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Original Article

Predictive value of serum alkaline phosphatase, tumor-specific growth factor, and macrophage migration inhibitory factor for the efficacy of immunotargeted therapy in osteosarcoma patients

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Abstract: Objective: To assess the predictive value of serum alkaline phosphatase (ALP), tumor-specific growth factor (TSGF), and macrophage migration inhibitory factor (MIF) for the efficacy of combined immunosuppressive and targeted therapy in osteosarcoma (OS). Methods: We retrospectively analyzed clinical data from 161 OS patients treated at Xi'an Honghui Hospital from October 2020 to October 2022. Patients received 12 weeks of therapy with interferon-α (IFN-α) and bevacizumab. Serum levels of ALP, TSGF, and MIF were measured before and after treatment. Based on treatment efficacy, patients were categorized into effective and ineffective groups. Both univariate and logistic regression analyses were utilized to evaluate the influence of these biomarkers on therapy outcomes. Results: A significant reduction in serum ALP, TSGF, and MIF levels post-treatment was found (all $P<0.001$). Higher pre-treatment levels of these biomarkers were associated with less effective outcomes ($P<0.001$). Conclusion: Pre-treatment levels of ALP, TSGF, and MIF are significant independent predictors of response to immunotargeted therapy in OS patients, suggesting their potential role in guiding treatment strategies.

Keywords: Osteosarcoma, immunotherapy, targeted therapy, alkaline phosphatase, tumor-specific growth factor, macrophage migration inhibitory factor

Introduction

Osteosarcoma (OS), a bone disease, is the third most prevalent cancer in children and adolescents, following lymphoma and brain tumors [1]. This bone cancer typically arises during periods of rapid growth, predominantly affecting adolescents with an incidence of approximately 0.8-1.1 per 100,000 per year among those aged 15-19, and also presenting a lesser peak in older adults [2, 3]. OS originates from mesenchymal cell lines capable of producing osteoid and/or immature bone [4]. The etiology of OS remains largely undefined, although its frequent occurrence in growing adolescents and localization to the metaphyses of long bones suggests a link with rapid bone growth [5].

In the evolution of treatment strategies, Rosen et al. developed a multidisciplinary approach in the 1970s that combines neoadjuvant and adjuvant chemotherapy with limb-sparing surgery, achieving approximately 70% five-year survival rates [6]. Recent advancements include the EURAMOS-1 trial by the Cooperative Osteosarcoma Study Group and the European Osteosarcoma Intergroup (EOI), which explored the effects of extended chemotherapy on event-free survival (EFS) [7]. This trial involved adding ifosfamide, etoposide, and pegylated interferon (IFN) α-2b, but showed only minimal improvement in EFS, indicating the limitations of traditional chemotherapy in enhancing survival, particularly in metastatic OS cases where survival rates were between 19%-30% [7].

Immunotherapy and targeted therapy represent emerging paradigms in OS treatment. Immunotherapy aims to enhance the body's antitumor immune response through increased

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cellular cytotoxicity and induction of tumor cell apoptosis [8, 9]. Concurrently, targeted therapy inhibits critical pathways essential for tumor growth, facilitating T-cell mediated tumor clearance and promoting apoptosis [10, 11]. Notably, the genetic instability in OS may generate novel epitopes that serve as potential targets for immune checkpoint inhibitors, despite the typically low tumor mutation burden (TMB) seen in childhood cancers [12, 13]. These therapies, particularly when combined, can significantly boost the host immune response and overall treatment efficacy in OS patients [14, 15].

Current clinical assessments of malignant tumors primarily rely on changes in lesion volume. While this method facilitates adjustments in treatment plans, it often fails to accurately evaluate therapeutic effects, demonstrating limitations in its clinical utility. Consequently, identifying reliable predictors of immunotargeted therapy efficacy in OS patients is crucial for optimizing treatment strategies and enhancing patient outcomes. Alkaline phosphatase (ALP), extensively present in the liver, bones, and kidneys, reflects potential liver, gallbladder, and skeletal disorders through serum level fluctuations, thus commonly used in clinical diagnosis and prognosis of these conditions [16]. Tumor-specific growth factor (TSGF), a product of tumor cells, increases in the peripheral blood due to enhanced capillary expansion and permeability associated with tumor growth and progression, serving primarily in the early detection, diagnosis, prognosis, and therapeutic efficacy evaluation of malignant tumors [17]. Macrophage migration inhibitory factor (MIF) functions as a pro-inflammatory cytokine involved in various pathological processes, including immune responses and inflammation, promoting cell proliferation and is prevalent in numerous malignancies [18]. The potential of these three biomarkers to predict the effectiveness of immunotargeted therapy in OS patients has not yet been explored. This study aims to evaluate their predictive value in enhancing the efficacy of such treatments in OS cases.

Methods

Study design and patients

Ethical approval for this retrospective study was granted by the Ethics Committee of Xi'an Honghui Hospital. We analyzed the clinical data of 161 OS patients who underwent immunotargeted therapy at the above hospital between October 2019 and October 2020. Inclusion criteria were: (1) OS diagnosis confirmed by CT, MRI, and pathological examination; (2) No prior tumor history; (3) Expected survival of more than 3 months; (4) No benefit from standard chemotherapy and initial treatment with immunotargeted therapy; (5) Completion of the prescribed immunotargeted therapy course; (6) Karnofsky performance status score of 60 or higher; (7) Availability of complete medical records. Exclusion criteria included: (1) Presence of other malignancies or bone tumors resulting from metastasis; (2) Significant comorbid conditions such as myocarditis or renal failure; (3) Prior surgical or immunotargeted therapy; (4) Immune system disorders or contraindications to immunotherapy; (5) Cognitive impairments or mental disorders; (6) Discontinuation of treatment or death due to natural disease progression; (7) Incomplete medical records. The details of the selection of patients included in the study were shown in **Figure 1**.

Data collection

We collected baseline data from the medical records of the patients, including gender, age, duration of disease, primary tumor location, Enneking classification, ALP, TSGF and MIF levels before treatment and 12 weeks after initiation of therapy. Treatment efficacy was also documented.

Treatment methods and efficacy evaluation

Treatment involved a regimen of subcutaneous IFN-α at 9 million units every three weeks and intravenous bevacizumab at 10 mg/kg every two weeks for 12 weeks. Efficacy was assessed four weeks post-treatment using the revised Response Evaluation Criteria in Solid Tumors. Outcomes were categorized as: Complete Response (CR) - disappearance of all target lesions and normalization of tumor markers for at least four weeks; Partial Response (PR) - at least 30% reduction in the sum of diameters of target lesions for a minimum of four weeks; Stable Disease (SD) - less than 30% decrease or less than 20% increase in the sum of diameters of target lesions; Progressive Disease (PD) - 20% or more increase in the sum of diameters of target lesions or appearance of new

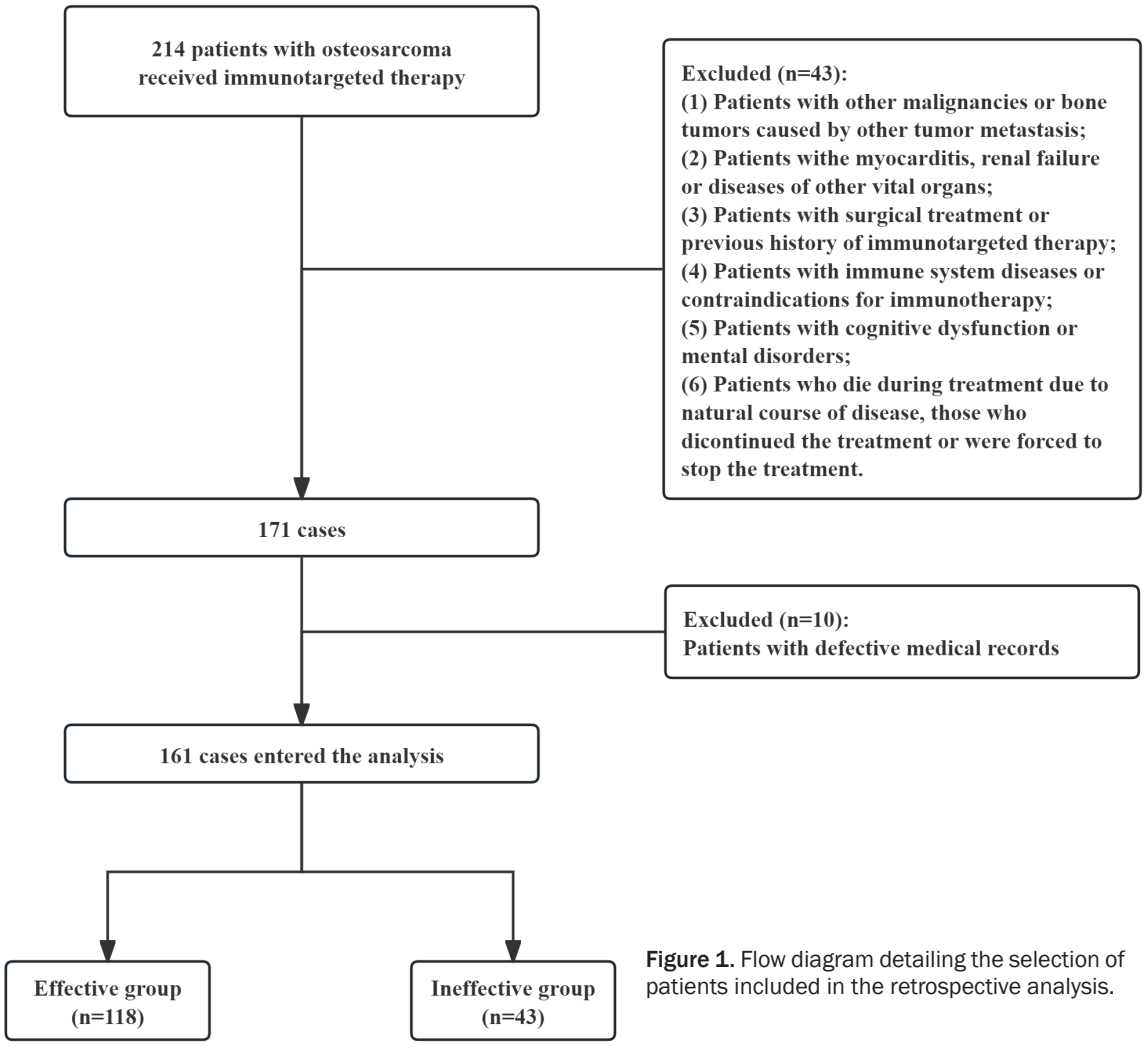

Figure 1. Flow diagram detailing the selection of patients included in the retrospective analysis.

lesions. Patients achieving CR or PR were classified as responding effectively to treatment, while those with SD or PD were deemed non-responders.

Outcome measures

(1) For each patient, 5 mL of fasting cubital venous blood was collected before and at the end of treatment. Samples were centrifuged for 10 minutes at 3000 rpm with a centrifuge radius of 10 cm to obtain the supernatant. Serum levels of ALP were determined using disodium phenylphosphate colorimetry, TSGF levels were measured by immunoturbidimetric assay, and MIF levels were assessed through enzyme-linked immunosorbent assay according to the respective kit instructions. ALP kits were sourced from Nanjing Jiancheng Bioengineering Institute, China (A059-2-2); TSGF kits from Shanghai Yushao Biotechnology Co., Ltd., China (YS-F10035); and MIF kits from Upingbio Technology Co., Ltd., China (SYP-H0341).

(2) Disease-free survival within three years post-treatment was considered indicative of a favorable prognosis. Univariate prognostic factors were initially analyzed, followed by inclusion in a multivariate regression analysis to identify independent prognostic factors.

Statistical methods

Data analyses were conducted using SPSS version 25.0. Categorical variables, expressed as counts and percentages, were compared between groups using the chi-square test. Continuous variables were tested for normality; those fitting a normal distribution were described statistically as mean ± standard deviation and compared between groups using

Table 1. Clinical data of all patients

Clinical features		n = 161
Gender		
Male		84 (52.2)
Female		77 (47.8)
Age		19.24±1.23
Duration of disease (months)		6.05±1.01
Primary tumor location		
Tibia		38 (23.6)
Femur		58 (36.0)
Humerus		53 (32.9)
Other parts		12 (7.5)
Enneking classification		
IIA		64 (39.8)
IIB		72 (44.7)
III		25 (15.5)
Before treatment	ALP (IU/L)	399.5 (338.8, 444.9)
	TSGF (U/mL)	84.6 (76.3, 90.6)
	MIF (ng/mL)	1.7 (1.4, 2.1)
After 12 weeks of treatment	ALP (IU/L)	276.4 (242.4, 305.5)*
	TSGF (U/mL)	61.5 (55.0, 68.2)*
	MIF (ng/mL)	1.4 (1.1, 1.7)*
Treatment efficacy	Complete response	39 (24.2)
	Partial response	79 (49.1)
	Stable disease	35 (21.7)
	Progressive disease	8 (5.0)

Note: ALP, serum alkaline phosphatase; TSGF, tumor-specific growth factor; MIF, macrophage migration inhibitory factor; *P<0.001 vs. before treatment.

the t-test. Variables with skewed distributions were expressed as medians and interquartile ranges; inter-group and intra-group comparisons were performed using the Mann-Whitney U test and the Wilcoxon test, respectively. Logistic regression was employed to determine factors influencing the efficacy of immunotargeted therapy in OS patients. Receiver operating characteristic (ROC) curves were plotted, and areas under the curves (AUCs) were calculated to evaluate the predictive value of serum ALP, TSGF, and MIF levels for immunotargeted therapy efficacy. A *P*-value<0.05 was considered statistically significant.

Results

General information of patients and therapeutic efficacy

After screening, 161 patients qualified for the study. The cohort consisted of 84 males and 77 females, ages ranging from 9 to 23 years (mean: 19.24±1.23), and the duration of disease spanned 3 to 8 months (mean: 6.05± 1.01). The primary tumor sites were the tibia (38 cases), femur (58 cases), humerus (53 cases), and other locations (12 cases). Based on the Enneking staging system, patients were classified as Stage IIA (64 patients), IIB (72 patients), and III (25 patients). After 12 weeks of treatment, significant decreases were observed in serum levels of ALP, TSGF, and MIF (all P<0.001). There were no deaths or treatment discontinuations. The treatment was considered effective CR and PR in 118 patients (73.3%) and ineffective SD and PD in 43 patients (26.7%). See **Table 1**.

Comparison of baseline data of ineffective and effective groups

The effective group exhibited higher pre-treatment levels of ALP, TSGF, and MIF compared to the ineffective group (all P<0.001). No significant differences were found in sex, age, disease duration, primary tumor location, or Enneking stage between the groups (all P>0.05), as shown in **Table 2**.

Analysis of factors affecting the efficacy of immunotargeted therapy

In the univariate analysis, ALP, TSGF, and MIF levels showed statistical significance and were thus selected as independent variables. The optimal cutoff values for efficacy, determined by the Youden index, were 409.9 for ALP, 87.5 for TSGF, and 1.7 for MIF. The efficacy of immunotargeted therapy was modeled as a dependent variable in a binary logistic regression analysis (ineffective = 1, effective = 0), with pre-treatment serum levels of ALP, TSGF, and MIF identified as significant predictors of thera-

Table 2. Comparison of clinical data between effective and ineffective groups

Clinical features	Effective group (n = 118)	Ineffective group (n = 43)	X^2/t	P
Sex			0.754	0.385
Male	64 (54.2)	20 (46.5)		
Female	54 (45.8)	23 (53.5)		
Age	18.17±1.97	18.37±2.60	0.521	0.603
Duration of disease (months)	6.08±0.94	6.24±1.05	0.926	0.356
Primary tumor location			4.404	0.221
Tibia	27 (22.9)	11 (25.6)		
Femur	43 (36.4)	15 (34.8)		
Humerus	42 (35.6)	11 (25.6)		
Others	6 (5.1)	6 (14.0)		
Enneking classification			0.988	0.610
IIA	49 (41.5)	15 (34.8)		
IIB	50 (42.4)	22 (51.2)		
III	19 (16.1)	6 (14.0)		
ALP (IU/L)	379.4 (327.5, 430.15)	444.9 (415.1, 459.2)	5.227	<0.0001
TSGF (U/mL)	81.6 (73.5, 86.8)	92.6 (88.3, 97.3)	7.106	<0.0001
MIF (ng/mL)	1.6 (1.4, 1.9)	2.1 (1.8, 2.3)	4.008	<0.0001

Note: ALP, serum alkaline phosphatase; TSGF, tumor-specific growth factor; MIF, macrophage migration inhibitory factor.

Table 3. Multi-factor analysis of the factors affecting the efficacy of immunotargeted therapy in osteosarcoma patients

Factors	β	SE	Wald	P	HR	95% CI
ALP (1 = >409.9, 0 = ≤409.9)	2.796	0.609	21.108	0.000	16.387	4.970-54.026
TSGF (1 = >87.5, 0 = ≤87.5)	2.392	0.542	19.465	0.000	10.935	3.779-31.647
MIF (1 = >1.7, 0 = ≤1.7)	2.380	0.583	16.643	0.000	10.810	3.445-33.922
Constant	-5.286	0.793	44.425	0.000	0.005	-

Note: ALP, serum alkaline phosphatase; TSGF, tumor-specific growth factor; MIF, macrophage migration inhibitory factor.

peutic outcome (all P<0.0001), as shown in **Table 3**.

Predictive value of serum ALP, TSGF and MIF for the efficacy of immunotargeted therapy in OS patients

Based on logistic regression, the risk score for therapy efficacy, Logit (P), is calculated as follows: Logit (P) = 22.087 + 0.017 * ALP (0 = ≤409.9, 1 = >409.9) + 0.124 * TSGF (0 = ≤87.6, 1 = >87.6) + 1.941 * MIF (0 = ≤1.7, 1 = >1.7). The probability of efficacy for each patient is then derived from the equation: $P = \frac{e^{Logit}(P)}{1 + e^{Logit}(P)}$.

The AUC values for ALP, TSGF, and MIF alone are 0.761, 0.838, and 0.733, respectively. When combined, the AUC reaches 0.905, demonstrating enhanced predictive accuracy (**Figure 2**; **Table 4**).

Correlation of serum ALP, TSGF and MIF with Ennecking staging

A correlation exists between serum levels of ALP, TSGF, and MIF and Enneking staging in OS patients. As staging advances, levels of these biomarkers increase, with only ALP showing statistically significant differences (P<0.05, **Table 5**).

Analysis of single factor influencing prognosis

Univariate analysis reveals that post-treatment levels of ALP, TSGF, and MIF are associated with prognosis (all P<0.05). However, gender, age, and pathology type do not show significant prognostic influence (all P>0.05) (**Table 6**).

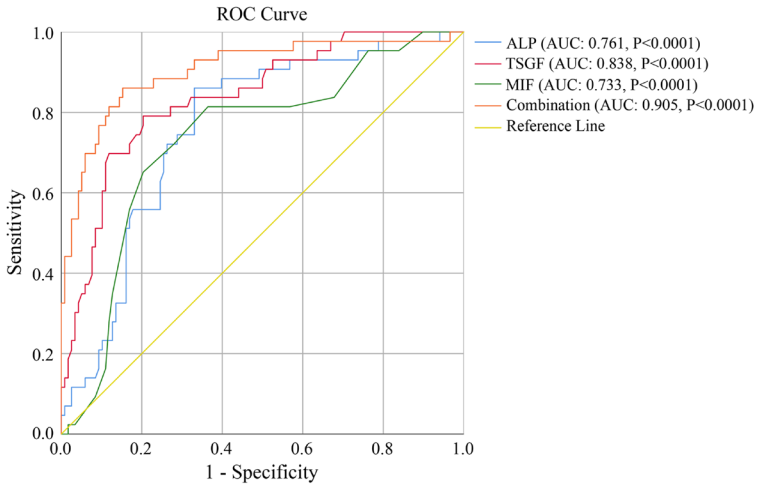

Figure 2. ROC curves of serum ALP, TSGF and MIF for predicting the efficacy of immunotargeted therapy in osteosarcoma patients. Note: ALP, serum alkaline phosphatase; TSGF, tumor-specific growth factor; MIF, macrophage migration inhibitory factor.

Table 4. Predictive value of serum ALP, TSGF and MIF for immunotargeted therapy efficacy in OS patients

Factors	AUC	SE	P	95% CI	Specificity (%)	Sensitivity (%)
ALP	0.761	0.041	0.000	0.681-0.841	66.9	86.0
TSGF	0.838	0.035	0.000	0.769-0.907	79.7	79.1
MIF	0.733	0.045	0.000	0.645-0.821	63.6	81.4
Combination	0.905	0.030	0.000	0.846-0.963	84.7	86.0

Note: ALP, serum alkaline phosphatase; TSGF, tumor-specific growth factor; MIF, macrophage migration inhibitory factor.

Table 5. Correlation of serum ALP, TSGF, and MIF with Ennecking staging

Staging	ALP	TSGF	MIF
IIA	410 (358.9, 447.1)	85.5 (76.1, 92.2)	1.8 (1.4, 2.1)
IIB	393.1 (333.6, 439.4)	83.4 (74.8, 89.2)	1.7 (1.4, 2.0)
III	426.6 (379.4, 453.0)	90.45 (83.1, 95.8)	2.1 (1.8, 2.3)
F	3.410	1.923	2.997
P	0.036	0.149	0.053

Note: ALP, serum alkaline phosphatase; TSGF, tumor-specific growth factor; MIF, macrophage migration inhibitory factor.

Multivariate regression analysis of factors influencing prognosis

Using the Youden index to establish optimal cutoff values, the thresholds are set at 312.4 for ALP, 62.4 for TSGF, and 1.9 for MIF. In a multivariate regression analysis, these post-treatment levels serve as independent variables, with prognosis categorized as poor (1) or favorable (0). Results indicate that post-treatment levels of ALP, TSGF, and MIF independently affect prognosis (all P<0.05) (**Table 7**).

Discussion

IFNs are glycoproteins secreted by foreign pathogens and tumor cells, classified under a broader category of biomolecules known as cytokines [19]. IFNs possess antiviral, immunomodulatory, and proliferation-inhibiting properties, and can disrupt various signaling pathways including those involving vascular endothelial growth factor (VEGF), interleukin (IL)-8, IL-10, transforming growth factor-β, and tumor necrosis factor-α [20]. Targeted therapies not only exert direct anti-tumor effects but also modulate immune responses by enhancing dendritic cell (DC) antigen presentation and cytotoxic T lymphocyte activation [21]. Despite advancements in medical technology and the growing experience with immunotargeted therapies, which have improved the 5-year survival rate for OS patients, outcomes remain suboptimal for some, underscoring the need for reliable therapeutic efficacy predictors to refine clinical protocols and enhance patient outcomes [22].

Post-treatment analysis revealed statistically significant reductions in serum levels of ALP, TSGF, and MIF compared to baseline. ALP, primarily sourced from the liver and bone, plays a crucial role in bone formation by hydrolyzing phosphate to provide phosphoric acid for hydroxyapatite deposition and breaking down pyrophosphate to facilitate bone salt forma-

Table 6. Univariate analysis of prognostic influences

Clinical features	Favourable prognosis (n = 127)	Poor prognosis (n = 34)	X^2/t	P
Sex			1.589	0.208
Male	63	21		
Female	64	13		
Age	18.77±2.24	18.41±1.78	0.866	0.388
Duration of disease (months)	6.11±1.00	6.20±0.84	0.481	0.631
Primary tumor location			3.842	0.279
Tibia	32	6		
Femur	42	12		
Humerus	46	11		
Others	7	5		
Enneking classification			0.096	0.953
IIA	51	13		
IIB	56	16		
III	20	5		
ALP (IU/L)	274.00 (241.65, 300.05)	294.50 (267.35, 330.95)	2.409	0.017
TSGF (U/mL)	60.60 (53.80, 66.70)	66.30 (60.35, 72.55)	3.770	0.0002
MIF (ng/mL)	1.40 (1.00, 1.70)	1.50 (1.20, 2.10)	2.640	0.009

Note: ALP, serum alkaline phosphatase; TSGF, tumor-specific growth factor; MIF, macrophage migration inhibitory factor.

Table 7. Multivariate regression analysis of factors influencing prognosis in OS patients

Factors	β	SE	Wald	P	HR	95% CI
ALP (1 = >312.4, 0 = ≤312.4)	1.327	0.461	8.282	0.004	3.769	1.527-9.302
TSGF (1 = >62.4, 0 = ≤62.4)	1.322	0.446	8.769	0.003	3.751	1.564-8.999
MIF (1 = >1.9, 0 = ≤1.9)	1.576	0.518	9.253	0.002	4.833	1.751-13.340
Constant	-2.704	0.422	41.043	0.000	0.067	-

Note: OS, osteosarcoma; ALP, serum alkaline phosphatase; TSGF, tumor-specific growth factor; MIF, macrophage migration inhibitory factor.

tion, thus reflecting the osteoblast differentiation rate [23]. ALP is highly specific as a tumor marker in OS, pivotal for assessing treatment responsiveness and tumor burden correlation [24]. Notably, Mialou et al. identified serum ALP levels exceeding 500 IU/L as an independent risk factor for diminished disease-free and overall survival [25]. TSGF, secreted by tumor cells, fosters the proliferation of tumors and adjacent capillaries, sustaining tumor growth. Recognized internationally as a broad-spectrum marker, TSGF correlates with malignancy growth and suggests high tumor aggressiveness and treatment complexity when overexpressed [26, 27]. TSGF levels can rise significantly during the early stages of malignant tumor formation, inducing genes associated with malignant transformation [28]. TSGF also impacts the differentiation of certain T lymphocyte clones by inhibiting the production of immunoglobulins IgG and IgM, enhancing tumor cell resistance to natural killer cells, and promoting angiogenesis [29]. MIF is a multifunctional cytokine expressed in immune, endocrine, and epithelial cells exposed to the external environment [30]. Recent findings indicate that MIF plays a crucial role in both innate immunity and tumor progression, including malignant transformation [31]. Studies by Han et al. have demonstrated that increased MIF expression correlates with poorer prognosis in advanced OS [32]. Given the roles of ALP, TSGF, and MIF in tumor growth and prognosis assessment, we hypothesize that these markers can predict the efficacy of immunotargeted therapy in OS.

Binary logistic regression analysis confirmed that pre-treatment levels of serum ALP, TSGF, and MIF are predictive of the efficacy of immu-

notargeted therapy in OS patients. Specifically, high expression levels of these biomarkers were associated with reduced therapy effectiveness. Receiver operating characteristic curves further demonstrated that these markers, particularly when combined, offer substantial predictive value for treatment outcomes. Accordingly, we recommend that OS patients with elevated pre-treatment levels of ALP, TSGF, and MIF may benefit from additional therapies, such as radiotherapy or other immunosuppressants, alongside regular monitoring of serum levels to adjust treatment plans appropriately.

Additionally, our analysis identified a significant relationship between serum ALP levels and Enneking staging in OS patients, with stage III patients exhibiting higher levels than those in stage II. No similar associations were observed for TSGF and MIF, potentially due to their lower sensitivity compared to ALP.

This study has several limitations. Firstly, besides the three biomarkers analyzed (ALP, TSGF, and MIF), other potential indicators might also influence the efficacy of immunotargeted therapy in OS patients. Secondly, this investigation, being a single-center retrospective analysis, is constrained by its scope and scale. Thus, a multicenter, large-sample, prospective study is essential to rigorously validate the predictive value of these biomarkers. Consequently, a well-designed randomized controlled trial is necessary to confirm our findings through prospective data collection and sample size calculations, and to further elucidate the relationship between these serum biomarkers and treatment outcomes.

In conclusion, OS patients exhibiting high serum levels of ALP, TSGF, and MIF pre-treatment may receive limited benefit from immunotargeted therapy. These biomarkers are independent predictors of therapy efficacy, with their combined assessment offering superior predictive value. Future research should focus on integrating ALP, TSGF, and MIF as key indices to evaluate the effectiveness of immunotargeted treatments in OS patients.

Disclosure of conflict of interest

None.

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