

§ T_{TE} is defined as CD45RA+ and CD45RO–.

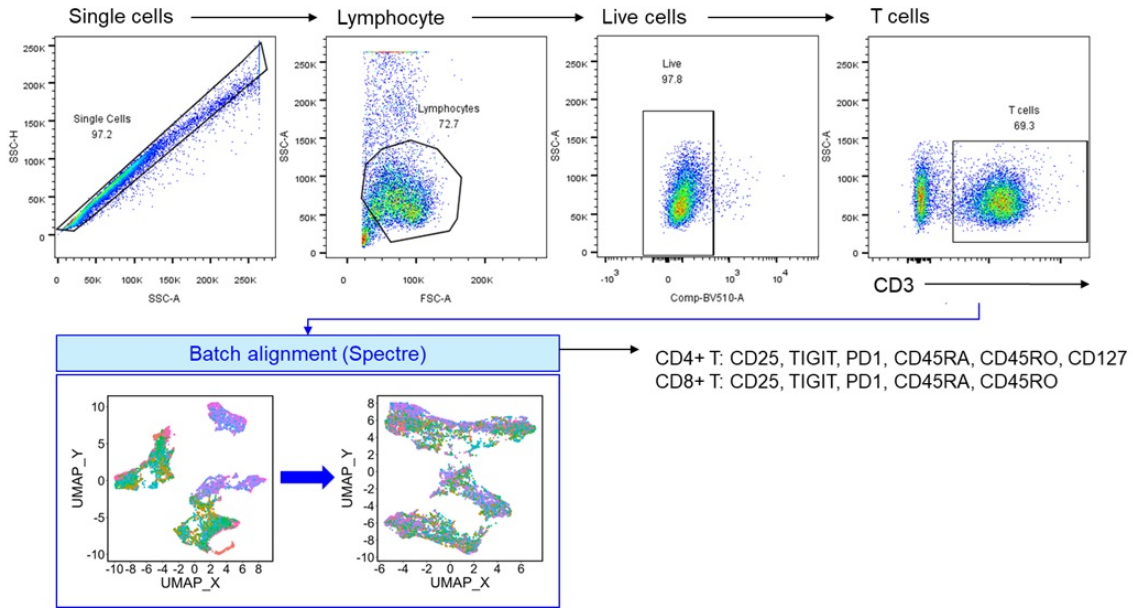

Fig. 1 Representative gating strategy for assessing T-cell subpopulations in patients' blood. After differentiating the T-cell subsets, batch alignment was performed using the Spectre package, as described in the methods section. Results are expressed as a percentage of the total T-cell population.

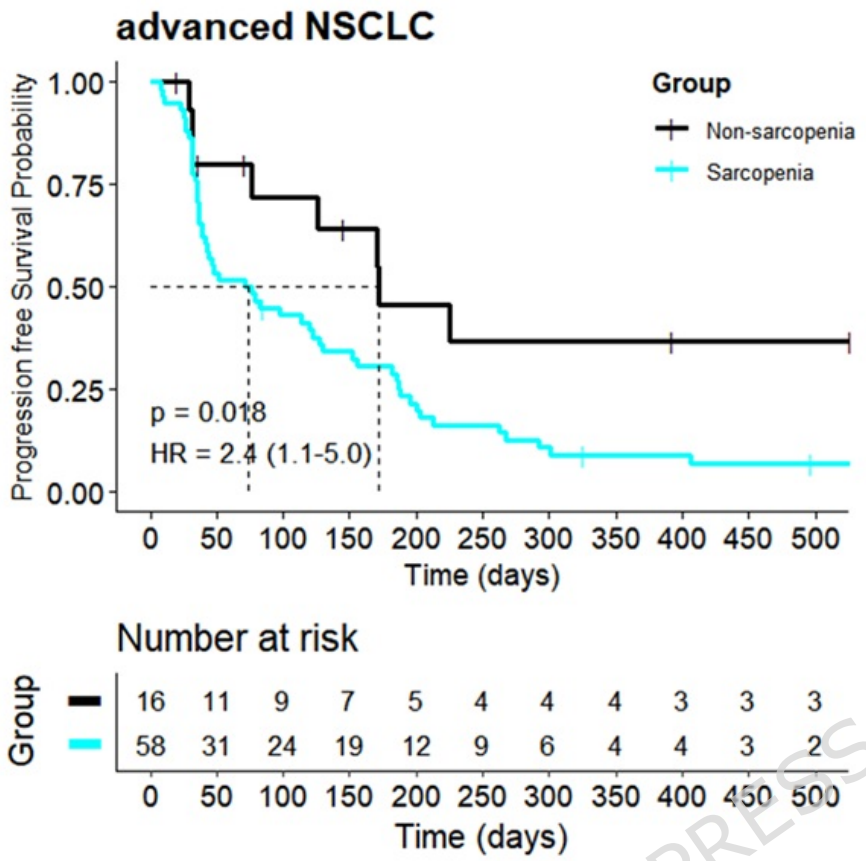

Fig. 2 Kaplan–Meier survival curve for PFS. Censored data points are indicated by bars on the survival curves. Abbreviations: PFS, progression-free survival.

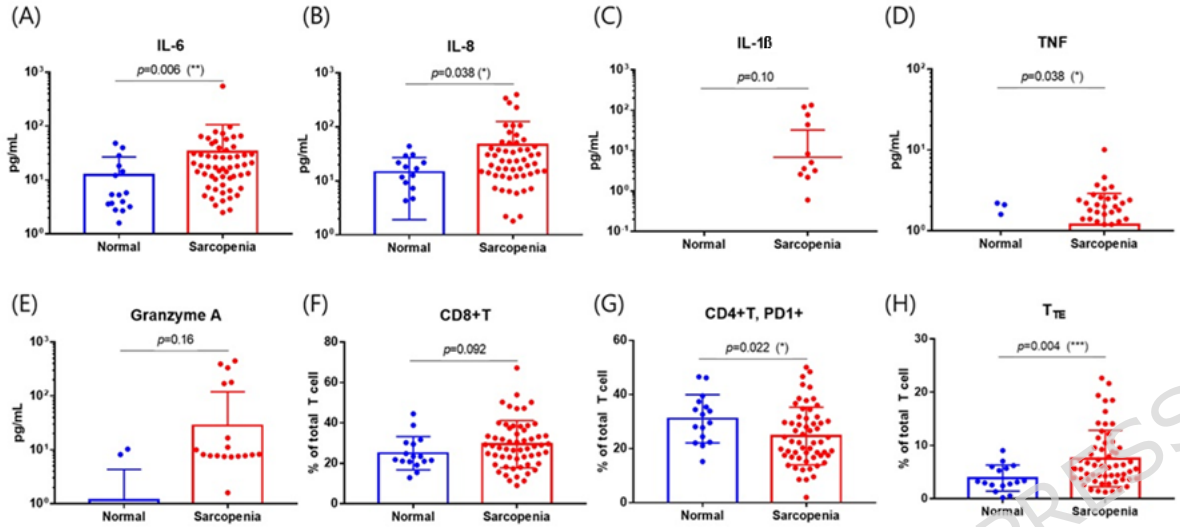

Fig. 3 Box plot comparing variables between the sarcopenia and non-sarcopenia groups (a) IL-6, (b) IL-8, (c) IL-1b, (d) TNF, (e) Granzyme A, (f) CD8+ T cells, (g) CD4+ T cells with PD1+, and (h) T_{TE}. For plots with a log-scaled y-axis, data points with a value of 0 were excluded. Abbreviations: IL, interleukin; TNF, tumor necrosis factor; T_{TE}, terminally differentiated effector T cells.

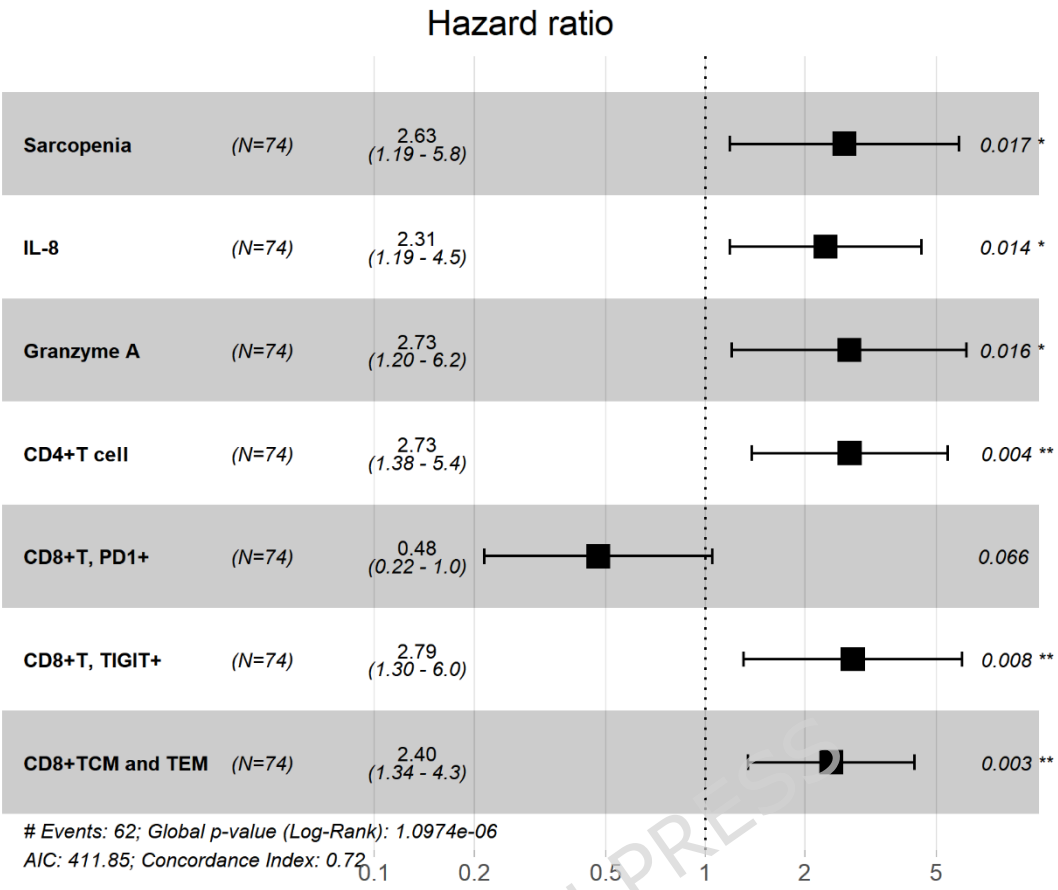

Fig. 4 Forest plot of HR for PFS. Abbreviations: HR, hazard ratio; PFS, progression-free survival.

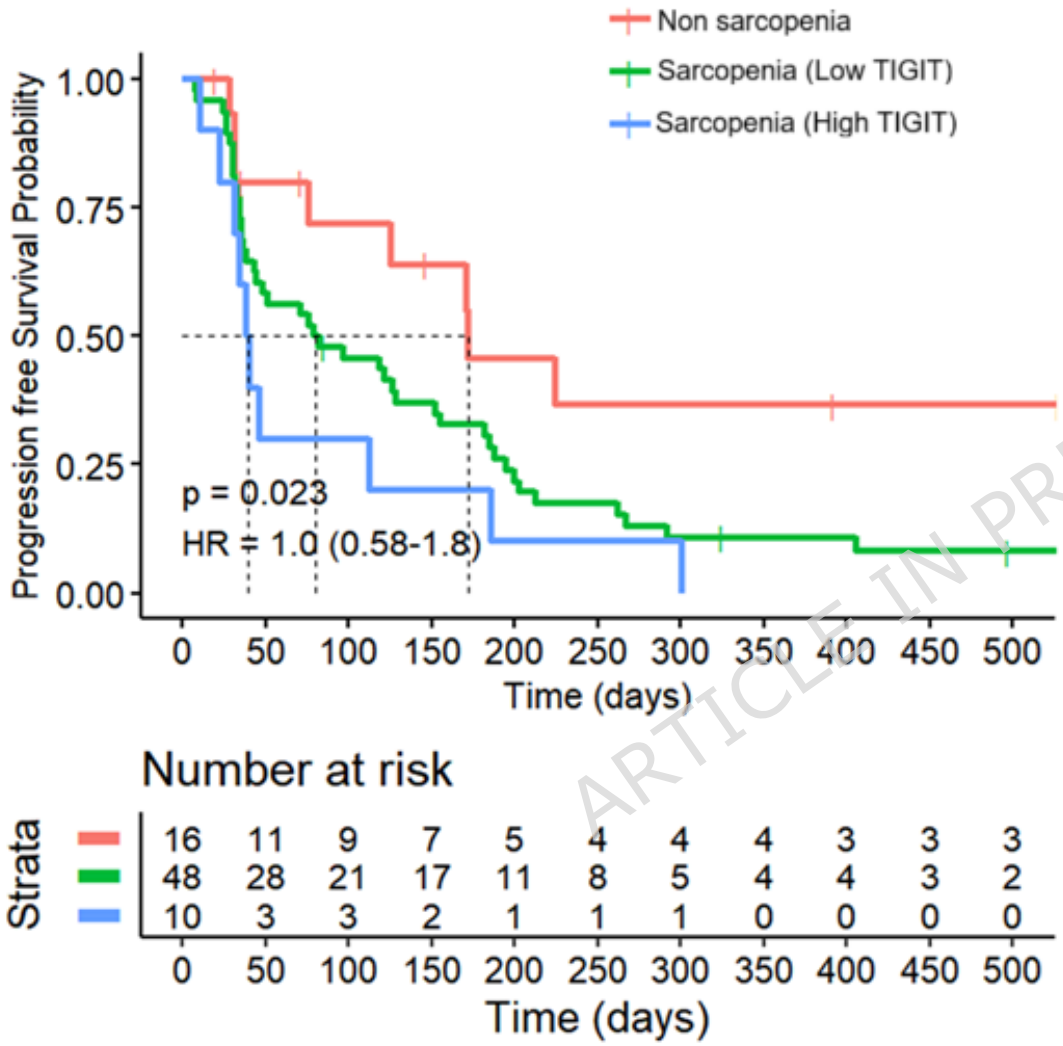

Fig. 5 PFS analysis according to TIGIT expression and sarcopenia. Abbreviations: PFS, progression-free survival.