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β-glucan combined with Envafolimab and Endostar as immune rechallenge for metastatic non-small cell lung cancer

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Abstract

Background Immune checkpoint inhibitor rechallenge has emerged as a prominent study area in non-small cell lung cancer (NSCLC). β-glucan was reported to reverse resistance to anti-PD-1/PD-L1 inhibitors by regulating the tumor microenvironment. In this self-initiated clinical trial (ChiCTR2100054796), NSCLC participants who have previously failed anti-PD-1 therapy received β-glucan (500 mg, bid, d1-21), Envafolimab (300 mg, d1) and Endostar (210 mg, civ72h) every 3 weeks until disease progression or unacceptable toxicity. The clinical efficacy and adverse events were observed, while serum samples were collected for proteomic analysis.

Results Twenty Three patients were enrolled from January 2022 to March 2023 (median age, 65 years; male, $n=18$ [78.3%]; squamous NSCLC, $n=9$ [39.1%]; mutant type, $n=13$ [56.5%]). The overall response rate (ORR) was 21.7% and disease control rate (DCR) was 73.9%. Median progression-free survival (mPFS) and median overall survival (mOS) was 4.3 months [95% CI: 2.0–6.6] and 9.8 months [95% CI: 7.2–12.4], respectively. The mPFS between PD-L1 positive and negative subgroup has significant difference (6.3 months vs. 2.3 months, $p=0.002$). Treatment-related adverse events (TRAEs) occurred in 52.2% of patients. The most common TRAEs were hypothyroidism (26.1%) and fatigue (26.1%). 2 (8.7%) grade 3 adverse events were reported. No adverse reaction related deaths have been observed. Proteomic analysis revealed that the levels of CASP-8, ARG1, MMP12, CD28 and CXCL5 correlated with resistance to the treatment while the levels of CD40-L and EGF related to the favorable response.

Conclusion β-glucan combined with Envafolimab and Endostar has considerable efficacy and safety for immune rechallenge in metastatic NSCLC patients who failed of anti-PD-1 treatment previously, especially for PD-L1 positive patients.

Keywords β-glucan, Envafolimab, Immune checkpoint inhibitors, Rechallenge, Non-small cell lung cancer

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Introduction

Non-small cell lung cancer (NSCLC) is the most common type of lung cancer [1]. With the development of immunotherapy, immune checkpoint inhibitors (ICIs) typified by anti-programmed cell death-1 (PD-1) /anti-programmed cell death ligand-1 (PD-L1) inhibitors have become the main treatment option for advanced NSCLC due to their sustaining anti-tumor efficacy and tolerable safety profile [2]. However, ICIs only benefits a small number of patients and a significant proportion of patients could develop primary or acquired drug resistance to ICIs [3], which has become an important issue that cannot be ignored. At present, there is a lack of consistent guidelines or consensus on the evaluation criteria for immunotherapy resistance and the choice of intensive treatment after immune resistance. To solve this dilemma, multiple combination therapies have been explored, including ICIs combined with chemotherapy or targeted drugs, dual immunotherapy, systemic immunotherapy in conjunction with local radiotherapy, exploration of other immune checkpoints and tumor vaccines [4–6]. However, the outcomes of current clinical trials regarding immune rechallenge were less than satisfactory. The reason was most likely that they ignored the important role played by the regulation of the tumor immune microenvironment (TME) in anti-tumor treatment.

β-glucan, the main component of microbial cell walls, is a linear polysaccharide linked by β-glucoside bond of D-glucose monomeris [7]. As an immune adjuvant, β-glucan has been used in clinical practice alongside other anti-tumor medications for tumor treatment [8]. Following oral administration of β-glucan, there was an increase in dendritic cells (DCs) within the TME of wild-type mice with colitis-associated colorectal cancer (CAC), resulting in enhanced production of $CD8^+$T cells and related cytokines, ultimately leading to a delay in CAC development [9]. β-glucan could also enhance the efficacy of immunotherapy by regulating the composition of intestinal microbiota and adjusting the intestinal microenvironment [10]. Furthermore, for patients with advanced tumors resistant to anti-PD-1 inhibitors, 69.2% obtained stable disease (SD) when added with β-glucan on the basis of maintaining the original treatmennt regimen in our previous study, suggesting that β-glucan had the potential to reverse immune resistance [11].

Envafolimab is a single domain anti-PD-L1 inhibitor administered subcutaneously, which can block the PD-1/PD-L1 pathway, reactivate the suppressed T cells to induce anti-tumor immune response and recover the antigen presentation function of DCs [12–14]. Envafolimab monotherapy has been proved the anti-tumor activity and safety in the second and above line treatment for a variety of MSI-H/dMMR advanced solid tumors in phase II clinical studies [15, 16]. Nowadays, the conversion of anti-PD-1 /PD-L1 antibodies as immune rechallenge is also a treatment option for some patients with NSCLC [17, 18].

Anti-angiogenic drugs play a very important role in the treatment of advanced NSCLC, especially in lung adenocarcinoma [19]. Previous studies have shown that the abnormal blood vessels in the TME were associated with drug resistance [20]. As a novel recombinant human endostatin, Endostar can strongly inhibit VEGF expression and angiogenesis while also inducing vascular normalization [21]. By inducing vascular normalization of tumor blood vessels, it can promote the infiltration of T cells into the tumor, thereby transforming the tumor microenvironment into "hot tumor". The combination of anti-PD-1 inhibitors and Endostar exhibited a significant synergistic effect in suppressing tumor growth in preclinical lung tumor models as well as NSCLC patients [22–24].

Totally, our study aims to evaluate the efficacy and safety of β-glucan combined with Envafolimab (an anti-PD-L1 antibody) and Endostar (an angiogenesis inhibitor) in the treatment of metastatic NSCLC patients who have previously failed anti-PD-1 therapy, while also investigating potential biomarkers associated with treatment response and resistance.

Materials and methods

Study design and ethics

This is a single-arm, Phase II clincal trial (ChiCTR2100054796, Registration Date: 2021–12-27) performed at the Affiliated Changzhou No.2 People's Hospital of Nanjing Medical University, Changzhou city, to evaluate the efficacy and safety of β-glucan combined with Envafolimab and Endostar in mNSCLC patients failed of anti-PD-1 therapy previously. Participants received β-glucan (500 mg, bid, d1-21), Envafolimab (300 mg, d1) and Endostar (210 mg, civ72h) every 3 weeks until disease progression or unacceptable toxicity. Tumor imaging and objective response assessment were performed every 2 cycles according to the Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1. Patients were followed up for safety 30 days after the last cycle and followed for survival every 12 weeks after disease progression or the last cycle. The study was approved by the Ethics Committee of Changzhou No.2 People's Hospital (Approval number: [2021YLJSD002]) and conducted in accordance with the principles of the Declaration of Helsinki. All registered patients have signed informed consent forms to participate.

Inclusion and exclusion criteria for participants

All enrolled patients were in line with the following conditions: (1) aged from 18 to 80 years old; (2) confirmed as stage IV NSCLC histologically or cytologically; (3) Eastern Cooperative Oncology Group Performance Status (ECOG PS) 0 or 1; (4) with at least one measurable target lesion; (5) failed of anti-PD-1 therapy and never treated with PD-L1 inhibitors; (6) with sufficient organ and bone marrow function; (7) with an expected survival of at least 3 months or the ability to tolerate a minimum of 2 treatment cycles.

Patients with any of the following conditions were excluded: (1) participating in other clinical studies; (2) with invasion of large blood vessels confirmed by imaging; (3) with symptoms of central nervous system metastases; (4) with grade III-IV (New York Heart Association classification) congestive heart failure; (5) allergic to the drugs used in this trial; (6) requiring long-term systemic application of corticosteroids.

Characteristics of all enrolled patients were collected at baseline, including age, sex, pathology, driver gene mutation, ECOG PS score, number of treatment lines and expression of PD-L1. Clinical data were collected from patients receiving treatment until disease progression or death. Enrolled patients provided serum samples at baseline, when achieved best response and after disease progression. The samples were centrifuged at 2000 rpm for 10 min and stored at -80 °C for proteomic analysis.

Clinical outcomes

The primary endpoints were objective response rate (ORR) and safety. The secondary endpoints included disease control rate (DCR), progression-free survival (PFS) and overall survival (OS). The best response (BOR) is evaluated by RECIST version 1.1, classified as complete response (CR), partial response (PR), stable disease (SD), and progressive disease (PD). ORR is defined as the percentage of all patients who achieved CR and PR. DCR is defined as the percentage of all patients who achieved CR, PR and SD. The NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 was used to evaluate the rating of adverse events. SPSS 25.0 and Graphpad Prism 9.0.0 were used for statistical analysis and image drawing of the data. Chi-square test, Fisher's exact test and rank sum test were used to compare the differences in efficacy and survival outcomes between subgroups. Kaplan–Meier curves were drawn. The log-rank test was used to for the significance of survival curves in subgroups. $p < 0.05$ indicated that the difference was statistically significant. The safety-related results were summarized by descriptive statistics.

Proteomic analysis

Each protein was quantified by using multiple proximity extension assays. A double-recognition immunoassay mechanism was applied in which two pairs of antibodies (each with a unique DNA oligonucleotide marker) simultaneously bind to a target protein in a liquid medium, bringing the two antibodies in close proximity, allowing hybridization and serving as a template for DNA polymerase-dependent extension steps. Double-stranded DNA was unique to a particular antigen and was amplified using primers in quantities proportional to the sample concentration of the target protein. The target of amplification was quantified by RT-PCR. Protein abundance was reported as normalized protein expression (NPX), which was on the Log2 scale. In order to convert several correlated proteins into a number of uncorrelated variables and visualize the dataset, PCA for K-means clustering was performed. Double-tailed Welcht-test was performed to identify proteins which were significantly enriched or consumed in serum samples. The immune cytokines of different response groups were compared by Mann–Whitney U test and evaluated by log-rank test. R statistical program (version 3.6.3) was used for data analysis. Graphpad Prism 9.0.0 was used for further data visualization. $p < 0.05$ was considered statistically significant.

Results

Clinical baseline characteristics of patients

From January 2022 to March 2023, 23 patients were enrolled in this study with median age of 65 years. Among the recruited patients, 18 (78.3%) were male, 14 (60.9%) patients were adenocarcinoma, 5 (21.7%) were with ECOG PS 0. PD-L1 expression was found to be positive in 15 (65.2%) patients. Patients received β-glucan combined with Envafolimab and Endostar as 2 (10/23, 43.5%), 3 (6/23, 26.1%) or > 3 (7/23, 30.4%) lines of therapy (Table 1).

Efficacy evaluation

As of the data cutoff date (April 1, 2024), 5 patients achieved PR, 12 patients achieved SD and 6 patients were evaluated as PD (Fig. 1). No patients achieved CR. The overall ORR was 21.7% and DCR was 73.9% (Table 2). The median PFS was 4.3 months [95% CI: 2.0–6.6] (Fig. 2A) and the median OS was 9.8 months [95% CI: 7.2–12.4] (Fig. 2D).

According to pathology and expression of PD-L1, 23 patients were categorized into different subgroups for analysis. The results showed that the ORR of patients in adenocarcinoma group and squamous cell carcinoma group were 35.7% and 0% ($p = 0.116$). The DCR of adenocarcinoma group was significantly higher than that of squamous cell

Table 1 Baseline clinical characteristics of patients

Characteristics	*n* = 23
Age, median (range)	65 (55, 80)
Gender, n (%)	
Male	18 (78.3)
Female	5 (21.7)
Histopathology, n (%)	
Adenocarcinoma	14 (60.9)
Squamous cell carcinoma	9 (39.1)
Gene mutation status, n (%)	
Mutant-type	13 (56.5)
Wild-type	10 (43.5)
ECOG PS, n (%)	
0	5 (21.7)
1	18 (78.3)
Lines of therapy, n (%)	
2	10 (43.5)
3	6 (26.1)
> 3	7 (30.4)
PD-L1 expression, n (%)	
Positive	15 (65.2)
Negative	8 (34.8)

ECOG PS Eastern Cooperative Oncology Group performance status, *PD-L1* Programmed cell death ligand-1

carcinoma group (85.7% vs. 55.6%, $p=0.162$). Additionally, the ORR of PD-L1 positive and negative groups were 26.7% and 12.5% ($p=0.621$) and the DCR were 86.7% and 50.0%, respectively ($p=0.131$) (Table 2).

Patients with adenocarcinoma tended to have better mPFS compared to those with squamous cell carcinoma, but the difference was not statistically significant (5.8 months [95% CI: 2.9–7.7] vs. 3.0 months [95% CI: 0.4–5.6], $p=0.223$) (Fig. 2B). However, there was no significant difference in mOS between the two subgroups (8.2 months [95% CI: 7.0–9.0] vs. 12.3 months [95% CI: 1.6–23.0], $p=0.724$) (Fig. 2E). Apart from this, the mPFS was significantly improved in PD-L1 positive patients than PD-L1 negative patients (6.3 months [95% CI: 4.7–7.9] vs. 2.3 months [95% CI: 0.3–2.9], $p=0.002$) (Fig. 2C). The mOS had the same trend (9.9 months [95% CI: 3.8–16.0] vs. 8.1 months [95% CI: 5.0–10.6], $p=0.398$) (Fig. 2F) between the two subgroups, though the difference was not statistically significant.

Safety

In safety analysis, 12/23 (52.2%) patients experienced treatment-related adverse events (TRAEs) during treatment. The most common TRAEs were hypothyroidism (26.1%) and fatigue (26.1%), followed with anemia (13.0%), rash (13.0%) and fever (9.7%). 2/23 (8.7%) grade 3 adverse events were reported. Up to April 2024, no immune-associated pneumonia or adverse reaction related deaths have been observed (Table 3).

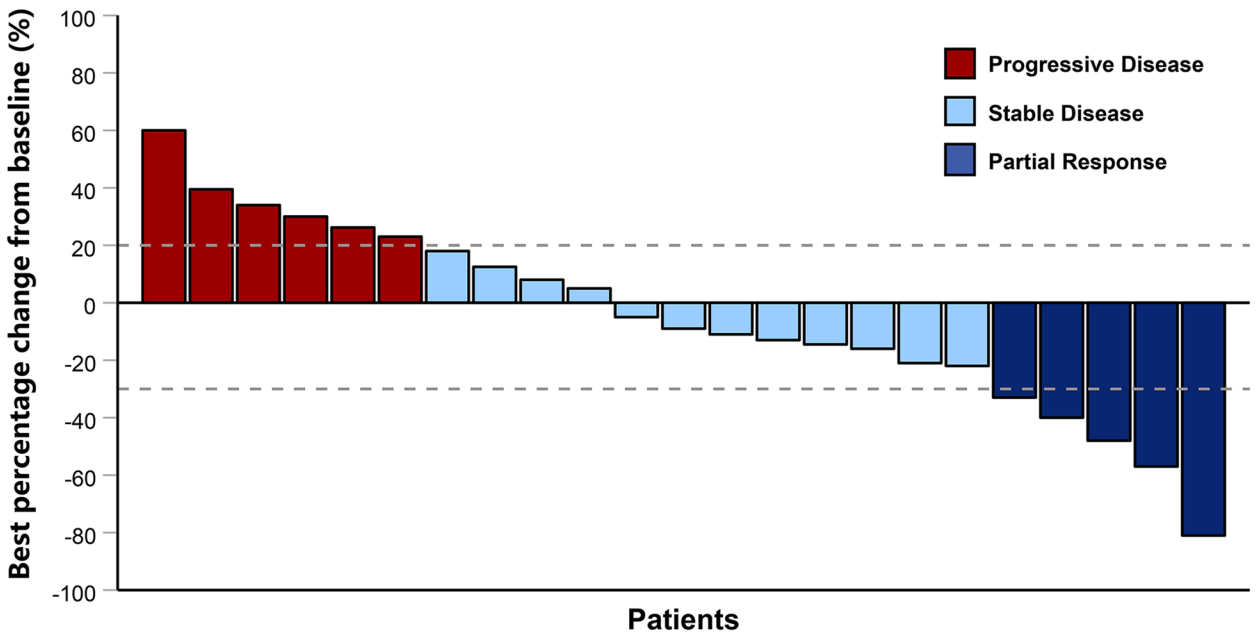

Fig. 1 Waterfall plot of all patients ($n=23$). As of the data cutoff date (April 1, 2024), 5 patients achieved PR, 12 achieved SD and 6 patients were evaluated as PD. PR, partial response; SD, stable disease; PD, progressive disease

Table 2 Best response of all patients and subgroups

Response	All patients (*n* = 23)	LUAD (*n* = 14)	LUSC (*n* = 9)	χ^2 value	*P* value	PD-L1 positive (*n* = 15)	PD-L1 negative (*n* = 8)	χ^2 value	*P* value
BOR, n (%)									
CR	0	0	0			0	0		
PR	5 (21.7)	5 (35.7)	0			4 (26.7)	1 (12.5)		
SD	12 (52.2)	7 (50.0)	5 (55.6)			9 (60.0)	3 (37.5)		
PD	6 (26.1)	2 (21.3)	4 (44.4)			2 (13.3)	4 (50.0)		
ORR (%)	21.7	35.7	0	-	0.116	26.7	12.5	-	0.621
DCR (%)	73.9	85.7	55.6	-	0.162	86.7	50.0	-	0.131

BOR Best overall response, *CR* Complete response, *PR* Partial response, *SD* Stable disease, *PD* Progression of disease, *ORR* Objective response rate, *DCR* Disease control rate, *PD-L1* Programmed cell death-ligand 1, *LUAD* Lung adenocarcinoma, *LUSC* Lung squamous cell carcinoma

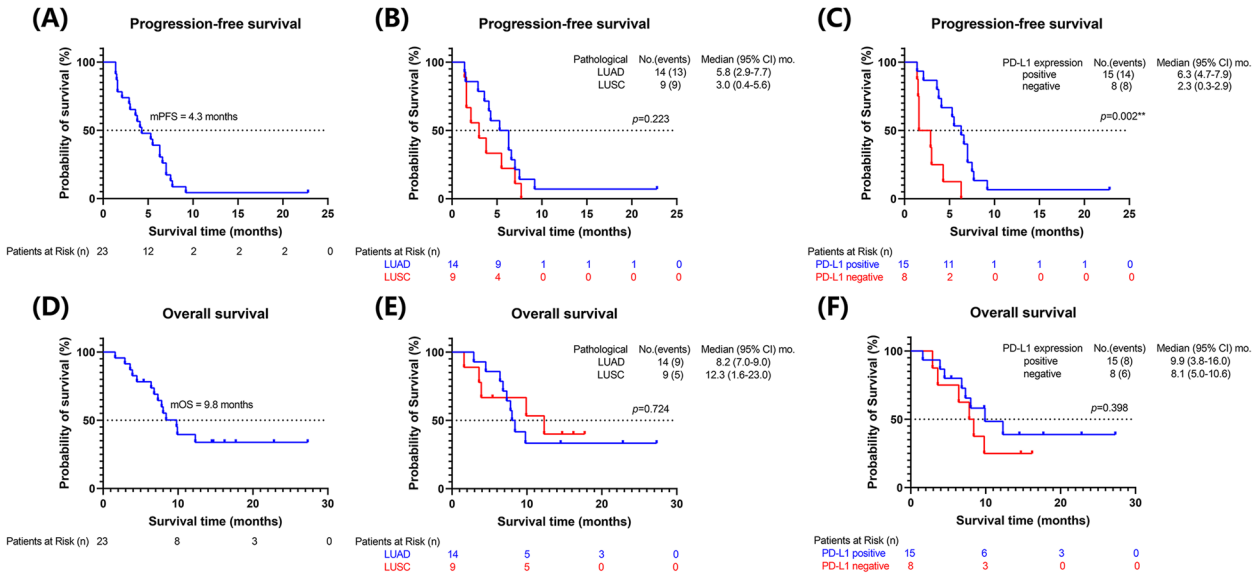

Fig. 2 Kaplan–Meier curves of progression-free survival (PFS) and overall survival (OS). **A** PFS of all patients (*n* = 23). **B** PFS of LUAD (*n* = 14) and LUSC (*n* = 9) subgroups. **C** PFS of PD-L1 positive (*n* = 15) and PD-L1negative (*n* = 8) subgroups. **D** OS of all patients (*n* = 23). **E** OS of LUAD (*n* = 14) and LUSC (*n* = 9) subgroups. **F** OS of PD-L1 positive (*n* = 15) and PD-L1 negative (*n* = 8) subgroups. Use log-rank test to compare survival curves of subgroups. $p < 0.05$ is considered to be statistically significant. LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; PD-L1, programmed cell death ligand-1

Table 3 Profiles of adverse events

TRAEs, n (%)	Grade			
	1	2	3	4
Fatigue	4 (17.4)	1 (4.3)	1 (4.3)	0
Hypothyroidism	6 (26.1)	0	0	0
Rash	3 (13.0)	0	0	0
Anemia	1 (4.3)	1 (4.3)	1 (4.3)	0
Fever	2 (8.7)	0	0	0

TRAE Treatment-related adverse events

Protemic expression and analysis

Serum samples were collected at baseline, when achieved response and/or disease progression. Proteomic tests were performed on serum samples of 9 patients ultimately (Fig. 3A, B). A total of 5 cytokines were found to be significantly different before and after tumor progression, including CASP-8, ARG1, MMP12, CD28 and CXCL5 (Fig. 3C, D, Figure S2A).

Nine patients were divided into a response group (*n* = 6, PR + SD) and a non-response group (*n* = 3, PD) according to the best response. The results showed that IL-6, CD83 and ICOSLG had significant differences at baseline between response group and non-response group (Fig. 3E, F, Figure S1A, Figure S2B).

In response group, 2 patients achieved PR and 4 patients achieved SD. We then compared the cytokines of patients in response group at baseline and after response to the treatment and found that the levels of CD40-L and EGF relate to the favorable response (Fig. 3G, H, Figure S1B, Figure S2C). Univariate and multivariate cox

regression analysis was used to explore the correlation between cytokines with PFS or OS at baseline in the two groups, but the results showed no significant differences.

Discussion

Recently, there has been a growing body of research dedicated to the investigation of immune rechallenge [25]. For NSCLC, several studies with small samples of immune rechallenge were reported while the efficacy varied widely. The ORR ranged from 5.9% to 14.3%, and DCR ranged from 45.8% to 85.7% for single immunotherapy [17, 18, 26]. Dual immunotherapy achieved an ORR of 47.3% and a DCR of up to 81.5% [27]. A comprehensive meta-analysis encompassing 49 studies was conducted to access the efficacy and safety of immune rechallenge in patients with solid tumors. The findings revealed an ORR rate of 21.8%, a mPFS of 4.9 months and a mOS of 15.6 months while the incidence of adverse events was 52.2%, including 21.5% for grade 3 and above [28]. For our study, the ORR rate, mPFS was 21.7%, 4.3 months, which is similar to the above study. However, the mOS was 9.8 months, which is significantly lower than the above study. This may be attributed to the preponderance of patients with 3 lines or above in our study. Meanwhile, the incidence of adverse events in our study was 52.2%, with no grade 4 adverse events or new specific immune-related adverse events occurred, indicating that this treatment regimen (β-glucan combined with Envafolimab and Endostar) is feasible and safe as immune rechallenge for mNSCLC patients.

Modulating TME from an immunosuppressive environment to an inflammatory environment holds great promise for achieving immune rechallenge [29]. In this process, β-glucan offers a new treatment perspective. Previous studies have demonstrated that β-glucan can activate DCs and macrophages, inhibit MDSCs and TEDCs, thereby eliminating tumor-induced immunosuppression [30, 31]. It also regulates nature killer (NK) cell function by altering macrophage phenotype, enhancing antigen presentation, promoting initiation and differentiation of cytotoxic T lymphocytes [32–35]. The addition of β-glucan is of great value in improving the immunosuppressive TME, especially in combination with ICIs. For example, Hu et al. discovered that β-glucan can enhance the antitumor effects of anti-PD-L1 inhibitors in melanoma models [36]. Wang et al. developed an antibody-β-glucan conjugate (AGC) by linking β-glucan with anti-PD-L1 inhibitors, the AGC-mediated bridging effect enhanced the interaction between tumor cells and DCs. Compared to anti-PD-L1 inhibitors monotherapy, AGC induced the infiltration of DCs and the activation of T cells in TME, resulting in an earlier immune response [37].

Anti-PD-1 inhibitors and anti-PD-L1 inhibitors have been regarded as the same drug for a long time. Typically, anti-PD-L1 inhibitors are usually not applied after resistance to anti-PD-1 inhibitors. As research progresses, many studies indicated mechanistic differences between anti-PD-L1 and anti-PD-1 inhibitors. In addition to inhibiting the PD-1/PD-L1 pathway, anti-PD-L1 inhibitors can also inhibit the overexpression of PD-L1 on the surface of DCs in the TME, thus activating the antigen presentation function of DCs and recruiting T cells for the unique killing of tumor cells [38–40]. In the past few years, many studies found that after the progress of anti-PD-1 treatment, the usage of anti-PD-L1 antibody could also obtain a fairly controllable therapeutic efficacy [41–43]. Therefore, conversion of anti-PD-1/PD-L1 antibody is also an emerging therapeutic regimen as immune rechallenge strategy.

In the subgroup analysis, there were no significant differences observed in ORR between adenocarcinoma and squamous cell carcinoma subgroups or between PD-L1 positive and negative subgroups. Patients with adenocarcinoma tended to have better mPFS compared to those with squamous cell carcinoma, but the difference was not statistically significant. This may be attributed to the limited sample size. At the same time, there was no difference in mOS. It is important to note that PFS reflects short-term treatment effects, whereas OS can be influenced by various factors such as ECOG PS status, site of metastasis, lines of treatment, etc. Therefore, it was not uncommon to see differences in PFS but often no differences in OS in clinical studies. The high expression of PD-L1 at baseline has been associated with enhanced efficacy of anti-PD-1/PD-L1 therapy in advanced NSCLC

(See figure on next page.)

Fig. 3 Data preprocessing for DEGs and protemic analysis results of 9 patients. **A** A Boxplot of data preprocessing for different expressed proteins (DEPs). **B** Heat map of DEPs in all patients ($n=9$). Red or blue represents high or low expression. **C** Volcano plot of DEPs before and after progression in all patients ($n=9$). Red dots represent significantly associated proteins at FDR ≤ 5%. **D** Boxplot of significantly different DEPs in plasma of all patients ($n=9$) before and after progression. **E** Volcano plot of DEPs of RG ($n=6$) and NRG ($n=3$) at baseline. Red dots represent significantly associated proteins at FDR ≤ 5%. **F** Boxplot of significantly different DEPs in RG and NRG at baseline. **G** Volcano plot of DEPs in RG ($n=6$) before and after response. Red dots represent significantly associated proteins at FDR ≤ 5%. **H** Boxplot of significantly different DEPs in plasma of RG before and after response. The DEPs were compared by Mann–Whitney U test and evaluated by Log-rank test. *$p<0.05$, **$p<0.01$. RG, response group. NRG, non-response group. DEPs, different expressed proteins. NPX, normalized protein expression

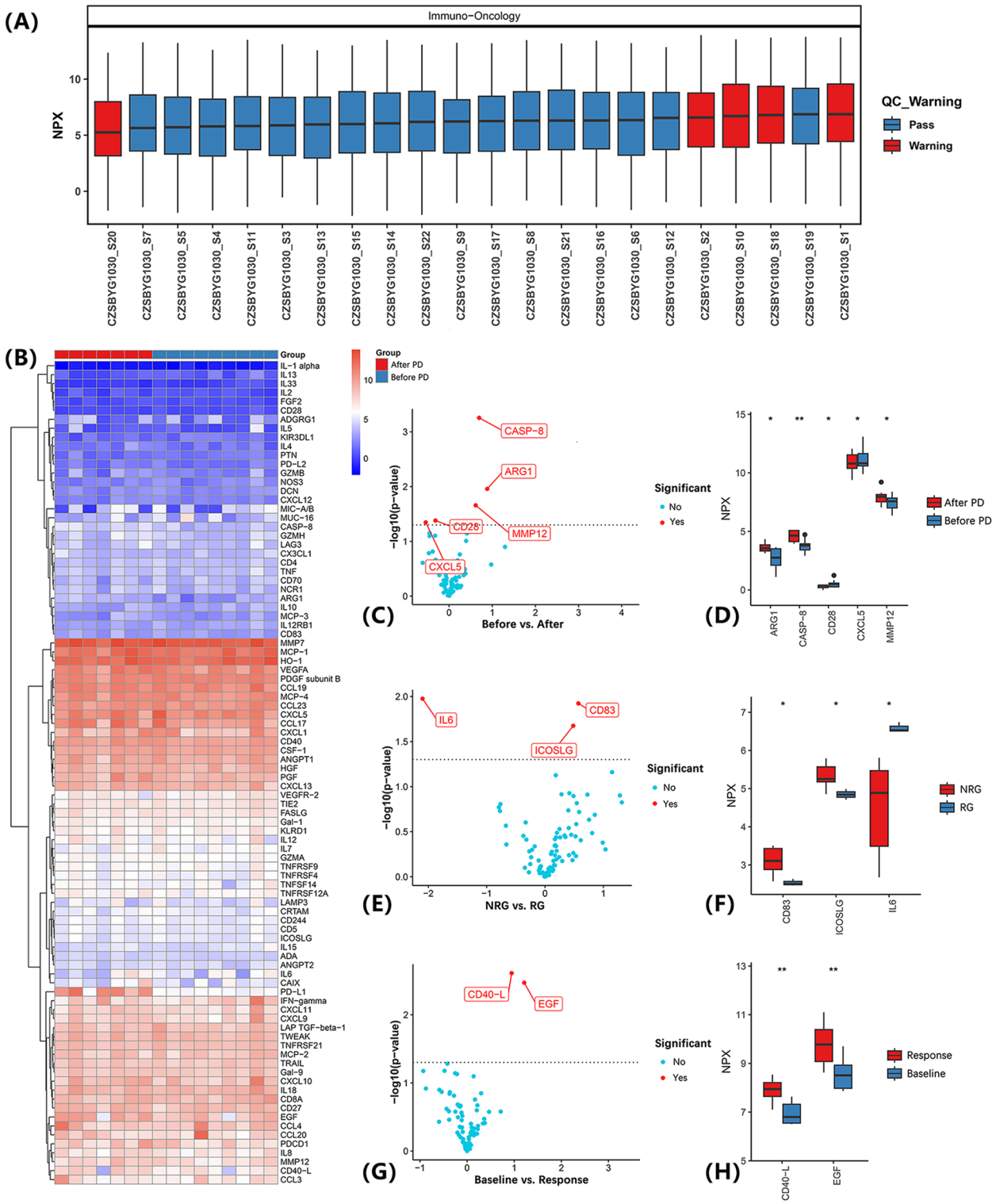

Fig. 3 (See legend on previous page.)

[44, 45]. However, it is not clear whether PD-L1 levels change during the previous immunotherapy. At present, the redetection of PD-L1 expression before immune rechallenge is still rare. Whether PD-L1 can be used as a therapeutic marker for immune rechallenge is controversial [46, 47]. In our study, the PFS of PD-L1 positive

subgroup was significantly better than that of PD-L1 negative subgroup. The OS data showed the same trend with no significant difference, which we thought was also related to the small sample size. These results indicated that the expression of PD-L1 at baseline might also serve as a prognostic biomarker for immune rechallenge.

Admittedly, effective biomarkers for immune rechallenge are currently lacking. Therefore, we collected peripheral blood samples from patients at different treatment time points and conducted proteomic analysis in an attempt to identify potential markers for therapeutic efficacy and prognosis. A total of 5 cytokines, including CASP-8, ARG1, MMP12, CD28 and CXCL5, were found to be significantly different before and after tumor progression and we considered this might be related to the treatment resistance. Among the cytokines, previous studies have shown that MMP12 and ARG1 were associated with poor prognosis in various tumors [48, 49] and CASP-8 promoted melanoma progression [50]. The increase of $CD8^+CD28^+$ T cells in advanced NSCLC patients who received chemo (radio) therapy predicted treatment efficacy and prognosis [51, 52]. However, the results of CXCL5 in our study could not be confirmed by current studies, as CXCL5 tended to be associated with poor prognosis in many solid tumors [53].

Comparing between response and non-response groups, mNSCLC patients with lower ICOSLG and CD83 at baseline responded better to the treatment, but the specific thresholds remains unknown [54, 55]. However, high expression of IL-6 associated with poor efficacy with immunosuppression in many tumors [56], which differed from our results. Furthermore, CD40-L can activate NK cells, $CD4^+$ and $CD8^+$ T cells and inhibit tumor progression [57]. EGF signaling is the core in regulating tumor cell proliferation [58]. The levels of CD40-L and EGF might relate to the favorable response. However, due to the limited number of patients enrolled in proteomic analysis, the results should be interpreted with caution. Further investigation is required in a larger cohort to validate these proteins' association with resistance or response to immune rechallenge, thus providing more reliable predictive biomarkers for guiding clinical work.

The main limitation of this study was that the patients enrolled in the study were not enough, leading to insufficient statistical power in the analysis. Enrollment should be expanded or a multi-center clinical study could be conducted in the follow-up studies. Secondly, as a single-arm study, it was difficult to accurately compare the efficacy and side effects with other treatment regimens in immune rechallenge studies. Despite these limitations, this study was the first attempt to demonstrate that β-glucan combined with Envafolimab and Endostar has considerable efficacy in patients who have failed previous anti-PD-1 treatment. As a "chemo-free" treatment regimen, attempting to regulate the TME for the purpose of immune rechallenge, this is more acceptable for cancer patients.

Conclusion

In summary, β-glucan combined with Envafolimab and Endostar has considerable efficacy and safety for immune rechallenge in metastatic NSCLC patients who failed of anti-PD-1 treatment previously. Especially, PD-L1 positive patients can benefit more from immune rechallenge than PD-L1 negative patients.

Abbreviations

AGC	Antibody-β-glucan conjugate
BOR	Best overall response
CAC	Colitis associated colorectal cancer
CR	Complete response
CTCAE	Common Terminology Criteria for Adverse Events
DC	Dendritic cell
DCR	Disease control rate
DEP	Different expressed proteins
ECOG PS	Eastern Cooperative Oncology Group performance status
ICI	Immune checkpoint inhibitor
irAE	Immune-related Adverse Event
LUAD	Lung adenocarcinoma
LUSC	Lung squamous cell carcinoma
MDSC	Myeloid-derived suppressor cell
mNSCLC	Metastatic non-small cell lung cancer
mPFS	Median progression-free survival
mOS	Median overall survival
NK	Natural killer
NPX	Normalized protein expression
NRG	Non-response group
NSCLC	Non-small-cell lung cancer
ORR	Objective response rate
OS	Overall survival
PCA	Principal components analysis
PD	Progression of disease
PD-1	Programmed cell death-1
PD-L1	Programmed cell death ligand-1
PFS	Progression-free survival
PR	Partial response
RECIST	Response Evaluation Criteria in Solid Tumors
RG	Response group
SD	Stable disease
TEDC	Tumor-educated dendritic cell
TME	Tumor microenvironment
TRAE	Treatment-related adverse events

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12865-024-00651-x.

Supplementary Material 1: Supplemental Figure 1. Heat maps of different groups. (A) Heat map of DEPs in RG (n=6) and NRG (n=3) at baseline. Red or blue represents high or low expression. (B) Heat map of DEPs in RG (n=6) before and after response. Red or blue represents high or low expression. DEPs, different expressed proteins. RG, response group. NRG, non-response group.

Supplementary Material 2: Supplemental Figure 2. PCA results of different groups. (A) PCA of 9 patients before and after response. (B) PCA of RG (n=6) and NRG (n=3) at baseline. (C) PCA of RG (n=6) before and after response. PCA shows no significant differences. PCA, principal components analysis. RG, response group. NRG, non-response group.

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Authors' contributions
QG, HJ, DL and CQ, conceptualization. XP, YZ, YZ, QZ, and GW, investigation. YL, LQ, original draft writing and formal analysis. QG, HJ, DL, review and editing. All authors contributed to the article and approved the submitted version.

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Availability of data and materials
The datasets generated and analyzed during the current study are not publicly available due to limitations of ethical approval involving the patient data but are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
The study was approved by the Ethics Committee of Changzhou No.2 People's Hospital (Approval number: [2021YLJSD002]) and conducted in accordance with the principles of the Declaration of Helsinki. All registered patients have signed informed consent forms to participate.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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