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Pretreatment levels of serum alkaline phosphatase are associated with the prognosis of patients with non-small cell lung cancer receiving immune checkpoint inhibitors

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Abstract. Immune checkpoint inhibitors (ICIs) have been an encouraging treatment method in non-small cell lung cancer (NSCLC). However, bone and liver metastases are considered to restrain immunotherapy efficacy. Since serum alkaline phosphatase (ALP) is associated with bone and liver metastases, it was investigated whether serum ALP could be a novel biomarker to predict the efficacy of ICIs treatment. In the present study, 143 patients with NSCLC receiving ICIs treatment were retrospectively analyzed. The objective response rate (ORR) was compared between the ALP high and low groups, bone metastasis and non-bone metastasis groups, and liver metastasis or non-liver metastasis groups. The associations between clinical characteristics, including ALP level, bone or liver metastasis and median progression-free survival (mPFS) time were analyzed by univariate and multivariate Cox regression analysis. It was found that bone metastasis was associated with a lower ORR (24 vs. 43%; P<0.05) and shorter mPFS (10.2 vs. 17.3 months; P=0.010) in patients with NSCLC receiving ICIs. Liver metastasis was associated with lower ORR (22 vs. 38%; P<0.05), but not with mPFS (P=0.119). The ALP level was higher in patients with bone or liver metastasis than in those without (119.6 or 103.6 vs. 83.3 U/l, respectively; P<0.05). Higher ALP levels were also associated with bone or liver metastasis, lower ORR (20 vs. 39%; P<0.05) and shorter mPFS (8.5 vs. 15.4 months; P=0.009). Cox regression analysis demonstrated that ALP was an independent prognostic indicator of mPFS (hazard ratio, 1.856; 95% confidence interval, 1.030-3.343; P=0.040). In conclusion, pretreatment levels of serum ALP might be a predictive indicator of clinical outcome in patients with NSCLC after ICIs treatment.

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Abbreviations: ALP, alkaline phosphatase; CTLA-4, cytotoxic T lymphocyte-associated antigen-4; ICIs, immune checkpoint inhibitors; MSI, microsatellite instability; NSCLC, non-small cell lung cancer; ORR, objective response rate; PD-1, programmed cell death-1; PD-L1, programmed cell death-ligand 1; PFS, progression-free survival; TMB, tumor mutational burden

Key words: ALP, biomarker, ICIs, NSCLC

Introduction

Lung cancer is the leading cause of cancer-related mortality worldwide (1). Non-small cell lung cancer (NSCLC) accounts for ~85% of all histological types (2). The treatment plan for patients with advanced NSCLC depends on their genotype, and treatments include targeted therapy, chemotherapy and immunotherapy (3). Immune checkpoint inhibitors (ICIs), such as programmed cell death-1 (PD-1)/programmed cell death-ligand 1 (PD-L1) or cytotoxic T lymphocyte-associated antigen-4 (CTLA-4) antibodies, have been approved for the treatment of patients with advanced NSCLC based on their encouraging efficacy in a series of clinical trials (4,5). Although combination with chemotherapy or antiangiogenic agents improves the clinical outcome of ICIs, a considerable fraction of patients does not benefit from ICIs (6-8). Therefore, it is important to identify biomarkers that can be used to predict the response to ICIs in patients with NSCLC.

At present, PD-L1 expression, tumor mutational burden (TMB) and microsatellite instability (MSI) status have been recognized as reliable indicators to predict the response to ICIs in NSCLC, hepatocellular carcinoma, urothelial carcinoma and other tumors (9,10). As the most important target of immunotherapy, the infiltration, T cell receptor repertoire diversity and gene signatures of $CD8^+$ T cells can also predict the clinical benefit of ICIs treatment in advanced NSCLC (11-13). Additionally, several routine blood parameters, including absolute eosinophil count (14), neutrophil-lymphocyte ratio (15) and lactate dehydrogenase levels (16), have been found to be valuable in the prediction of the clinical outcome of ICIs

treatment. However, the role of serum alkaline phosphatase (ALP) in the prediction of ICIs treatment efficacy remains unclear.

ALP is a glycoprotein that catalyzes hydrolytic and phospho-transfer reactions. Elevated serum ALP has been reported in bone and liver-related diseases (17,18). Additionally, serum ALP has been found to be an independent prognostic factor in NSCLC, gastric cancer, breast cancer and other cancer types (19-21). Furthermore, elevated ALP levels have been associated with bone or liver metastasis in patients with lung cancer, prostate cancer, breast cancer and other cancer types (22). In recent studies, bone and liver metastases have been reported to restrain immunotherapy efficacy via regulation of the tumor immune microenvironment (23,24). Therefore, an investigation was conducted to determine whether ALP could be a predictive indicator of ICIs treatment efficacy in patients with NSCLC.

In the present study it was found that bone or liver metastasis was associated with poor prognosis in patients with NSCLC receiving ICIs. Furthermore, elevated serum ALP levels were associated with bone and liver metastases, indicating that ALP levels might be an independent prognostic indicator of ICIs treatment efficacy in patients with NSCLC.

Patients and methods

Patients and data collection. Between January 2018 and December 2020, patients with NSCLC receiving ICIs treatment were investigated at the Institute of Cancer, Xinqiao Hospital, Third Military Medical University (Chongqing, China). Patients who met the following criteria were included in the present study: i) Pathologically diagnosed lung squamous cancer or adenocarcinoma without receiving an operation; ii) not harboring driver gene mutations, including EGFR, ALK, ROS1 and MET; iii) receiving ICIs treatment (including PD-1, PD-L1 or CTLA-4 antibody) for at least 2-3 cycles (6 weeks); iv) complete image examination of evaluable foci pretreatment and post-treatment; and v) complete record of pretreatment ALP levels and complete record of follow-up. Patients with mutations of driver genes, or without records of pretreatment ALP levels, or receiving ICIs treatment <6 weeks were excluded. Overall, 143 patients were included and data regarding their clinical characteristics [age, sex, histology type, TNM stage (according to the 8th AJCC staging system) (25), metastatic sites, treatment lines (first-line or ≥2nd line) and treatment methods] were collected from medical records. The treatment methods included ICIs monotherapy or combined therapy with other treatments (chemotherapy, anti-angiogenic agents or both). The present study was approved by the Ethics Committee of Xinqiao Hospital, Third Military Medical University (approval no. AMUWEC20210386; Chongqing, China). All patients or their legal representatives gave written informed consent for the treatment and inquiries related to the present study.

ALP detection and cut-off value selection. ALP was detected using the endpoint spectrophotometric method in the peripheral blood of patients <1 week before treatment with ICIs, and the ALP level data were collected from medical records. The normal level of ALP is between 0 and 110 U/l (26). In the present study, 110 U/l, which is the normal upper limit of ALP used at Xinqiao Hospital, was selected as the cut-off value to divide patients into high and low ALP level groups.

Follow-up evaluation and definition of progression-free survival (PFS). Patients were evaluated during follow-up by CT of the lungs and abdomen or MRI of the brain. The response to ICIs treatment was classified according to iRECIST criteria (27) as complete response (iCR), partial response (iPR), stable disease or progressive disease. Treatment response was evaluated every 6 weeks.

The ORR was defined as the proportion of patients with response of iPR and iCR. PFS was defined as the time from the beginning of immunotherapy until recurrence of disease or death due to any cause. Telephone follow-up was performed every 6 months, and the last follow-up date was August 31, 2021.

Statistical analysis. The ALP level data are presented as the median and interquartile range. The mPFS data are presented as Kaplan-Meier curves. The composition of patients or response rate data are presented as n (%), n or the proportion. The results of univariate and multivariate Cox regression analyses are presented as the hazard ratio and 95% CI. The difference in ALP levels was analyzed using an unpaired Student's t-test or one-way ANOVA with a Bonferroni post hoc test. The associations between ALP levels and clinical characteristics were analyzed using a χ^2 test and Fisher's exact test if the expected count was <5. The percentages of clinical response of patients to ICIs treatment in two groups were compared using a χ^2 test. Kaplan-Meier survival curves and the log-rank test were used to estimate mPFS, and univariate and multivariate Cox regression analyses were used to determine the associations between clinicopathological factors and survival. A forest plot was used to show the results of multivariate Cox regression analysis using Prism GraphPad 9.0 software (GraphPad Software, Inc.). All statistical analyses were conducted using SPSS 20.0 software (IBM Corp.). P<0.05 was considered to indicate a statistically significant difference.

Results

Patient characteristics. A total of 143 patients with NSCLC receiving ICIs were included, and their baseline characteristics are presented in Table I. There were 61 (42.7%) older patients (age ≥60 years), and the mean age was 57.26±9.86 years (range, 35-76 years). Most patients were male (79.0%) and had stage IV disease (87.4%). In addition, 51.7% of patients had squamous cancer, 59.4% of patients received first-line ICIs treatment, and 45.5% of patients were treated with combination therapy of ICIs and other treatments. The median follow-up time was 30.2 months (range, 2.7-52.4 months).

Bone and liver metastases predict poor prognosis of patients with NSCLC receiving ICIs. Previous preclinical and clinical evidence has demonstrated that bone or liver metastases can restrain immunotherapy efficacy (23,24). Therefore, the predictive value of bone or liver metastasis in the prognosis of patients with NSCLC receiving ICIs was investigated. There

Table I. Baseline characteristics of 143 patients with lung cancer.

Characteristics	No. (%)
Age, years	
≥60	61 (42.7)
<60	82 (57.3)
Sex	
Male	113 (79.0)
Female	30 (21.0)
Histology type	
SC	74 (51.7)
AD	69 (48.3)
TNM stage	
III	18 (12.6)
IV	125 (87.4)
Metastatic sites	
Bone only	45 (31.4)
Liver only	10 (7.0)
Both bone and liver	17 (11.9)
Neither bone or liver	71 (49.7)
Treatment lines	
1st	85 (59.4)
≥2nd	58 (40.6)
Combined therapy	
Yes	65 (45.5)
No	78 (54.5)

AD, adenocarcinoma; SC, squamous cancer.

Table II. Association between clinical variables and serum ALP levels.

	Pretreatment ALP levels		
Characteristics	≥110 U/l, n	<110 U/l, n	P-value
Age, years			0.809
≥60	16	45	
<60	23	59	
Sex			0.402
Male	29	84	
Female	10	20	
Histology type			0.931
SC	21	53	
AD	18	51	
Stage			0.100
III	2	16	
IV	37	88	
Bone metastasis			<0.001
Yes	34	28	
No	5	76	
Liver metastasis			0.036
Yes	10	17	
No	29	87	
Bone and liver metastasis			<0.001
Both	10	7	
Neither	4	67	
Treatment lines			0.945
1st	23	62	
≥2nd	16	42	
Combined therapy			0.304
Yes	15	50	
No	24	54	

AD, adenocarcinoma; ALP, alkaline phosphatase; SC, squamous cancer.

was a significant association between the response and bone metastasis (P<0.05; Fig. 1A). Furthermore, bone metastasis was associated with a shorter mPFS time in patients with NSCLC receiving ICIs treatment (10.2 vs. 17.3 months; P=0.010; Fig. 1B). Similarly, there was also a significant association between the response and liver metastasis (P<0.05; Fig. 1C). However, liver metastasis was not associated with mPFS time (P=0.119; Fig. 1D).

Elevated ALP levels are associated with bone and liver metastases in patients with NSCLC. ALP has been recognized as a valuable marker for skeletal and hepatobiliary disorders, including bone and liver metastases in patients with cancer (22). Therefore, the relationship between ALP levels and bone or liver metastasis in patients with NSCLC was investigated. ALP levels were higher in patients with bone or liver metastasis than in those without (119.6 or 103.6 vs. 83.3 U/l, respectively; P<0.05; Fig. 1E). Furthermore, the ALP levels of patients with both bone and liver metastases were higher compared with the 'None' group (135.7 vs. 83.3 U/l; P<0.01; Fig. 1E). In addition, higher levels of ALP were also associated with bone or liver metastasis, but not with age, sex, stage and other clinical characteristics (Table II).

Elevated ALP levels are an independent prognostic indicator for patients with NSCLC receiving ICIs. To determine the predictive value of ALP, the ALP levels in responders and non-responders to ICIs treatment were first compared. It was found that the levels of ALP were higher in non-responders than in responders (110.1 vs. 88.0 U/l; P<0.01; Fig. 2A). There was a significant association between the response and ALP levels (P<0.05; Fig. 2B). Furthermore, high ALP levels were associated with shorter mPFS (8.5 vs. 15.4 months; P=0.009; Fig. 2C). Additionally, multivariate analysis revealed that ALP level, stage and histology type were independent prognostic indicators for mPFS in the present study (Table III). Patients with high ALP levels or stage IV had a shorter mPFS than those with low ALP levels or stage III, while patients with squamous cancer had a longer mPFS than those with adenocarcinoma (Fig. 3). However, associations between bone metastasis and mPFS were not observed in the multivariate analysis (Table III).

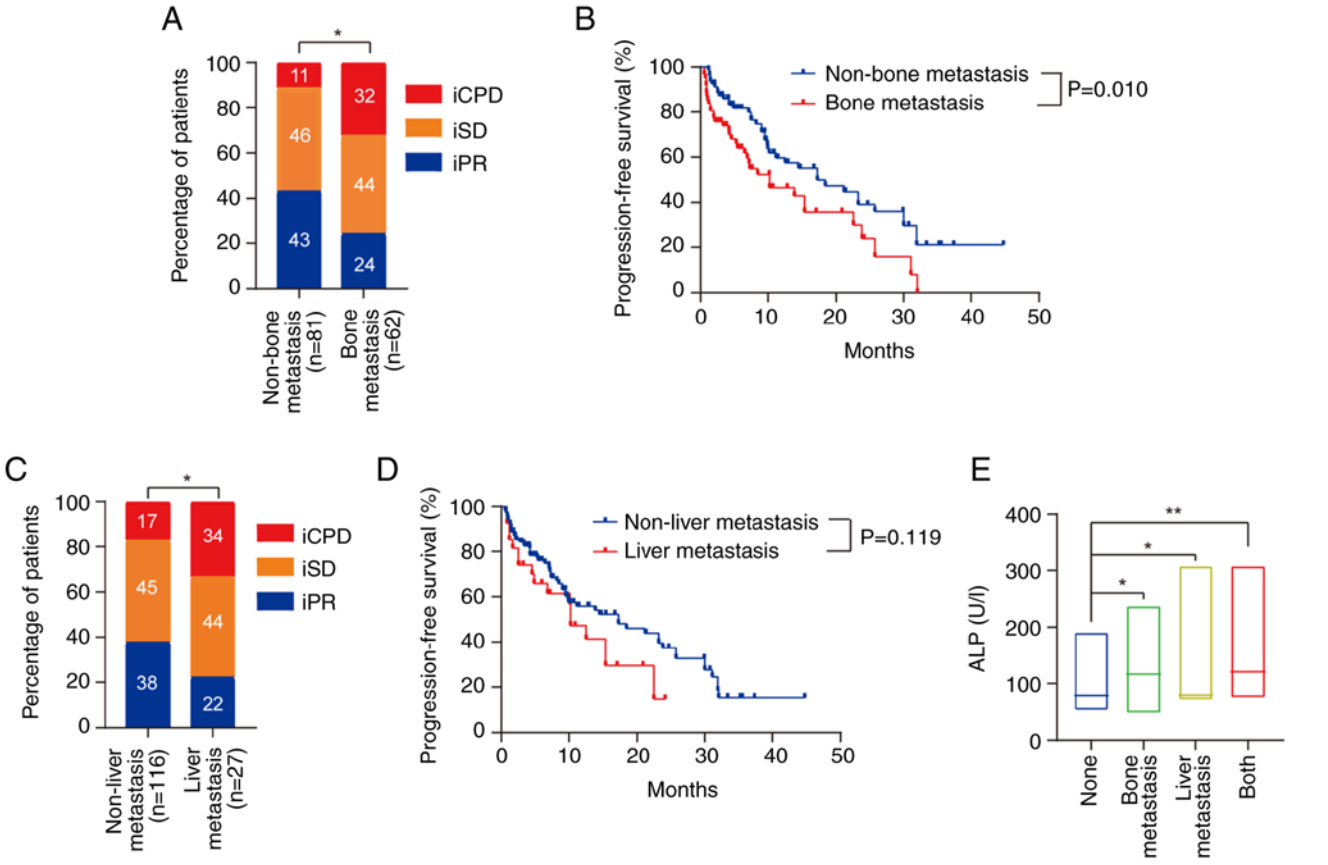

Figure 1. Bone and liver metastasis restrain efficacy of ICIs treatment. (A) Response to ICIs treatment of patients with NSCLC with or without bone metastasis. (B) mPFS of patients with NSCLC with or without bone metastasis after ICIs treatment. (C) Response to ICIs treatment of patients with NSCLC with or without liver metastasis. (D) mPFS of patients with NSCLC with or without liver metastasis after ICIs treatment. (E) ALP levels in patients with NSCLC with or without bone metastasis and liver metastasis. *P<0.05, **P<0.01. ALP, alkaline phosphatase; ICIs, immune checkpoint inhibitors; iCPD, progressive disease; iPR, partial response; iSD, stable disease; NSCLC, non-small cell lung cancer; mPFS, median progression-free survival.

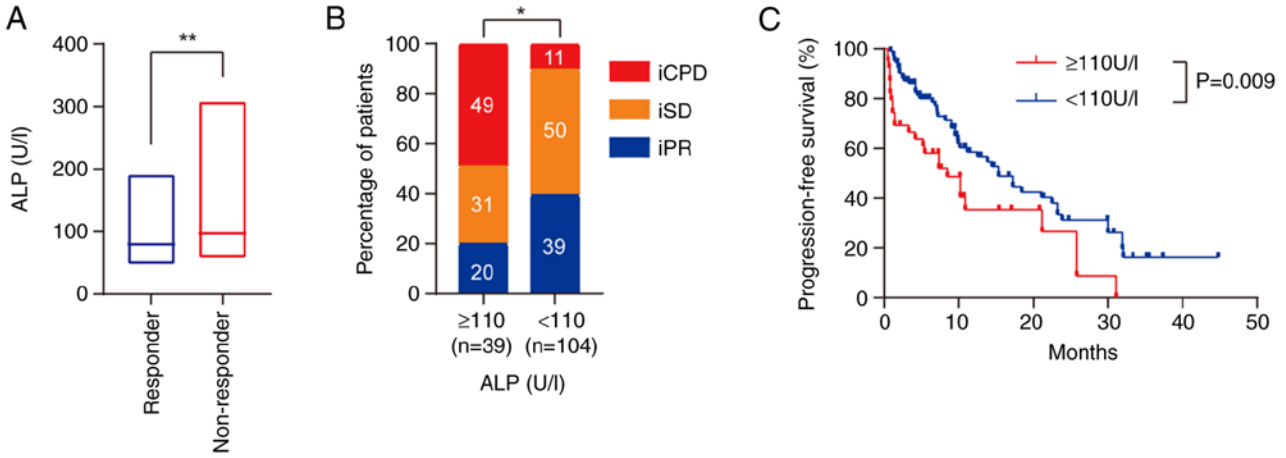

Figure 2. ALP levels predict efficacy of ICIs treatment in patients with NSCLC. (A) ALP levels in responders and non-responders to ICIs treatment. (B) Response to ICIs treatment of patients with NSCLC with or without high ALP levels. (C) Progression-free survival of patients with NSCLC with or without high ALP levels after ICIs treatment. *P<0.05, **P<0.01. ALP, alkaline phosphatase; ICIs, immune checkpoint inhibitors; iCPD, progressive disease; iSD, stable disease; NSCLC, non-small cell lung cancer; iPR, partial response.

Discussion

Immunotherapy, especially ICIs treatment, has been one of the most promising treatment approaches in NSCLC (4,5). ICIs monotherapy or combined therapy has been approved as a first-line treatment and has improved the prognosis of patients with advanced lung cancer (28,29). Although the ORR has been increased by combined therapy independent of the PD-L1 level measured, not all patients benefit from the ICIs treatment, and this is termed a so-called primary resistance (30).

Table III. Univariate and multivariate analyses of different parameters for progression-free survival.

	Univariate			Multivariate		
Variables	HR	95% CI	P-value	HR	95% CI	P-value
Sex (male vs. female)	1.274	0.731-2.219	0.417			
Age (≥60 vs. <60 years)	1.028	0.653-1.617	0.903			
Histology (SC vs. AD)	1.402	0.889-2.209	0.134	0.511	0.302-0.863	0.012
Stage (III vs. IV)	0.343	0.195-0.603	0.006	3.104	1.213-7.944	0.018
Bone metastasis (no vs. yes)	0.563	0.349-0.907	0.010	1.174	0.856-2.641	0.103
Liver metastasis (no vs. yes)	0.658	0.356-1.217	0.119			
Treatment lines (1st vs. ≥2nd)	0.898	0.548-1.472	0.662			
Combined therapy (yes vs. no)	1.453	0.804-2.262	0.199			
ALP (<110 vs. ≥110 U/l)	1.838	1.609-3.191	0.009	1.856	1.030-3.343	0.040

AD, adenocarcinoma; ALP, alkaline phosphatase; HR, hazard ratio; SC, squamous cancer.

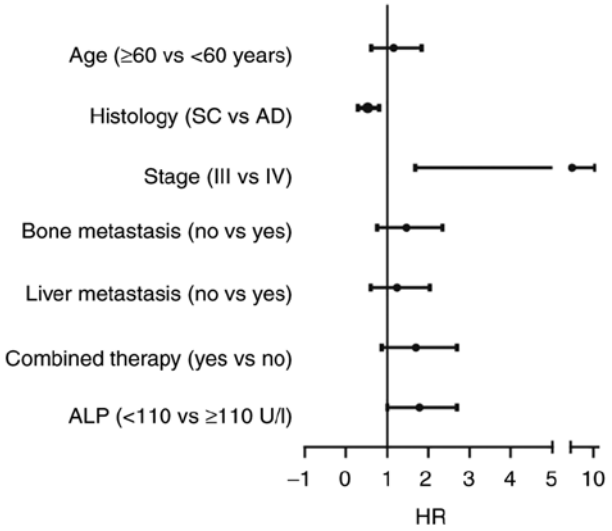

Figure 3. Forest plot of univariate analyses of different parameters for progression-free survival. AD, adenocarcinoma; ALP, alkaline phosphatase; HR, hazard ratio; SC, squamous cancer.

Numerous studies have demonstrated the mechanisms of primary resistance in ICIs treatment (31-33), which include the absence of tumor-specific antigens, downregulation of antigen presentation molecules and suppression of T cell infiltration. Emerging evidence has indicated that bone and liver metastases might induce primary resistance to immunotherapy by regulating the immune microenvironment (23,24). Subgroup analysis of clinical trials revealed that patients with prostate cancer and bone metastasis exhibited primary resistance to ICIs treatment (34). Mechanistically, bone metastatic loci could release TGF-β to restrain Th1 lineage development to further inhibit antitumor immunity (34). Furthermore, a recent study reported that the presence of liver metastasis is associated with poor response to immunotherapy in patients with melanoma or NSCLC, which is consistent with the findings of the present study (23). An animal experiment revealed that liver metastasis could recruit immunosuppressive macrophages to promote antigen-specific T cell apoptosis, resulting in a systemic loss of T cells and diminished immunotherapy efficacy (23). However, a recent meta-analysis reported that patients with lung cancer with and without liver metastasis obtained comparable efficacy after ICIs treatment (35). Therefore, prospective studies might be required to further identify the relationship between liver metastasis and the efficacy of ICIs treatment.

Primary resistance cannot be completely explained by currently recognized biomarkers, such as PD-L1 expression, MSI status or TMB level. Thus, studies are increasingly focusing on other biomarkers to predict the response to ICIs treatment (14,36). As aforementioned, elevated serum ALP levels have been demonstrated to be associated with bone and liver-related diseases. In the present study, it was found that high levels of serum ALP were associated with bone or liver metastasis in patients with NSCLC, which is consistent with previous studies (22,37). Although numerous studies have demonstrated that a high ALP level predicted poor prognosis in various types of cancer, such as NSCLC, gastric cancer and breast cancer (19-21), there is little research on the relationship between serum ALP levels and efficacy of ICIs treatment. In the present study, serum ALP levels were identified as an independent prognostic indicator of ICIs treatment in patients with NSCLC. Recently, it was also reported that high levels of serum ALP predicted poor prognosis in patients with HER-2-negative gastric cancer receiving immunotherapy (38), which might support the findings of the present study. However, the cut-off value of ALP was not the same in the previous study and the present study (225 U/l in the previous study and 110U/l in the present study). The cut-off value in the present study was based on the normal upper limit of ALP, and was consistent with another study (26). Therefore, 110 U/l might be a more suitable cut-off value. There are four isoenzymes of ALP in human serum, including tissue non-specific, germ cell, placental and intestinal ALP, but the standard test could not detect the tissue non-specific isoenzymes, which account for 26-34% of total ALP (22). Thus, whether the undetectable isoenzymes of ALP could also predict the efficacy of ICIs treatment remains to be further studied.

As aforementioned, bone or liver metastasis, and high ALP levels were associated with poor prognosis in patients

with NSCLC receiving ICIs treatment in the present study. However, in the present study, it was found that only high ALP levels, rather than bone or liver metastasis, were an independent prognostic factor using multivariate analysis, rather than bone or liver metastasis. This difference indicated that other factors affecting ALP levels, such as benign liver and bone diseases, might also restrain the efficacy of ICIs treatment. Furthermore, it was found that higher ALP levels were associated with a shorter PFS time in patients without bone metastasis (data not shown). However, this hypothesis should be verified in further studies.

There were a number of limitations in the present study. Firstly, this was a retrospective study in a single center with a small number of cases. Secondly, the lack of overall survival data might affect the reliability of the conclusions made. Thirdly, the PD-L1 expression, TMB and MSI status in patients might also affect the outcomes of ICIs treatment (9,10), which should be explored further in the future.

In conclusion, serum ALP levels might be an independent predictor of the response to ICIs treatment in patients with NSCLC. Due to a number of limitations of the present study, prospective studies are required to determine the actual prognostic significance of ALP levels in patients with NSCLC.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

FL and JY conceived and designed the study. KY and TS developed the methodology. TY, JC and SF acquired the data. TY and JC confirmed the authenticity of all the raw data. TY and JC analyzed and interpreted the data. TY and JC wrote the original draft. JY and FL reviewed and revised the manuscript. FL and JY supervised the study. TY, JC and TS were involved in funding acquisition. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The study was approved by the Ethics Committee of Xinqiao Hospital, Third Military Medical University (Chongqing, China). All patients or their legal representatives gave written informed consent for the treatment and inquiries related to this study.

Patient consent for publication

The patients provided written informed consent for publication of any associated data.

Competing interests

The authors declare that they have no competing interests.

References

1. Barta JA, Powell CA and Wisnivesky JP: Global epidemiology of lung cancer. Ann Glob Health 85: 8, 2019.
2. Molina JR, Yang P, Cassivi SD, Schild SE and Adjei AA: Non-small cell lung cancer: Epidemiology, risk factors, treatment, and survivorship. Mayo Clin Proc 83: 584-594, 2008.
3. Li T, Kung HJ, Mack PC and Gandara DR: Genotyping and genomic profiling of non-small-cell lung cancer: Implications for current and future therapies. J Clin Oncol 31: 1039-1049, 2013.
4. Socinski MA, Jotte RM, Cappuzzo F, Orlandi F, Stroyakovskiy D, Nogami N, Rodríguez-Abreu D, Moro-Sibilot D, Thomas CA, Barlesi F, *et al*: Atezolizumab for First-Line Treatment of Metastatic Nonsquamous NSCLC. N Engl J Med 378: 2288-2301, 2018.
5. Reck M, Rodriguez-Abreu D, Robinson AG, Hui R, Csőszi T, Fülöp A, Gottfried M, Peled N, Tafreshi A, Cuffe S, *et al*: Updated analysis of KEYNOTE-024: Pembrolizumab versus platinum-based chemotherapy for advanced non-small-cell lung cancer with PD-L1 tumor proportion score of 50% or greater. J Clin Oncol 37: 537-546, 2019.
6. Rodriguez-Abreu D, Powell SF, Hochmair MJ, Gadgeel S, Esteban E, Felip E, Speranza G, De Angelis F, Dómine M, Cheng SY, *et al*: Pemetrexed plus platinum with or without pembrolizumab in patients with previously untreated metastatic nonsquamous NSCLC: Protocol-specified final analysis from KEYNOTE-189. Ann Oncol 32: 881-895, 2021.
7. Paz-Ares L, Vicente D, Tafreshi A, Robinson A, Soto Parra H, Mazières J, Hermes B, Cicin I, Medgyasszay B, Rodríguez-Cid J, *et al*: A randomized, placebo-controlled trial of pembrolizumab plus chemotherapy in patients with metastatic squamous NSCLC: Protocol-Specified final analysis of KEYNOTE-407. J Thorac Oncol 15: 1657-1669, 2020.
8. Socinski MA, Nishio M, Jotte RM, Cappuzzo F, Orlandi F, Stroyakovskiy D, Nogami N, Rodríguez-Abreu D, Moro-Sibilot D, Thomas CA, *et al*: IMpower150 final overall survival analyses for atezolizumab plus bevacizumab and chemotherapy in first-line metastatic nonsquamous NSCLC. J Thorac Oncol 16: 1909-1924, 2021.
9. Yarchoan M, Albacker LA, Hopkins AC, Montesion M, Murugesan K, Vithayathil TT, Zaidi N, Azad NS, Laheru DA, Frampton GM and Jaffee EM: PD-L1 expression and tumor mutational burden are independent biomarkers in most cancers. JCI Insight 4: e126908, 2019.
10. Chang L, Chang M, Chang HM and Chang F: Microsatellite instability: A predictive biomarker for cancer immunotherapy. Appl Immunohistochem Mol Morphol 26: e15-e21, 2018.
11. Jiang P, Gu S, Pan D, Fu J, Sahu A, Hu X, Li Z, Traugh N, Bu X, Li B, *et al*: Signatures of T cell dysfunction and exclusion predict cancer immunotherapy response. Nat Med 24: 1550-1558, 2018.
12. Hurkmans DP, Kuipers ME, Smit J, van Marion R, Mathijssen RHJ, Postmus PE, Hiemstra PS, Aerts JGJV, von der Thüsen JH and van der Burg SH: Tumor mutational load, CD8(+) T cells, expression of PD-L1 and HLA class I to guide immunotherapy decisions in NSCLC patients. Cancer Immunol Immunother 69: 771-777, 2020.
13. Han J, Duan J, Bai H, Wang Y, Wan R, Wang X, Chen S, Tian Y, Wang D, Fei K, *et al*: TCR repertoire diversity of peripheral PD-1(+)CD8(+) T cells predicts clinical outcomes after immunotherapy in patients with non-small cell lung cancer. Cancer Immunol Res 8: 146-154, 2020.
14. Soyano AE, Dholaria B, Marin-Acevedo JA, Diehl N, Hodge D, Luo Y, Manochakian R, Chumsri S, Adjei A, Knutson KL and Lou Y: Peripheral blood biomarkers correlate with outcomes in advanced non-small cell lung Cancer patients treated with anti-PD-1 antibodies. J Immunother Cancer 6: 129, 2018.

15. Diem S, Schmid S, Krapf M, Flatz L, Born D, Jochum W, Templeton AJ and Früh M: Neutrophil-to-Lymphocyte ratio (NLR) and Platelet-to-Lymphocyte ratio (PLR) as prognostic markers in patients with non-small cell lung cancer (NSCLC) treated with nivolumab. Lung Cancer 111: 176-181, 2017.
16. Banna GL, Signorelli D, Metro G, Galetta D, De Toma A, Cantale O, Banini M, Friedlaender A, Pizzutillo P, Garassino MC and Addeo A: Neutrophil-to-lymphocyte ratio in combination with PD-L1 or lactate dehydrogenase as biomarkers for high PD-L1 non-small cell lung cancer treated with first-line pembrolizumab. Transl Lung Cancer Res 9: 1533-1542, 2020.
17. Siddique A and Kowdley KV: Approach to a patient with elevated serum alkaline phosphatase. Clin Liver Dis 16: 199-229, 2012.
18. Vimalraj S: Alkaline phosphatase: Structure, expression and its function in bone mineralization. Gene 754: 144855, 2020.
19. Namikawa T, Ishida N, Tsuda S, Fujisawa K, Munekage E, Iwabu J, Munekage M, Uemura S, Tsujii S, Tamura T, *et al*: Prognostic significance of serum alkaline phosphatase and lactate dehydrogenase levels in patients with unresectable advanced gastric cancer. Gastric Cancer 22: 684-691, 2019.
20. Chen B, Dai D, Tang H, Chen X, Ai X, Huang X, Wei W and Xie X: Pre-treatment serum alkaline phosphatase and lactate dehydrogenase as prognostic factors in triple negative breast cancer. J Cancer 7: 2309-2316, 2016.
21. Li D, Yu H and Li W: Albumin-to-alkaline phosphatase ratio at diagnosis predicts survival in patients with metastatic non-small-cell lung cancer. Onco Targets Ther 12: 5241-5249, 2019.
22. Đokic-Lisanin M, Pantovic V, Jovanovic Z, Samardz G and Jurisic V: Values of alkaline phosphathase and their isoenzyme profiles in patients with cancer in respect to bone and liver metastasis. Arch Oncol 21: 14-16, 2013.
23. Yu J, Green MD, Li S, Sun Y, Journey SN, Choi JE, Rizvi SM, Qin A, Waninger JJ, Lang X, *et al*: Liver metastasis restrains immunotherapy efficacy via macrophage-mediated T cell elimination. Nat Med 27: 152-164, 2021.
24. Landi L, D'Inca F, Gelibter A, Chiari R, Grossi F, Delmonte A, Passaro A, Signorelli D, Gelsomino F, Galetta D, *et al*: Bone metastases and immunotherapy in patients with advanced non-small-cell lung cancer. J Immunother Cancer 7: 316, 2019.
25. Detterbeck FC, Boffa DJ, Kim AW and Tanoue LT: The eighth edition lung cancer stage classification. Chest 151: 193-203, 2017.
26. He S, Wang Y, Peng H, Yang L, Chen H, Liang S, Lu L and Chen Y: Pretreatment alkaline phosphatase and epstein-barr virus DNA predict poor prognosis and response to salvage radiotherapy in patients with nasopharyngeal carcinoma and metachronous bone-only metastasis. J Cancer 8: 417-424, 2017.
27. Seymour L, Bogaerts J, Perrone A, Ford R, Schwartz LH, Mandrekar S, Lin NU, Litière S, Dancey J, Chen A, *et al*: iRECIST: Guidelines for response criteria for use in trials testing immunotherapeutics. Lancet Oncol 18: e143-e152, 2017.
28. Pai-Scherf L, Blumenthal GM, Li H, Subramaniam S, Mishra-Kalyani PS, He K, Zhao H, Yu J, Paciga M, Goldberg KB, *et al*: FDA Approval Summary: Pembrolizumab for treatment of metastatic non-small cell lung cancer: First-Line therapy and beyond. Oncologist 22: 1392-1399, 2017.
29. Saxena P, Singh PK, Malik PS and Singh N: Immunotherapy alone or in combination with chemotherapy as first-line treatment of non-small cell lung cancer. Curr Treat Options Oncol 21: 69, 2020.
30. Sharma P, Hu-Lieskovan S, Wargo JA and Ribas A: Primary, adaptive, and acquired resistance to cancer immunotherapy. Cell 168: 707-723, 2017.
31. Isazadeh A, Hajazimian S, Garshasbi H, Shadman B, Baghbanzadeh A, Chavoshi R, Taefehshokr S, Farhoudi Sefidan Jadid M, Hajiasgharzadeh K and Baradaran B: Resistance mechanisms to immune checkpoints blockade by monoclonal antibody drugs in cancer immunotherapy: Focus on myeloma. J Cell Physiol 236: 791-805, 2021.
32. Bai R, Chen N, Li L, Du N, Bai L, Lv Z, Tian H and Cui J: Mechanisms of cancer resistance to immunotherapy. Front Oncol 10: 1290, 2020.
33. Li YZ and Zhang HM: Recent advances in primary resistance mechanisms against immune checkpoint inhibitors. Curr Opin Oncol 34: 95-106, 2022.
34. Jiao S, Subudhi SK, Aparicio A, Ge Z, Guan B, Miura Y and Sharma P: Differences in tumor microenvironment dictate T helper lineage polarization and response to immune checkpoint therapy. Cell 179: 1177-1190 e13, 2019.
35. Qin BD, Jiao XD, Liu J, Liu K, He X, Wu Y, Ling Y, Duan XP, Qin WX, Wang Z and Zang YS: The effect of liver metastasis on efficacy of immunotherapy plus chemotherapy in advanced lung cancer. Crit Rev Oncol Hematol 147: 102893, 2020.
36. McKean WB, Moser JC, Rimm D and Hu-Lieskovan S: Biomarkers in precision cancer immunotherapy: Promise and challenges. Am Soc Clin Oncol Educ Book 40: e275-e291, 2020.
37. Ramaswamy G, Rao VR, Krishnamoorthy L, Ramesh G, Gomathy R and Renukadevi D: Serum levels of bone alkaline phosphatase in breast and prostate cancers with bone metastasis. Indian J Clin Biochem 15: 110-113, 2000.
38. Hu J, Yang S, Wang J, Zhang Q, Zhao L, Zhang D, Yu D, Jin M, Ma H, Liu H, *et al*: Blood alkaline phosphatase predicts prognosis of patients with advanced HER2-negative gastric cancer receiving immunotherapy. Ann Transl Med 9: 1316, 2021.