

Efficacy and Biomarkers of Advanced Non-small Cell Lung Carcinoma (NSCLC) Patients Receiving Different PD-1 Agents in Northern China: A Real-world Clinical Study

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Research article

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Abstract

Aim and Methods: From May 2019 to January 2020, 116 advanced NSCLC patients with programmed death ligand-1 (PD-L1) expression > 1% were treated with nivolumab (44), pembrolizumab (21), and toripalimab (51) as a mono-therapy, respectively. The primary endpoints were defined as the objective response rate (ORR) after 3 and 6 months of therapy, and the progression-free survival (PFS). The observed efficacy of the different PD-1 antibodies was also compared. The gene mutation statuses of tumor protein p53 (TP53), epidermal growth factor receptor (EGFR), and anaplastic lymphoma kinase (ALK), the tumor mutational burden (TMB), along with the expression of CD47 were analyzed.

Results: Toripalimab had a higher ORR and a longer PFS than the other two PD-1 agents after 3 months of evaluation ($P = 0.0178$). Thenon-classical mutations of EGFR (EGFRG719C and EGFRE709V) did not significantly influence the efficacy of the PD-1 inhibitors, while TP53 mutation, high TMB and elevated PD-L1 expression showed benefits. EGFR co-mutated genes were enriched in the “Response to osmotic stress”, “response to oxidative stress” and “myeloid leukocyte activation” pathways. CD47 expression showed a negative correlation to the prognosis of anti-PD-1 therapy. Participants with ALK rearrangement exhibited poor clinical outcomes.

Conclusions: Toripalimab seemed to have a more rapid effect and provide a longer PFS than pembrolizumab and nivolumab. The PD-1 blockade therapy was benefited by TP53 mutation, high TMB, and elevated PD-L1 expression. CD47 expression is a potential biomarker to predict the efficacy of PD-1 blockade treatment in patients with EGFR mutations.

Clinical Trail : Real-World Study of Four PD-1 Agents in China(May/22/2019)

Trial Registration number: NCT03966456

URL:<https://register.clinicaltrials.gov/prs/app/action/SelectProtocol?sid=S0008Y4C&selectaction=Edit&uid=U000450C&ts=3&cx=z2uldc>

Introduction

Global cancer statistics from 2018 revealed that lung cancer is still the most common malignant tumor (11.6% of all cancer patients) and the leading cause of death from cancer (18.4% of all cancer deaths) [1]. Due to inadequate tobacco control and a sharp increase in the number of young smokers, the incidence of lung cancer in China is increasing. Non-small cell lung cancer (NSCLC) accounts for 85% of the total lung cancer cases [2]. According to previous studies, the 5-year survival rate of NSCLC is 17%, and that of advanced NSCLC is only < 5%. Surgical resection is the most effective treatment for patients with stage I, II, or IIIA NSCLC. However, 70% of patients in the late stage cannot undergo surgery, and patients with metastatic or recurrent disease have a relatively limited response to conventional treatments. Therefore, it is necessary to explore novel therapies to meet the clinical needs of this patient group.

Traditional cancer immunotherapy relies mainly on direct activation of the immune system. Recently, however, the focus of immunotherapy has moved toward reducing immune tolerance of tumor cells [3]. The abnormal expression of immune checkpoint molecules is one of the mechanisms of tumor immune escape, which is caused by ligand-receptor interactions on the surface of immune and tumor cells [4]. Cytotoxic T lymphocyte-associated protein 4 (CTLA-4), programmed cell death protein 1 (PD-1), and their corresponding ligands B7-1/2 and PD-L1, have received the most clinical attention. Researchers have also developed monoclonal antibodies targeting these molecules, namely immune checkpoint inhibitors (ICIs). These ICIs deliver anticancer effects by suppressing and blocking the action of inhibitory receptors, such as PD-1 and CTLA-4, so that the immune system can effectively recognize and attack cancer cells [5, 6].

From 2018 to 2020, 6 monoclonal antibody agents against PD-1, including nivolumab, pembrolizumab, toripalimab, sintilimab, camrelizumab, and tislelizumab, have been approved by the China Food and Drug Administration and marketed in China for a variety of cancer indications. Currently, only nivolumab monotherapy and the combination therapy of pembrolizumab plus chemotherapeutics have been approved as second-line and first-line therapies, respectively, for NSCLC patients in China. Since different monoclonal PD-1 antibodies have different pharmacokinetic profiles related to the unique complementarity-determining region sequences in the heavy and light chains, we conducted a clinical trial (Real-World Study of Four PD-1 Agents in China: NCT03966456) to compare the clinical efficacies of 3 anti-PD-1 monoclonal antibodies, including nivolumab, pembrolizumab, and toripalimab. Here, we report the therapeutic outcomes and potential predictive biomarkers for the different PD-1 antibodies analyzed in this study.

Participants And Methods

Study population

As part of an ongoing clinical trial (Real World Study of Four PD-1 Agents in China, NCT03966456), this clinical assessment was carried out using data from 116 NSCLC patients admitted to the Affiliated Hospital of Qingdao University between May 2019 and January 2020. The inclusion conditions were patients 18–85 years old who were diagnosed with advanced NSCLC with positive PD-L1 expression (tumor proportion score > 1%) by pathological examination. All patients were treated with a PD-1 monoclonal antibody as a mono-therapy. Patients with a classical epidermal growth factor receptor (EGFR) mutation were excluded, excepting EGFRG719C (18/29) and EGFR E709V (19/29). The Response Evaluation Criteria in Solid Tumors (RECIST version 1.1) were followed, and the patients must have had ≥ 1 measurable lesion per response. The Eastern Cooperative Oncology Group (ECOG) performance status was 0 or 1 for all participants. The study protocol and all amendments were approved by the institutional ethics committee of the Affiliated Hospital of Qingdao University (QYFYKYL 471311920).

Treatments and endpoints

All of the enrolled participants received anti-PD-1 treatment with nivolumab (3 mg/kg, Q2W), pembrolizumab (200 mg, Q3W), or toripalimab (240 mg, Q3W) as a mono-therapy. Radiographic imaging was performed before treatment and every 4 weeks following treatment. The objective response rate (ORR) after 3 and 6 months of therapy and the progression-free survival (PFS) were selected as the primary endpoints. Exploratory endpoints included the correlation between clinical efficacy and biomarkers, such as EGFR G719C (18/29) and EGFR E709V (19/29), variant frequencies of tumor protein p53 (TP53), and anaplastic lymphoma kinase (ALK) genes, along with the tumor mutation burden (TMB).

Biomarker studies

Pretreatment tumor biopsies from all participants were examined for PD-L1 expression by immunohistochemical (IHC) staining with the anti-human PD-L1 monoclonal antibody SP142 [7]. The PD-L1 positivity evaluated by certified pathologists was defined as a strong PD-L1 staining on the cell membrane detected in $\geq 1\%$ of tumor cells or any staining intensity observed in tumor-infiltrating immune cells. The number of deduced somatic mutations per megabase (Mb) was used to evaluate the TMB. Using 12 mutations/Mb as the cutoff value, $TMB \geq 12$ mutations/Mb was defined as a high TMB, and $TMB < 12$ mutations/Mb was defined as a low TMB. Whole-exome sequencing of tumor tissue and matched peripheral blood mononuclear cells were profiled using a commercially available capture-based lung plasma sequencing panel (Burning Rock Biotech Ltd., Guangzhou, China) targeting 168 genes and spanning 160,000 human genomic regions. Briefly, fragmented DNA captured by probe baits was purified with magnetic beads, and the library was indexed and amplified by polymerase chain reaction (PCR). A bio-analyzer assay was then used to assess the quality and size range of the library. The pool of 30 normalized libraries was finally loaded onto a NextSeq 500 system (Illumina, Inc., San Diego, CA, USA) for paired-end sequencing.

Gene ontology functional analysis and Kyoto Encyclopedia of Genes and Genomes pathway enrichment analysis of EGFR and co-mutation events

Each group was further separated into 2 subgroups by the median percent spliced in (psi) value. Then, univariate Cox regression analysis was conducted to explore independent prognostic factors with the significance set as a P value < 0.05 . Next, we selected the co-mutated genes with an EGFR mutation in our study as candidates for Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses using Metascape (<http://metascape.org/>). A P-value of < 0.05 indicated statistical significance.

Western blot analysis

Proteins were extracted from the the Formalin-Fixed and Paraffin-Embedded (FFPE) samples. The protein concentration was determined by a BCA kit (Pierce, Rockford, IL, USA). And then the proteins were separated by 10% SDS-PAGE, transferred to nitrocellulose membranes (Millipore, Bedford, MA, USA), blocked with 5% low fat dried milk for 2 h, and incubated overnight at 4 °C with corresponding primary antibodies directed against CD47 (Rabbit, 1:2000, ab175388, Abcam). Subsequently, the membranes were incubated with horseradish peroxidase (HRP)-conjugated donkey-anti-rabbit secondary antibody for

2 h at room temperature. Finally, the protein bands were visualized using an enhanced chemiluminescent reagent.

Statistical analysis

Data were analyzed by using the statistical software SPSS version 20.0 (IBM Corp, Armonk, NY, USA) and Graph Pad Prism 5.0 software (Graph Pad Software Inc., San Diego, CA, USA). Continuous variables with a normal distribution are presented as the mean \pm standard deviation and were compared by the Student's t-test. Continuous variables with a non-normal distribution are expressed as the median (inter quartile range) and were compared with the Mann–Whitney test. Categorical variables are expressed as a number and proportion and were analyzed with the Fisher's exact or chi-squared (χ^2) test. The variables with a statistically significant difference were further explored using binary logistic regression analysis. Irreversible organ injury and death were defined as poor outcome events. The survival time was defined as the period between the diagnosis of NSCLC and the occurrence of a poor outcome event. The Kaplan–Meier method and the Cox proportional hazards regression model were used to evaluate the prognostic factors. Differences were considered significant at a P-value of < 0.05 .

Results

Patient population

The baseline clinical characteristics of the 116 participants are summarized in Table 1. There were no significant differences in age among the 3 cohorts ($P > 0.1$), and the average age was 64 years old. More male than female patients were enrolled, which was consistent with a higher incidence of NSCLC in males than in females. All participants in this study received ≥ 1 prior line of treatment and anti-PD-1 mono-therapy as described above (nivolumab [3 mg/kg, Q2W], pembrolizumab [200 mg, Q3W] or toripalimab [240 mg, Q3W]).

Table 1
Baseline patients demographics and clinical characteristics

Characteristics	All Patients (N = 116)	Patients Treated with Toripalimab (N = 51)	Patients Treated with Nivolumab (N = 44)	Patients Treated with Pembrolizumab (N = 21)
Age at enrollment-yr				
Mean ± SD	63.6 ± 5.1	64.7 ± 8.3	62.0 ± 10.3	64.3 ± 6.7
Sex-No				
Female	46	21	16	9
Male	70	30	28	12
Clinical disease stage No.				
IV	35	29	3	3
III	81	22	41	18
Smoking status-No.				
Never	98	46	36	16
Former or current	18	5	8	5

Subgroup analysis for clinical efficacy

A total of 6 participants had died by January 23, 2020 including 4 who were treated with nivolumab, 1 who was treated with pembrolizumab, and 1 who was treated with toripalimab. The changes observed at 3 and 6 months from baseline were each analyzed (Fig. 1A and 1B). According to the results shown in Fig. 1C and 1D, the toripalimab cohort exhibited a higher ORR while the pembrolizumab cohort showed a higher disease control rate, but these differences were not statistically significant ($P > 0.05$). The PFS curves of the 3 anti-PD-1 agents were not significantly separated (Fig. 1E). The median PFS times for the nivolumab, pembrolizumab, and toripalimab groups were 9, 10, and 11 months, respectively (log rank $P = 0.990$). And there was no significant difference in efficacy between the 3 agents with respect to the histology (Fig. 2).

Efficacy for the adenocarcinoma subtype with or without a nonclassical EGFR mutation

Figure 3A-3G show the survival analysis (Fig. 3E) according to the adenocarcinoma subtype with (Fig. 3C and 3D) or without EGFR mutation (EGFRG719C (18/29) or EGFR E709V (19/29)) (Fig. 3A and 3B). According to the next-generation sequencing analysis, 8 patients displayed both of EGFRG719C (18/29) and EGFR E709V (19/29) mutations.

In the adenocarcinoma subtype without an EGFR mutation, the ORRs (Fig. 3C) of nivolumab, pembrolizumab, and toripalimab after 3 months of therapy were 30.0%, 50.0%, and 23.1%, respectively ($P = 0.0271$); and after 6 months of therapy, they were 20.0%, 50.0%, and 46.2%, respectively ($P = 0.2567$) (Fig. 3D). Survival analysis (Fig. 3F) indicated that the median PFS times of the nivolumab, pembrolizumab, and toripalimab cohorts were 9 [95% CI: 7.328–10.674] months, 10 [95% CI: 6.08–13.910] months, and 12 [95% CI: 10.522–13.478] months, respectively ($P > 0.05$). Toripalimab was shown to have a better efficacy.

In the adenocarcinoma subtype with a non-classical EGFR mutation, the ORRs (Fig. 3C and 3D) of nivolumab, pembrolizumab, and toripalimab after 3 months of therapy were 37.5%, 33.3%, and 50.0%, respectively ($P = 0.0183$) (Fig. 3A); and after 6 months of therapy, they were 43.8%, 25.0%, and 63.3%, respectively ($P = 0.3667$) (Fig. 3B). Survival analysis (Fig. 3G) indicated that the median PFS times of the nivolumab, pembrolizumab, and toripalimab cohorts were 9 (95% CI: 6.6–11.400), 10 (95% CI: 5.351–14.649), and 12 (95% CI: 7.702–16.298) months, respectively. Patients treated with pembrolizumab showed a higher ORR, while those treated with toripalimab enjoyed a longer PFS (Fig. 3D and 3G).

Functional enrichment analysis of co-mutated genes in patients with EGFR mutation and CD47 expression of the patients with EGFR mutation

According to the next-generation sequencing analysis, peripheral-extracellular signal-regulated kinase (P-MAPK1), neuroblastoma RAS (NRAS), TP53, tumor necrosis factor (TNF), and caspase-3 have high co-mutation frequencies with EGFR non-classical mutations (Fig. 3I). These genes were further assessed by functional and pathway enrichment analyses. The most significant enrichment results are shown in Fig. 3H. The commonly enriched terms were “proteoglycans in cancer,” “response to osmotic stress,” “response to oxidative stress,” and “myeloid leukocyte activation”. Western blot analysis showed that the patient with lower CD47 expression exhibited better efficacy to PD-1 therapy. But higher CD47 expression maybe associated with disease progression under PD-1 therapy (Fig. 3I).

Efficacy for the squamous cell carcinoma subtype

Analysis of the efficacy of the different PD-1 agents for treatment of the squamous cell carcinoma subtype is shown in Fig. 4A and 4B. In the squamous cell carcinoma subtype, the ORRs of nivolumab, pembrolizumab, and toripalimab after 3 months of therapy were 16.7%, 0.0%, and 50%, respectively ($P = 0.2062$); and after 6 months of therapy, they were 38.9%, 66.7%, and 50.0%, respectively ($P = 0.5878$). Survival analysis (Fig. 4C) indicated that the median PFS times of the 3 agents were 14 [95% CI: 4.398–23.602], 9 [95% CI: 5.882–12.128], and 6 [95% CI: 0–14.318] months, respectively. Nivolumab appeared to show a longer median PFS than the other 2 agents; however, due to the large difference in patient numbers, definite conclusions could not be deduced from the analysis.

Correlation of TP53 mutation, ALK rearrangement, and TMB with clinical efficacy

Among all 116 participants, just 78 had a TP53 mutation. The percentage of participants with a TP53 mutation was not significantly different between the 3 PD-1 antibody cohorts (Fig. 5A). According to our study, 80.77% (63/78) of the participants with a TP53 mutation also had a KRAS mutation. In addition, the participants with a TP53 mutation had a longer PFS (12 [95% CI: 10.887–13.113] and 8 [95% CI: 6.680–9.320] months for patients with a TP53 mutation and wild-type TP53, respectively $P = 0.019$) (Fig. 5B). Furthermore, the meta-analysis of our data showed that PD-1 therapy was more effective in patients with a TP53 mutation (Fig. 5C and 5D). Among the 3 PD-1 antibodies studied herein, the participants with a TP53 mutation appeared to be more sensitive to nivolumab after 6 months of therapy (Fig. 5D).

In our study, there were only 4 patients with ALK rearrangement, all of whom showed disease progression before the evaluation after 3 months of treatment. The receiver operating characteristic (ROC) curve analysis showed that the TMB was not associated with the efficacy of PD-1 treatment at the 3-month evaluation ($P > 0.1$) (Fig. 5E). However, at the 6-month evaluation, a higher TMB was significantly beneficial to the PD-1 therapy ($P < 0.05$) (Fig. 5E). At the 3-month evaluation, a higher PD-L1 expression level correlated with a better ORR, indicating that a high PD-L1 expression level is conducive to early treatment efficacy. However, at the 6-month evaluation, the difference was not significant (Fig. 5F).

Discussion

One of the aims of this study was to analyze the efficacies of different monoclonal anti-PD-1 antibodies in northern China. In a previous study, a comparison of the amino acid sequences of nivolumab and pembrolizumab showed that they are basically the same except for the variable region [8]. In addition, Fesses et al. compared the molecular, preclinical, and early clinical characteristics of nivolumab and pembrolizumab. They found significant molecular similarities between these drugs, indicating that the differences observed in the clinical data were not likely to be drug-dependent and likely to be drug-independent. The observed differences may be due to variations between the participant populations in the clinical trials. In our study, toripalimab was found to have a higher ORR and longer PFS than the other 2 PD-1 agents, especially at the 3-month evaluation.

The PD-L1 of tumor cells is currently the most widely studied protein and the only Federal Drug Agency–approved biomarker for clinical use. According to the KEYNOTE-042 study results published at the 2018 American Society of Clinical Oncology conference, for advanced NSCLC patients with PD-L1 $\geq 1\%$ and negative for EGFR or ALK mutations, the overall survival (OS) of those receiving first-line pembrolizumab therapy was higher than that of those receiving chemotherapy. This finding showed that pembrolizumab is more beneficial for NSCLC patients with PD-L1 $\geq 1\%$ [9]. The TMB is the sum of non-synonymous mutations in the coding region of the somatic genome. Non-synonymous somatic mutations can alter the amino acid sequence of the protein encoded by the affected gene, thereby forming a neoantigen and helping to enhance the immunogenicity of tumor cells [10]. There are various methods to calculate the TMB, and they generally require whole-exome sequencing of ≥ 200 cancer-related genes [11]. The TMB was first reported as a biomarker in the first edition of the 2019 National Comprehensive Cancer Network

guide for screening NSCLC patients for whom nivolumab combined with ipilimumab or nivolumab monotherapy may be effective. In the present study, higher TMB and PD-L1 expression levels were also demonstrated to benefit PD-1 blockade therapy. We found that the TMB influenced the ORR significantly after 6 months of therapy and that the PD-L1 expression influenced the ORR significantly after 3 months of therapy.

The correlation between mutations of cancer driver genes and the efficacy of PD-1 monoclonal antibody therapy has also attracted attention. An EGFR mutation in adenocarcinoma lung cancer is a good predictive factor for the efficacy of EGFR tyrosine kinase inhibitors [12–14]. In the KEYNOTE-001 study, the median OS of EGFR mutation–positive patients treated with pembrolizumab was significantly shorter than that in wild-type EGFR patients [15]. Gainor et al. also found that compared with wild-type EGFR NSCLC patients, EGFR mutation–positive NSCLC patients had a much lower ORR [16]. However, our study found that a non-classical EGFR mutation, such as G719C or E709V, did not significantly influence the efficacy of PD-1 therapy for NSCLC patients in northern China. According to the results of the functional enrichment analysis of co-mutated genes in patients with an EGFR mutation, the commonly enriched terms were “proteoglycans in cancer,” “response to osmotic stress,” “response to oxidative stress,” and “myeloid leukocyte activation” (Fig. 8B). And more the co-mutations, better the prognosis (Fig. 8A). These pathways associated activation of cell signaling pathways of proliferation, angiogenesis, and cell motility (Fig. 8C). The most enriched signal pathway of these mutations is “proteoglycans in cancer”. Proteoglycans (PGs) are heterogeneous glycoproteins, expressed in cells of the tumor microenvironment and on tumor cells, which could regulation immunosensitization [17]. “Response to osmotic stress”, “response to oxidative stress” and “myeloid leukocyte activation” pathways were also significantly enrichment herein. Both osmotic stress and oxidative stress are associated with inflammation. And inflammation was associated with immune activation [18]. Myeloid leukocyte activation may activate B cells [19] and improve the tumor microenvironment to make it sensitive to PD-1 agents [20]. Take above all, these gene characteristic maybe influence the patients response to PD-1 therapy. As we all known the expression of CD47 is involved in imparting resistance to programmed cell death (PD)-1/PD-ligand 1 inhibitors [21]. According to our study, the EGFR-mutated patients who were achieved PR after 6 m-therapy showed a lower CD47 expression. But the patients with disease progression exhibited a higher CD47 expression. Maybe CD47 expression is the potential biomarker to patients with EGFR mutation treated with PD-1 agents.

Another important driver gene of NSCLC is ALK. The ATLANTIC study showed that for patients with ALK rearrangement, if the PD-L1–positive cell expression rate is $\geq 25\%$, immunotherapy will still work [22]. Moreover, it has been reported that NSCLC patients with ALK gene fusion might benefit from PD-1 treatment [22–24]. However, according to our clinical observations, those with ALK gene fusion showed a poor response to PD-1 therapy. Previous studies have indicated that a high TMB and PD-L1 expression level were both independently correlated with survival benefits from anti-PD-1 therapy. In a recent study, researchers also observed that the median OS was longer in the TP53-mutated group than in the wild-type TP53 group (18.1 months vs. 8.1 months). Furthermore, the median PFS was significantly longer

and the ORR was higher in the TP53-mutated patients [24]. In accordance with these findings, we also found that a TP53 mutation was beneficial for PD-1 therapy.

This study had several limitations. The sample size of the group of participants treated with PD-1 antibodies in our study was small, especially when classified according to the therapy. Additionally, the OS was not determined for most of the participants, and the efficacy of PD-1 therapy was evaluated using only the ORR and the PFS. It can take years before an effective improvement or death occurs to enable further assessment of efficacy. When available, the final set of data, including the effectiveness and late toxicity endpoints, will provide valuable insight into the differences in survival and quality of life of the NSCLC patients.

Conclusions

Immune checkpoint blockade targeting PD-1 has dramatically changed the landscape for treatments in patients with NSCLC. Herein, we compared the efficacy of the 3 early accessible PD-1 agents for NSCLC patients in northern China, nivolumab, pembrolizumab, and toripalimab, and found that toripalimab conferred the most benefit. Mutation of TP53, high TMB, and elevated PD-L1 expression were beneficial to the PD-1 blockade therapy. The efficacy of PD-1 inhibitors was not significantly influenced by EGFR G719C or EGFR E709V. It is possible that proteoglycans, osmotic stress and oxidative stress, and myeloid leukocyte activation, which are regulated by EGFR, P-MAPK1, NRAS, TP53, TNF, and caspase-3, induced antitumor immunity in the tumor microenvironment leading to sensitization of PD-1 therapy. CD47 expression is the potential biomarker of EGFR-mutated NSCLC treated with anti-PD-1 therapy.

Abbreviations

NSCLC: non-small cell lung carcinoma

PD-1: programmed cell death protein 1

ORR: objective response rate

PFS: progression-free survival

TP53: tumor protein p53

EGFR: epidermal growth factor receptor

ALK: *anaplastic lymphoma kinase*

TMB: tumor mutational burden

P-MAPK1: peripheral-extracellular signal-regulated kinase

NRAS: neuroblastoma RAS

TNF: tumor necrosis factor

CTLA-4: cytotoxic T lymphocyte-associated protein 4

ICIS: immune checkpoint inhibitors

Declarations

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Authors' contributions

Man Jiang: acquisition of data; analysis and interpretation of data; drafting of the manuscript.

Xiaochun Zhang: study concept and design; acquisition of data.

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Availability of data and materials

The study protocol and all amendments were approved by the institutional ethics committee of the Affiliated Hospital of Qingdao University (QYFYKYL 471311920) and written informed consent was obtained from all the patients.

Consent for publication

Consent to publish has been obtained from all authors.

Competing interests

The authors declare that they have no competing interests.

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Figures

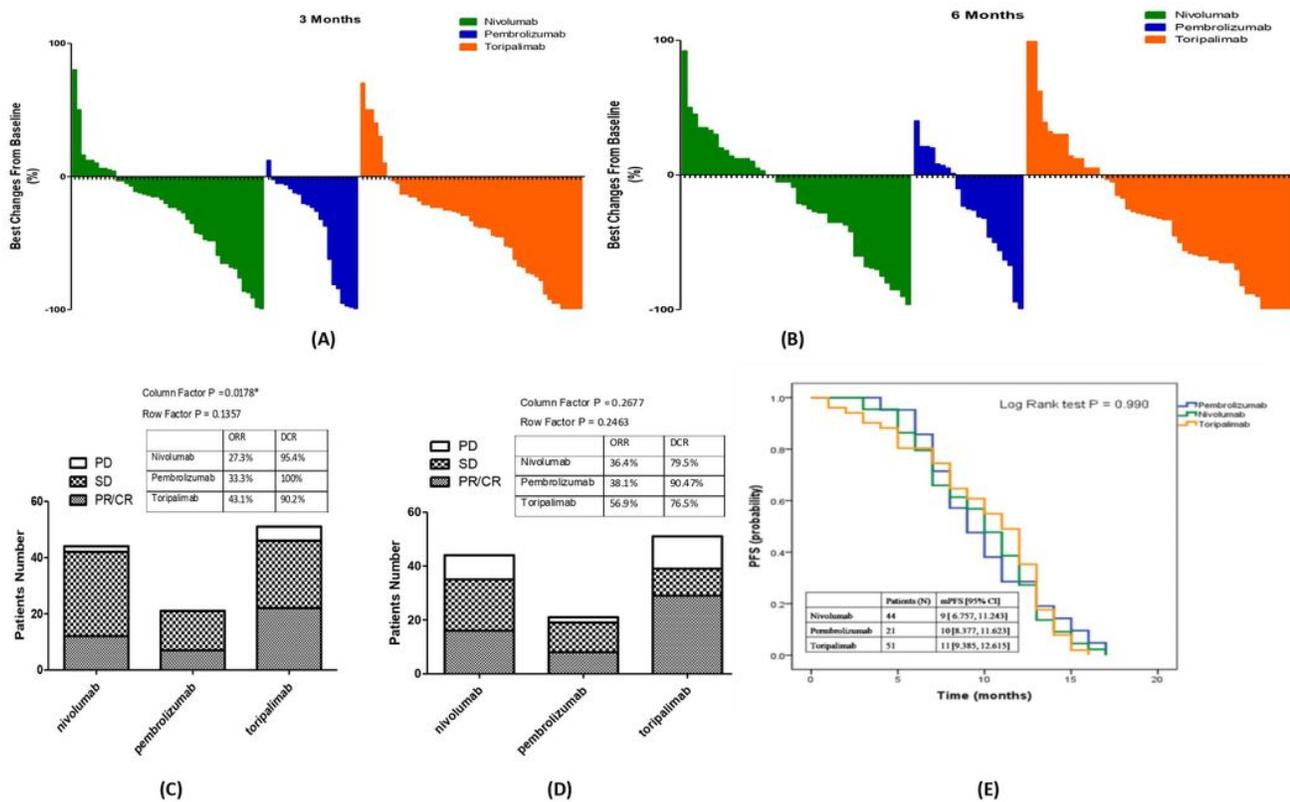
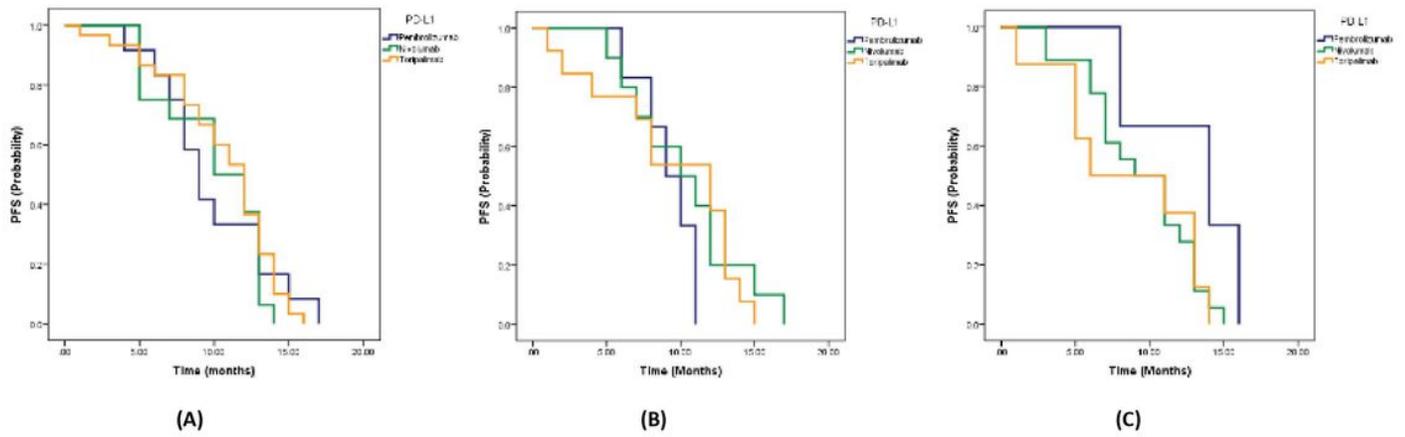


Figure 1

(A) and (B) efficacy analysis of Nivolumab, Pembrolizumab and Toripalimab in 3 and 6 months therapy, respectively; (C) and (D) ORR and DCR of three anti PD-1 antibodies after 3 months and 6 months therapy, respectively; (E) PFS of the three PD-1 agents.



	Adenocarcinoma without EGFR mutations		Adenocarcinoma with EGFR mutations		Squamous cell carcinoma	
	Patients (N)	mPFS [95% CI] (months)	Patients (N)	mPFS [95% CI] (months)	Patients (N)	mPFS [95% CI] (months)
Nivolumab	16	9 [6.6, 11.400]	10	9 [7.326, 10.674]	18	14 [4.398, 23.602]
Pembrolizumab	12	10 [5.351, 14.649]	6	10 [6.08, 13.910]	3	9 [5.882, 12.128]
Toripalimab	30	12 [7.702, 16.298]	13	12 [10.522, 13.478]	8	6 [0.00, 14.318]

Figure 2

Comparison of the PFSs of the three PD-1 agents in different histopathology, (A) (B) and (C) survival analysis to three PD-1 agents.

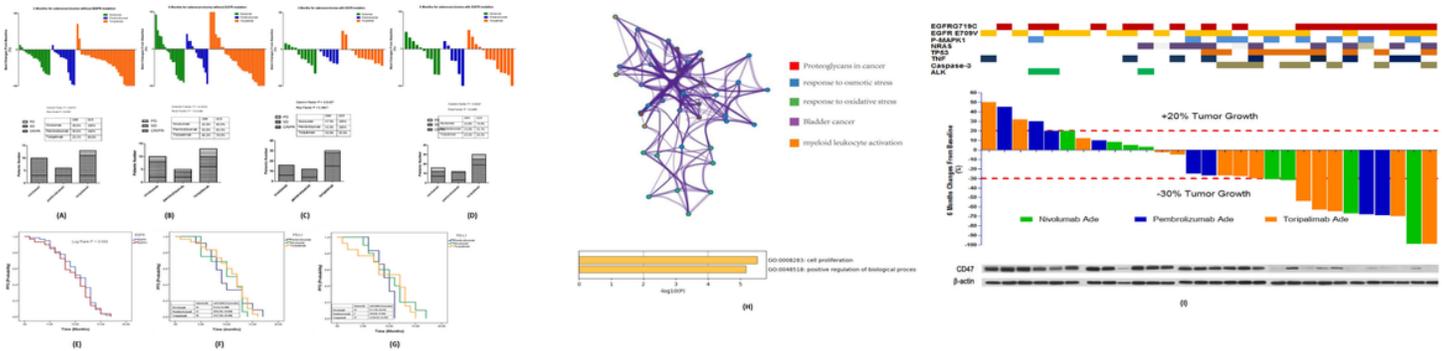


Figure 3

(A) (B) (C) and (D) efficacy analysis of Nivolumab, Pembrolizumab and Toripalimab in 3 and 6 months therapy to adenocarcinoma without and with EGFR mutations, respectively; (E) PFS of the adenocarcinoma patients with or without EGFR mutation; (F) PFS of the three PD-1 agents to adenocarcinoma patients without EGFR mutations, (G) PFS of the three PD-1 agents to adenocarcinoma patients with EGFR mutations; (H) Functional analyses on high frequency co-mutation gens with EGFR mutations ($P < 0.01$); (I) NGS analysis of the patients with EGFR mutation and the CD47 expression of the EGFR-mutant patient.

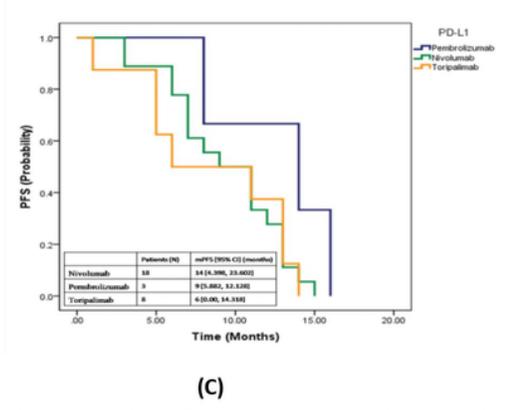
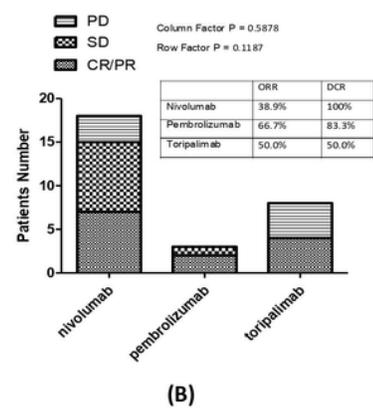
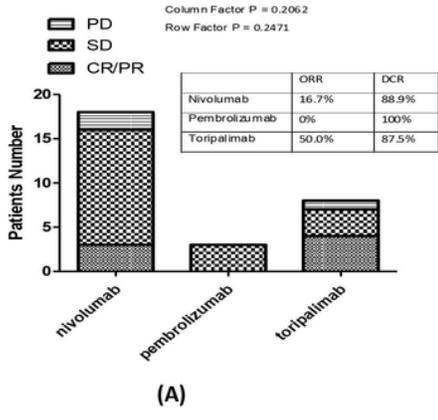
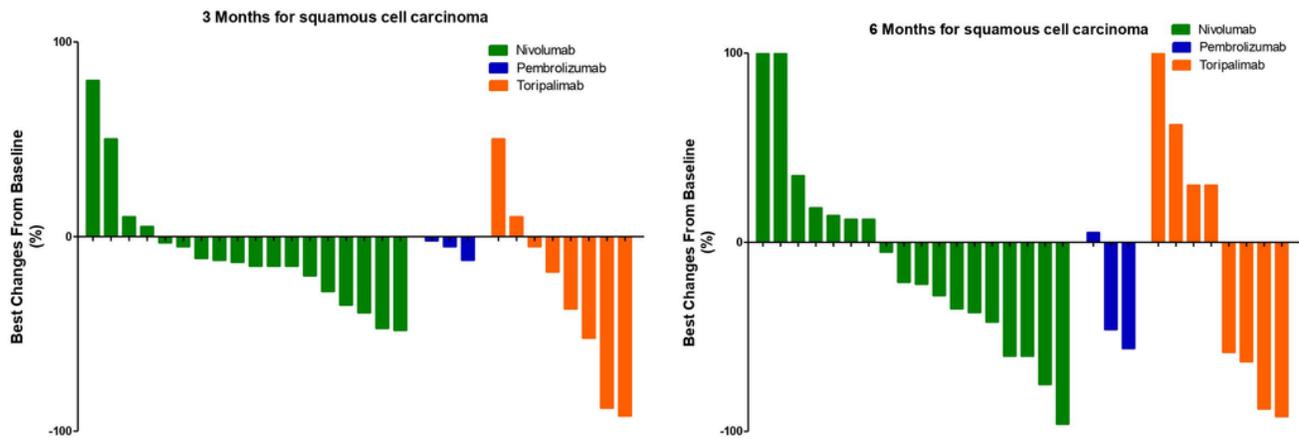


Figure 4

(A) and (B) efficacy analysis of Nivolumab, Pembrolizumab and Toripalimab in 3 and 6 months therapy to a squamous cell carcinoma patients, respectively; (C) PFS of the three PD-1 agents.

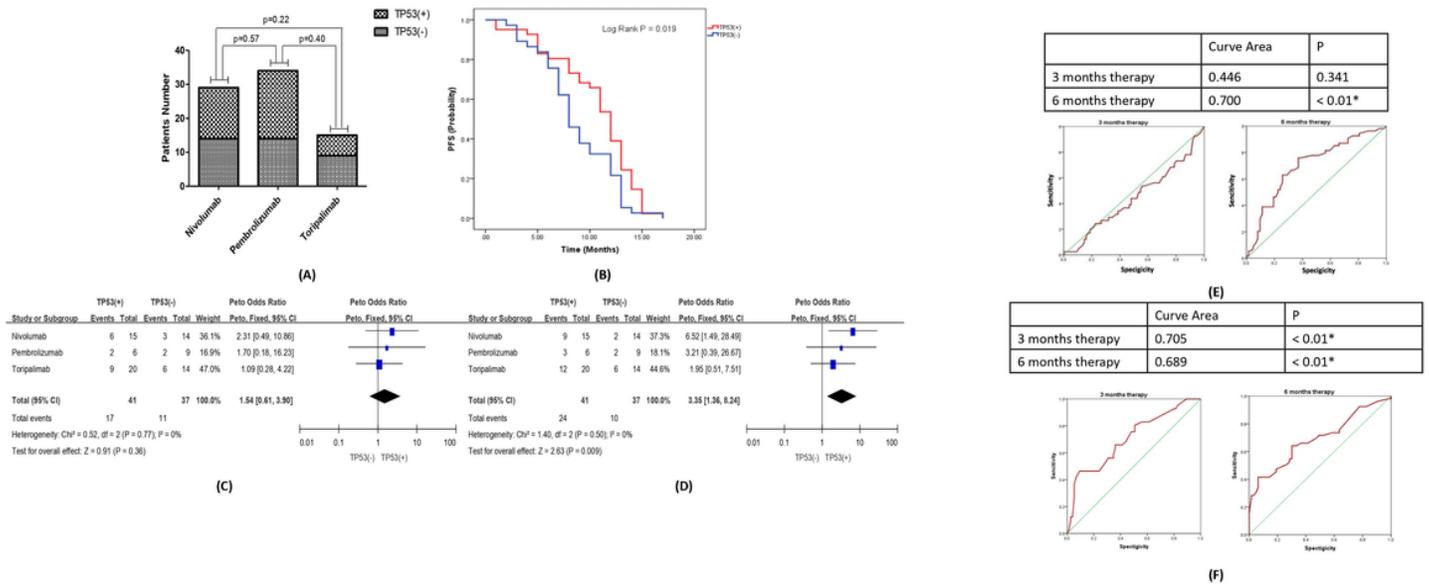


Figure 5

The influence of TP53 mutation to the efficacy of PD-1 therapy. (A) the TP53 mutation composition of the patients with the three agents; (B) survival analysis of patients with or without TP53 mutation; (C) The influence of TP53 mutation to the efficacy of PD-1 therapy in 3 months therapy (events stands for patients with PR); (D) The influence of TP53 mutation to the efficacy of PD-1 therapy in 6 months therapy (events stands for patients with PR); (E) ROC curves of TMB to PD-1 therapy efficacy; (F) ROC curves of PD-L1 expression to PD-1 therapy efficacy.