Efficacy, Safety, and Biomarkers of Toripalimab in Patients with Recurrent or Metastatic Neuroendocrine Neoplasms: A Multiple-Center Phase Ib Trial

Ming Lu[1], Panpan Zhang[1], Yanqiao Zhang[2], Zhongwu Li[3], Jifang Gong[1], Jie Li[1], Jian Li[1], Yan Li[1], Xiaotian Zhang[1], Zhihao Lu[1], Xicheng Wang[1], Jun Zhou[1], Zhi Peng[1], Weifeng Wang[4], Hui Feng[5], Hai Wu[5], Sheng Yao[5], and Lin Shen[1]

ABSTRACT

Purpose: Patients with recurrent or metastatic neuroendocrine neoplasms (NEN) had a poor prognosis and few treatment options. Toripalimab, a humanized IgG4 antibody specific for human PD-1 receptor, was first approved to treat second-line metastatic melanoma in China in 2018.

Patients and Methods: The multiple-center phase Ib trial enrolled patients with NENs (Ki-67 ≥ 10%) after failure of first-line therapy received 3 mg/kg toripalimab once every two weeks. The primary objective was objective response rate (ORR) and safety. PD-L1 expression and whole-exome sequencing were performed on tumor biopsies. Secondary objectives included duration of response (DOR), disease control rate (DCR), and progression-free survival and overall survival.

Results: Of 40 patients included from April 2017 to December 2018, 8 partial responses and 6 stable diseases were observed, for a 20% ORR and a 35% DCR. The median DOR was 15.2 months. Patients with PD-L1 expression (≥10%) or high tumor mutational burden (TMB) had better ORR than PD-L1 <10% (50.0% vs. 10.7%, $P = 0.019$) and TMB-low patients (75.0% vs. 16.1%, $P = 0.03$). Three of 8 (37.5%) responders harbored *ARID1A* mutations, whereas only 1 of 27 nonresponders had mutations ($P = 0.03$). Of note, 1 exceptional responder with TMB-L, microsatellite stable (MSS), and PD-L1–negative had multiple genomic arrangements with high prediction score for neoantigens.

Conclusions: Toripalimab had antitumor activity and safety in treating recurrent or metastatic NENs. Patients with positive PD-L1 expression, TMB-H (top 10%), and/or microsatellite instable (MSI-H) might preferentially benefit from the treatment. The genomic mutation of ARID1A and high genomic rearrangements might be correlated with clinical benefit.

Introduction

NENs are a highly heterogeneous group of tumors, with biological behaviors varying significantly from relatively inert to highly aggressive (1). Patients with recurrent or metastatic NENs have limited treatment options and poor prognosis. Platinum-based chemotherapy is the current standard first-line treatment for advanced NECs. After the failure of platinum-based regimen, the median PFS observed from the second-line treatments were less than 3–4 months, and median OS were less than 6 months (2–5). Tyrosine kinase inhibitors (sunitinib) and mTOR inhibitors (everolimus) have been shown to prolong progression-free survival for NETs (6–8), but these agents have a low response rate. Alternative second-line regimens include temozolomide combinations, FOLFIRI and FOLFOX (2, 3), but also with limited clinical efficacy. Unmet medical need for more effective and less toxic therapies remains in the second-line setting for advanced NENs, especially patients with high proliferation index (Ki-67 > 10%).

The programmed death 1 (PD-1) immune checkpoint pathway consists of PD-1, a coinhibitory receptor expressed on activated T-cell and its two ligands PD-L1 and PD-L2 expressed on tumor and immune cells (9). While PD-1 pathway is crucial for the induction and maintenance of peripheral tolerance, upregulation of PD-L1 in the tumor microenvironment leads to suppression of immune response in many solid tumors (10, 11). In recent years, PD-1 pathway blockade by mAbs has become an integral component of disease management for many cancer indications, especially in tumors with high PD-L1 expression and/or high lymphocyte infiltration (9). However, none of these agents have been approved for advanced NENs. Previous clinical studies have shown subgroups of NENs with frequent lymphocyte infiltration (12) and PD-L1 expression (13–15), indicating a therapeutic potential for PD-1 blockade therapy in NENs.

Toripalimab, also known as JS001 or TAB001, is a humanized IgG4κ mAb specific for human PD-1 receptor and blocks interactions of PD-1 with its ligands. Toripalimab contains the complementary-determining regions (CDR) of a murine antibody that binds to human PD-1 and human framework regions. A serine to proline substitution was introduced at amino acid 228 (S228P) to stabilize the IgG4 molecule. Toripalimab has shown an acceptably safety profile and promising clinical activities in patients with advanced solid tumors in several phase I/II studies (16, 17). Toripalimab was first approved to treat second-line metastatic melanoma in China on December 17, 2018.

Here we report the result from an open-label multiple-center single-arm phase Ib study evaluating the safety and efficacy of toripalimab in patients with recurrent or metastatic NENs.

[1]Department of Gastrointestinal Oncology, Key Laboratory of Carcinogenesis and Translational Research (Ministry of Education), Peking University Cancer Hospital and Institute, Beijing, China. [2]Harbin Medical University Cancer Hospital, Harbin, China. [3]Department of Pathology, Key Laboratory of Carcinogenesis and Translational Research (Ministry of Education), Peking University Cancer Hospital and Institute, Beijing, China. [4]OrigiMed, Shanghai, China. [5]Shanghai Junshi Biosciences Co., Ltd, Shanghai, China.

M. Lu and P. Zhang contributed equally to this article.

Clinicaltrials.gov Identifier: NCT03167853.

Corresponding Author: Lin Shen, Peking University Cancer Hospital and Institute, Fu-Cheng Road 52, Hai-Dian District, Beijing 100142, China. Phone: 8610-8819-6561; Fax: 86-10-88196561; E-mail: shenlin@bjmu.edu.cn

Clin Cancer Res 2020;26:2337–45

doi: 10.1158/1078-0432.CCR-19-4000

Translational Relevance

This multiple-center phase Ib study provides evidence for the efficacy and safety of an anti-PD-1 antibody toripalimab in treating recurrent or metastatic NENs with high proliferation index (Ki-67 ≥10%) after failure of first-line treatment. Patients with high PD-L1 expression (≥10%), high TMB (top 10%), and/or MSI-H might preferentially benefit from toripalimab (anti-PD-1) treatment. The genomic mutation of *ARID1A* might be correlated with clinical benefit to toripalimab. We also found one exceptional responder who had multiple genomic arrangements confirmed by messenger RNA expression with high prediction score for neoantigens. In recent years, combination strategy of tyrosine kinase inhibitors (TKI) with PD-1 checkpoint inhibitors has shown promising clinical benefit in patients with various solid tumors. Combination of toripalimab with VEGFR/FGFR inhibitor sulfatinib is currently being explored in metastatic NENs for safety and clinical efficacy (NCT03879057).

Patients and Methods

This study is a phase Ib multiple-center, open-label, clinical trial evaluating the safety and clinical activity of toripalimab in patients with recurrent or metastatic NEN with Ki-67 expression ≥10%, including well-differentiated neuroendocrine tumors and poorly differentiated neuroendocrine carcinomas. The study was approved by Peking University Cancer Hospital institutional review board and was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice. Each patient provided written informed consent. The study is registered with ClinicalTrials.gov (NCT03167853).

Patient eligibility

Eligible patients must be age 18 years or older with pathologically confirmed locally advanced or metastatic nonfunctional neuroendocrine tumor with Ki-67≥10%, which is incurable, and had failed standard therapy. Ki-67 IHC staining was performed, and Ki-67 index was calculated according to WHO 2010 classification in a central lab. Patient must have at least one measurable lesion per Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1 (18) at baseline, with Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1, and adequate organ and bone marrow function. Exclusion criteria included history of autoimmune diseases, diagnosis of immunodeficiency or received systemic steroid therapy within 4 weeks, prior treatment with bone marrow stimulating factors within past 2 weeks, prior mAb anticancer therapy within 4 weeks, and chemotherapy, targeted small-molecule therapy, or radiotherapy within 4 weeks; interstitial lung disease; history of HIV infection, and known active hepatitis B or hepatitis C virus infection; history with tuberculosis; known additional progressing malignancy; and active brain metastases (metastases that were stable for 4 weeks or more before the first dose of toripalimab were permitted), or carcinomatous meningitis or prior immune checkpoint blockade therapies.

Treatment and endpoints

Enrolled patients received 3 mg/kg toripalimab via intravenous infusion once every 2 weeks until confirmed disease progression, intolerable toxicity, withdrawal of patient consent, investigator's decision to discontinue treatment, or 24 months of therapy. Response was assessed every 8 weeks for the first 6 months and every 12 weeks thereafter according to RECIST v1.1 and Immune-related Response Evaluation Criteria in Solid Tumors (irRECIST; ref. 19) by investigator. Patients who initially developed progressive disease per RECISTv1.1 were allowed to continue therapy if the investigator considered patients to be gaining benefit from the treatment per irRECIST. Survival was assessed every 3 months after the discontinuation of a study drug.

Adverse events were assessed and graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events, version 4.0 and monitored throughout and for 30 days after treatment discontinuation (90 days for serious AEs). Immune-related AEs were defined as events with potentially drug-related immunologic causes that were consistent with an immune phenomenon.

The primary endpoint was objective response rate (ORR; defined as the percentage of patients with complete or partial response per RECIST1.1) and safety graded by CTCAE. Secondary endpoints were duration of response (DOR, defined as the time from the first documented response to radiologic progression), progression-free survival (PFS; time from enrollment to first documented PD or death from any cause, whichever occurred first), overall survival (OS, defined as the time from enrollment to death from any cause).

Efficacy and safety were assessed in the as-treated population, which was defined as all patients who received at least one dose of a study drug.

PD-L1 expression analysis in tumor biopsies

Tumor biopsy sample was obtained from each patient before treatment initiation. PD-L1 expression was detected by IHC staining with SP142 antibody. PD-L1 expression was evaluated on tumor cells and interstitial cells by certified pathologists. PD-L1–positive status in this study was defined as the presence of membrane staining of any intensity in ≥1% of tumor cells or interstitial cells (20).

Tumor mutational burden analysis

Whole-exome sequencing (WES) was performed with Sure Select Human All Exon V6 kit (Agilent) on tumor biopsies and matched peripheral blood mononuclear cells (PBMC) samples. Genomic alterations including microsatellite stability status, single base substitution (SNV), short and long insertions/deletions (INDEL), copy number variants (CNV), and gene rearrangement and fusions were assessed. The tumor mutational burden (TMB) was determined by analyzing somatic mutations including coding base substitution and INDELs per mega-base (Mb). Microsatellite stability status was determined by MANTIS with default parameter.

Sample size determination and statistical analysis

At a one-sided significance level of 0.05, a total of 40 patients could provide 80% power to show the efficacy of toripalimab at targeted ORR of 25% versus 10% for alternative second-line therapy using Clopper–Pearson method. A 40-patient enrollment size was thus planned for this study.

Safety and efficacy analyses included all patients who received at least 1 dose of study drug. ORR and its 95% exact confidence interval (CI) were determined by Clopper and Pearson methodology. PFS and OS were plotted using the Kaplan–Meier method, with median and corresponding two-sided 95% CIs reported. Duration of response was analyzed with Kaplan–Meier method with data from all responders.

The data cutoff for analysis was August 30, 2019. For biomarker analysis, PD-L1 ≥ 1% and PD-L1 ≥ 10% were used as cut-off points, while TMB top 90, 75, 50, and 25 percentiles were used as cut-off points for efficacy evaluation. Statistics analyses were performed with SAS version 9.4 or GraphPad Prism software.

Results

Patient population

From April 20, 2017 to December 11, 2018, a total of 53 patients with recurrent or metastatic NENs (Ki-67 ≥ 10%) were screened and 40 were enrolled. Baseline characteristics are summarized in **Table 1**. The median age was 58 years (range: 38–77). The majority were men (62.6%). By pathologic classification, there were 8 well-differentiated neuroendocrine tumors (WD-NET; 5 grade 2 and 3 grade 3) and 32 poorly differentiated neuroendocrine carcinomas (PD-NEC). As for the primary sites, 9 NENs were originated from pancreas and 31 were extrapancreatic NENs, including 10 colorectal, 6 stomach, 4 duodenums, 3 esophageal, and 8 other sites. The majority of patients (67.5%) had liver metastasis. 7 (17.5%) patients had received more than 3 prior lines of systemic treatments.

Treatment-related toxicity

As of August 30, 2019, 8.4 months after the last patient was enrolled, 40 patients had received 1 to 47 doses of toripalimab. 38 of 40 (95.0%) of patients experienced treatment-related adverse events (TRAE). The most frequently observed TRAE (≥20%) included proteinuria, elevated AST, hyperglycemia, elevated ALT, elevated direct bilirubin, elevated lipase, pruritus, elevated creatine kinase, anemia, and fatigue (**Table 2**). There was no treatment-related death. Grade 4 TRAE occurred in 1 (2.5%) patient (elevated ALT) and 10 (25.0%) patients experienced grade 3 TRAEs, including elevated lipase (6), hyperglycemia (3), elevated direct bilirubin (2), elevated AST (1), elevated creatine kinase (1), elevated LDH (1) and thrombocytopenia (1). 22 (55.0%) patients experienced immune-related AEs, including elevated AST (15), elevated ALT (13), elevated DBIL (12), and hypothyroidism (3). Permanent treatment discontinuation due to TRAE occurred in 4 (10%) patients, while 9 (22.5%) patients had treatment delayed due to TRAE.

Table 1. Baseline patient demographics and clinical characteristics.

Characteristics (*n* [%])	WD-NET *N* = 8 *n* (%)	PD-NEC *N* = 32 *n* (%)	Total *N* = 40 *n* (%)
Age, median (range) (y)	57 (47–71)	58 (38–77)	58 (38–77)
Sex			
Male	4 (50.0)	21 (65.6)	25 (62.5)
Female	4 (50.0)	11 (34.4)	15 (37.5)
Primary sites			
Pancreatic	4 (50.0)	5 (15.6)	9 (22.5)
Gastrointestinal	3 (37.5)	20 (62.5)	23 (57.5)
Others	1 (12.5)	7 (21.9)	8 (20.0)
Liver metastasis	6 (75.0)	21 (65.6)	27 (67.5)
PD-L1 expression			
≥1%	3 (37.5)	11 (34.4)	14 (35.0)
<1%	4 (50.0)	20 (62.5)	24 (60.0)
≥10%	3 (37.5)	7 (21.9)	10 (25.0)
<10%	4 (50.0)	24 (75.0)	28 (70.0)
Unknown	1 (12.5)	1 (3.1)	2 (5.0)
Prior lines of therapy			
1	4(50.0)	22 (68.8)	26 (65.0)
2	1 (12.5)	6 (18.7)	7 (17.5)
≥3	3 (37.5)	4 (12.5)	7 (17.5)

Note: PD-L1 positive status was defined as the presence of membrane staining of any intensity in ≥1% of tumor cells or interstitial cells by SP142 IHC staining.

Antitumor activity

By August 30, 2019, 29 (72.5%) patients deceased, 7 (17.5%) discontinued treatment due to disease progression, 1 withdrew consent, and 3 patients remained on study. The median treatment duration was 1.4 months (range 0.5 to 21.4 months). Among all 40 patients assessed by investigator by RECIST v1.1, 8 partial responses (PR) and 6 stable diseases (SD) were observed (**Fig. 1**). The ORR was 20% (95% CI, 9.1–35.7) and the DCR was 35% (95% CI, 20.6–51.7). Using the irRECIST criteria, two additional PRs were identified, resulting in a 25.0% ORR (95% CI, 12.7–41.2) and a 40% DCR (95% CI, 24.9–56.7). The median DOR was 15.2 months per RECIST v1.1 with 4 of 8 patients having ongoing responses by the cut-off date. The median PFS was 2.5 months (95% CI, 1.9–3.1) per RECIST v1.1 and the median OS was 7.8 months (95% CI, 5.0–10.8).

The ORR was 18.7% in the PD-NEC subgroup and 25.0% in the WD-NET subgroup. On the basis of the tissue origins, the ORR were 13.0%, 22.2%, and 37.5% for expancreatic GI-derived, pancreatic NENs and nondigestive NENs, respectively, per RECIST v1.1.

PD-L1 expression in tumor

Tumor PD-L1 expression results were obtained from 38 patients by SP142 IHC staining and 14 (36.8%) patients were identified as PD-L1$^+$ (≥1%). Among PD-L1$^+$ patients, 10 (26.3%) were identified as PD-L1 high expression (≥10%). PD-L1$^+$ patients responded significantly better to toripalimab treatment than PD-L1$^-$ patients in ORR (42.9% vs. 8.3%, unadjusted $P = 0.034$). PD-L1$^+$ patients also had numerically better median OS than PD-L1$^-$ patients, 9.1 vs. 7.2 months, but the difference was not statistically significant. Among PD-L1≥10% patients, the difference in ORR was more pronounced (50.0% vs. 10.7%, unadjusted $P = 0.019$; **Fig. 2A**). PD-L1 ≥ 10% patients had numerically better PFS and OS than PD-L1<10% patients, [median PFS 3.8 vs. 2.2 months, HR = 0.50 (95% CI, 0.24–1.06), $P = 0.07$; median OS: 9.1 vs. 7.2 months, HR = 0.55 (95% CI, 0.24–1.23), $P = 0.15$; **Fig. 2B** and **C**].

TMB analysis

Whole-exome sequencing was performed on both tumor biopsies and paired PBMCs. TMB was determined in 35 patients by analyzing somatic mutations within the coding region of the human genome. The TMB value was generally low in the cohort with median TMB at 2.4 mutations (muts) per million base pairs (Mb). Only one patient had TMB over 20 muts/Mb, who had a partial response. Two additional patients with TMB more than 10 muts/Mb who experienced a PR and a SD, respectively. A cut-off value of the top 10% of the TMB in this study (9.9 muts/Mb) was selected as the TMB high threshold as suggested by Samstein and colleagues (21). Patients with TMB ≥ 9.9 mutations/Mb ($n = 4$) had responded significantly better than patients with TMB fewer than 9.9 mutations/Mb ($n = 34$; ORR 75.0% vs. 16.1%, unadjusted $P = 0.03$; DCR 100% vs. 29.0%, unadjusted $P = 0.014$; **Fig. 2A**). Notably, TMB-high patients also had significant survival advantage in PFS than TMB-low patients [HR = 0.35 (95% CI, 0.15–0.84), $P = 0.019$ (**Fig. 2D**)]. Two of four TMB-high patients were also PD-L1 ≥ 10% and both experienced durable ongoing partial responses at 15.1 and

Table 2. Treatment-related adverse events (TRAE).

N (%)	All	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5
All TRAEs	38 (95.0)	12 (30.0)	15 (37.5)	10 (25.0)	1 (2.5)	0
Proteinuria	16 (40.0)	14 (35.0)	2 (5.0)	0	0	0
Elevated AST	15 (37.5)	12 (30.0)	2 (5.0)	1 (2.5)	0	0
Hyperglycemia	14 (35.0)	6 (15.0)	5 (12.5)	3 (7.5)	0	0
Elevated ALT	13 (32.5)	10 (25.0)	2 (5.0)	0	1 (2.5)	0
Elevated direct bilirubin	12 (30.0)	9 (22.5)	1 (2.5)	2 (5.0)	0	0
Elevated lipase	11 (27.5)	4 (10.0)	1 (2.5)	6 (15.0)	0	0
Pruritus	10 (25.0)	10 (25.0)	0	0	0	0
Elevated creatine kinase	10 (25.0)	8 (20.0)	1 (2.5)	1 (2.5)	0	0
Anemia	9 (22.5)	7 (17.5)	2 (5.0)	0	0	0
Fatigue	8 (20.0)	8 (20.0)	0	0	0	0
Elevated amylase	7 (17.5)	4 (10.0)	3 (7.5)	0	0	0
Rash	7 (17.5)	7 (17.5)	0	0	0	0
Nausea	6 (15.0)	6 (15.0)	0	0	0	0
Leukopenia	6 (15.0)	5 (12.5)	1 (2.5)	0	0	0
Hyponatremia	6 (15.0)	6 (15.0)	0	0	0	0
Fever	5 (12.5)	4 (10.0)	1 (2.5)	0	0	0
Hematuria	5 (12.5)	5 (12.5)	0	0	0	0
Diarrhea	5 (12.5)	4 (10.0)	1 (2.5)	0	0	0
Elevated blood uric acid	5 (12.5)	5 (12.5)	0	0	0	0
Neutropenia	4 (10.0)	4 (10.0)	0	0	0	0
Hearing loss	4 (10.0)	4 (10.0)	0	0	0	0
Elevated white blood cells	4 (10.0)	4 (10.0)	0	0	0	0
Hypochloremia	4 (10.0)	4 (10.0)	0	0	0	0
Elevated total bilirubin	4 (10.0)	3 (7.5)	1 (2.5)	0	0	0
Anorexia	4 (10.0)	4 (10.0)	0	0	0	0
Immune-related AE(irAE)	22 (55.0)	16 (40.0)	4 (10.0)	1 (2.5)	1 (2.5)	0
Elevated AST	15 (37.5)	12 (30.0)	2 (5.0)	1 (2.5)	0	0
Elevated ALT	13 (32.5)	10 (25.0)	2 (5.0)	0	1 (2.5)	0
Elevated direct bilirubin	12 (30.0)	9 (22.5)	1 (2.5)	2 (5.0)	0	0
Hypothyroidism	3 (7.5)	1 (2.5)	2 (5.0)	0	0	0

Note: Adverse events were graded according to National Cancer Institute Common Terminology Criteria (CTCAE) version 4.0. The most common TRAEs (≥10%) are listed.
Abbreviations: AST, aspartate aminotransferase; ALT, alanine aminotransferase.

21.9 months, respectively. TMB-high patients also had numerically better median OS than TMB low patients [not reached vs. 7.5 months; HR = 0.38 (95% CI, 0.13–1.12), $P = 0.08$ (**Fig. 2E**)].

Whole-exome sequencing and analysis

Valid whole-exome sequencing results were obtained from 35 patients. The most frequent genomic alterations included *TP53* (69%), *RB1* (37%), *CTNNB1* (20%), *CDKN2A* (14%), *KMT2D* (14%), *ARID1A* (11%), *CIC* (11%), and *TERT* (11%; **Fig. 3A**). NENs with different organ origins displayed distinctive signatures of genomic alterations. For instance, mutations of *MEN1* (37.5%) and *DAXX* (25%) were found in pancreatic NEN exclusively while mutations of *SMAD4* (15.8%), *PTEN* (15.8%), and *TERT* amplification (15.8%) were enriched in gastrointestinal NEN (**Fig. 3A**). The genomic mutation of *ARID1A* correlated with clinical benefit as 3/8 (37.5%) responders harbored *ARID1A* mutations while only 1 of 27 nonresponders were mutated ($P = 0.03$; **Fig. 3B**). Two patients were identified to be microsatellite instable (MSI-H) with TMB value at 25.2 and 8.3 muts/Mb, who experienced a PR and a progressive disease respectively. Interestingly, both MSI-H patients harbored *ARID1A* mutations.

Notably, one durable responder (PFS 15.8+ months) who was PD-L1 negative and with low mutational burden (1.0 muts/Mb) had multiple genomic arrangements confirmed by messenger RNA expression with high prediction score for neoantigens, which also correlated with high immune cell infiltrates in the tumor.

Other biomarkers and subgroup analysis

Additional biomarkers or subgroups analyzed for correlation with clinical efficacy included age, gender, baseline LDH level, prior line of treatment, and liver metastasis. Among the subgroups, female, LDH at normal range, and no liver metastasis had numerically better ORR than male, LDH above upper limit of normal and with liver metastasis. However, the differences were not statistically significant.

Discussion

Advanced neuroendocrine neoplasms have poor prognosis and limited therapeutic options after failure to first-line chemotherapy. Neuroendocrine tumors are recognized as relatively slow progressing tumors, and the efficiency of targeted drugs and somatostatin analogue is low. NEC resembles small-cell lung cancer (SCLC) in pathologic and biological features, such as rapid growth and relatively high sensitivity to platinum-based chemotherapy. Etoposide associated with platinum compounds such as cisplatin or carboplatin, is the current standard first-line treatment for both NEC and SCLC. However, unlike SCLC, there are no established effective options at second-line and later setting for NEC, thus those patients progressed rapidly with poor prognosis.

Regulatory approvals of checkpoint blockade therapy in recent years have revolutionize the disease management of solid tumors at

Table 3. Clinical activity of toripalimab in patients with advanced NENs per RECIST v1.1 and irRECIST criteria.

	PD-NEC ($n = 32$)	WD-NET ($n = 8$)	PD-L1 ≥1% ($n = 14$)	PD-L1 <1% ($n = 24$)	PD-L1 ≥10% ($n = 10$)	PD-L1 <10% ($n = 28$)	Pancreatic ($n = 9$)	Ex-pancreatic Gastrointestinal ($n = 23$)	Non-GI ($n = 8$)
RECIST									
PR	6	2	6	2	5	3	2	3	3
SD	3	3	0	5	0	5	3	2	1
PD	21	3	6	15	5	18	4	16	4
NE	2	0	0	2	0	2	0	2	0
ORR	18.7%	25.0%	42.9%	8.3%	50.0%	10.7%	22.2%	13.0%	37.5%
DCR	28.1%	62.5%	42.9%	29.2%	50.0%	28.6%	55.5%	21.7%	50.0%
irRECIST									
PR	8	2	7	3	5	5	2	4	4
SD	3	3	0	5	0	5	3	2	1
PD	19	3	7	14	5	16	4	15	3
NE	2	0	0	2	0	2	0	2	0
ORR	25.0%	25.0%	50.0%	12.5%	50.0%	17.9%	22.2%	17.4%	50.0%
DCR	34.4%	62.5%	50.0%	33.3%	50.0%	35.7%	55.5%	26.1%	62.5%

Note: PD-L1–positive status was defined as the presence of membrane staining of any intensity in ≥1% of tumor cells or interstitial cells by SP142 IHC staining.
Abbreviations: DCR, disease control rate; DCR, CR+PR+SD/total patients treated; ORR, CR+PR/total patients treated; NE, not evaluable, two patients with no posttreatment assessment; ORR, objective response rate; PR, partial response; SD, stable disease; PD, progressive disease.

different stage settings, including nivolumab (anti-PD-1) monotherapy for third-line SCLC (22) and atezolizumab (anti-PD-L1) in combination with chemotherapy for first-line SCLC (23).However, reports of clinical application of checkpoint blockade in recurrent or metastatic NEN are rare and of limited clinical efficacy. In the pancreatic neuroendocrine tumor (pNET) and carcinoid cohorts of the KEYNOTE-028 study, objective responses were observed in 6% (1/16) pNET patients and in 12% (3/25) carcinoid patients (24). Further clinical evaluation and biomarker analysis of checkpoint blockade therapy in the highly heterogeneous NENs are thus warranted.

Here we report the preliminary results of safety and efficacy of toripalimab in chemo-refractory advanced metastatic neuroendocrine neoplasms with Ki-67 index over 10%. Toripalimab demonstrated a manageable safety profile and durable antitumor activity with PD-L1 ≥ 10% and TMB-high patients preferentially benefiting from the treatment. The results showed that toripalimab had a much longer DOR than existing therapies, suggesting it is of value to identify the potential beneficial patients.

Among 40 patients enrolled, PD-NEC and WD-NET subgroups had similar response rates (ORR: 18.7% vs. 25.0%) while toripalimab appeared to be more efficacious treating pancreatic NENs (22.2% ORR) and nondigestive NENs (37.5% ORR) than GI-NENs (13.0% ORR), although the differences were not statistically significant. Consistently, subgroups of NENs, especially pNET are known to have frequent lymphocyte infiltration and positive PD-1 ligands expression (12). The PD-L1 expression of neuroendocrine tumors was reported as 8.3% (16/193), and that of the SCLC was 13.3% (10/75) by SP142 IHC staining (25). We evaluated the association of tumor PD-L1 expression with the clinical efficacy. PD-L1–positive patients responded significantly better than PD-L1–negative patients, ORR 42.9% vs. 8.3%, $P = 0.034$. This result is consistent with previous reports of PD-1 checkpoint blockade therapies for the treatment of SCLC, an indication shares pathologic and biological features with neuroendocrine neoplasms. Pembrolizumab observed a 33.3% ORR in patients with PD-L1^{+} SCLC, while nivolumab monotherapy had a 10% ORR in PD-L1 unselective SCLC (26).

The presence of MSI in the tumor genome had been associated with robust immune cell infiltrates and high response rate to checkpoint blockade therapy in a broad range of human malignancies (27).The FDA has granted accelerated approval to pembrolizumab and nivolumab as treatment options for patients with MSI-H or dMMR cancer regardless of tumor tissue origin. However, the prevalence of MSI in NENs is relatively low (∼3.6%; ref. 27). In our study, 2 patients were identified to be microsatellite instable (MSI-H) and they experienced a PR and a progressive disease, respectively.

TMB, which correlates with the presence of neoepitopes for T-cell recognition, has also been correlated with favorable responses to PD-1 pathway blockade monotherapies in solid tumors, including NSCLC (28) and advanced gastric cancer (29). The TMB value in our cohort is generally low with median TMB at 2.4 mutations per million base pairs. As TMB value varies greatly among different indications, it is necessary to explore the cut-off value for the TMB in each tumor type. Samstein and colleagues had suggested to use a top 10% or 20% of the TMB value of a certain indication as the TMB cutoff (21). In this study, when TMB was stratified into increasing percentiles, rates of responder improved with increasing TMB. Significantly higher objective response rate was only observed in those with TMB above the 90th percentile of 9.9 mutations/Mb (ORR, 75.0% vs. 16.1%, $P = 0.03$). Furthermore, TMB over 9.9 Muts/Mb patients also had significant better PFS than TMB low patients, median PFS 15.4 vs. 2.1 months (HR = 0.35 (95% CI, 0.15–0.84), $P = 0.019$; (**Fig. 2D**)].

In this study, PD-L1–positive expression in tumor and TMB high associated with favorable clinical response, as 6 of 8 responders were either PD-L1 positive or TMB above 9.9 Mutations/Mb (**Fig. 1A**). Notably, 2 of 4 TMB-high patients were also PD-L1≥10% and both experienced durable ongoing partial responses at 15.1 and 21.9 months, respectively. One partial responder with low TMB and lack of PD-L1 expression had a max reduction of 78.8% in target lesions and durable ongoing responses over 15 months. WES analysis revealed that this patient with particularly high genomic rearrangements. To determine whether these gene rearrangements created neoantigens, whole transcriptomic analysis was performed on the formalin-fixed paraffin-embedded tumor

tissue. RNA-seq demonstrated abundant rearranged transcripts with multiple high affinity neoantigens predicted by NetMHC software. Consistently, high immune cell infiltrations were found in the tumor biopsy from this patient that might contribute to the exceptional response to toripalimab.

In summary, this phase Ib study provides evidence for the efficacy of the toripalimab treating recurrent or metastatic NENs. The safety profile was manageable and comparable with other marketed PD-1 immune-checkpoint inhibitors. Of particular note, patients with PD-L1–positive expression, high TMB (top 10%) and/or MSI-H might

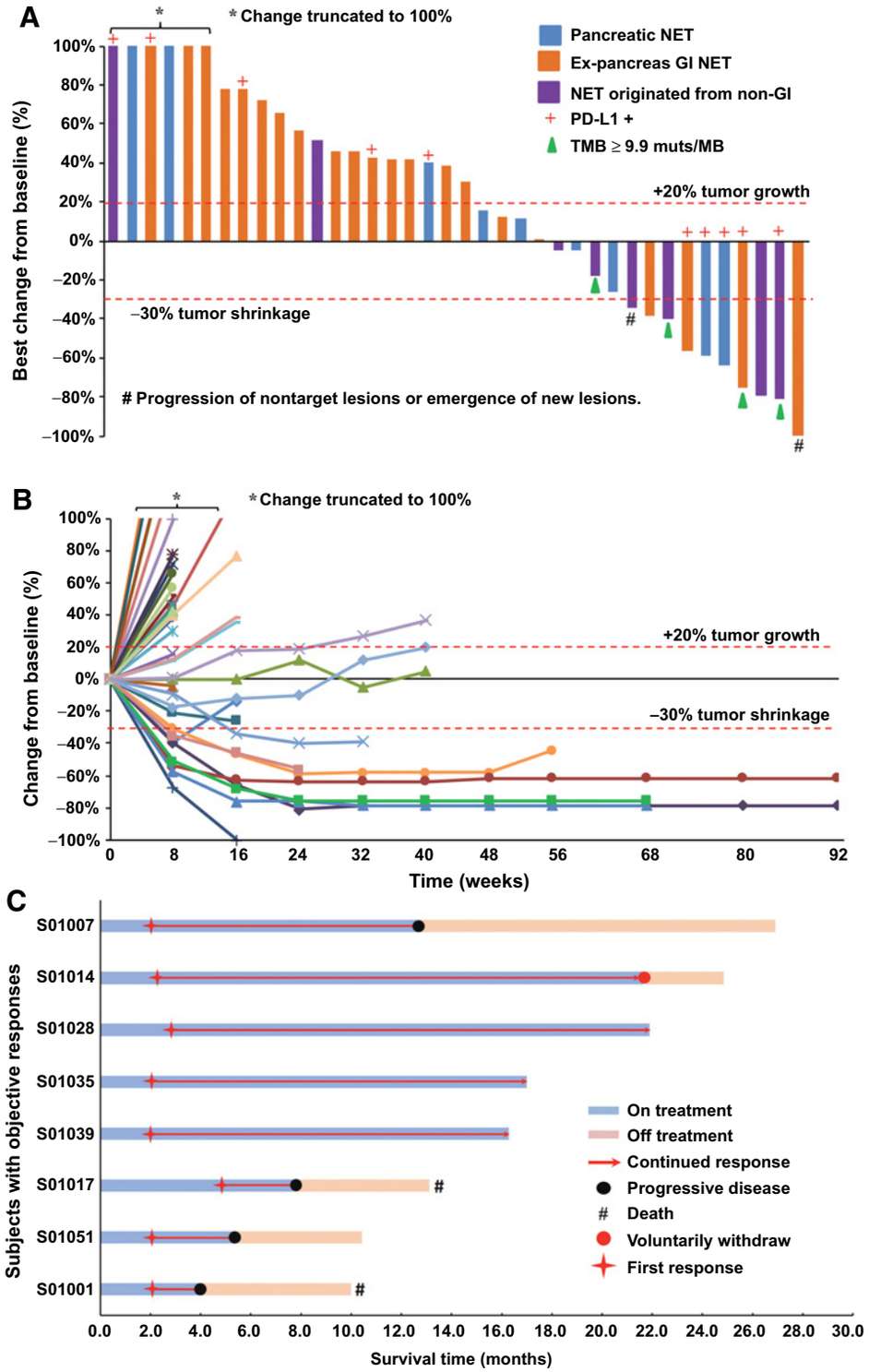

Figure 1.

A, Maximal change of tumor size from baseline assessed by investigator per RECIST v1.1 from patients with baseline and at least one posttreatment radiographic evaluation ($n = 38$). The length of the bar represents maximal decrease or minimal increase in target lesion(s). #, two patients with target lesion(s) reduced more than 30% but with new lesion(s) or progression of nontarget lesion(s) were classified as PD per RECIST v1.1, but both were classified as PR per irRECIST. Patients with tumor biopsy PD-L1 IHC positive are marked by red "+" on the top. Patients with TMB ≥ 9.9 muts/Mb are marked by green triangles at the bottom. **B,** Change of individual tumor burden over time from baseline, assessed by investigator per RECIST v1.1 ($n = 38$). **C,** Events and duration of response for patients with objective response per RECIST v1.1 ($n = 8$).

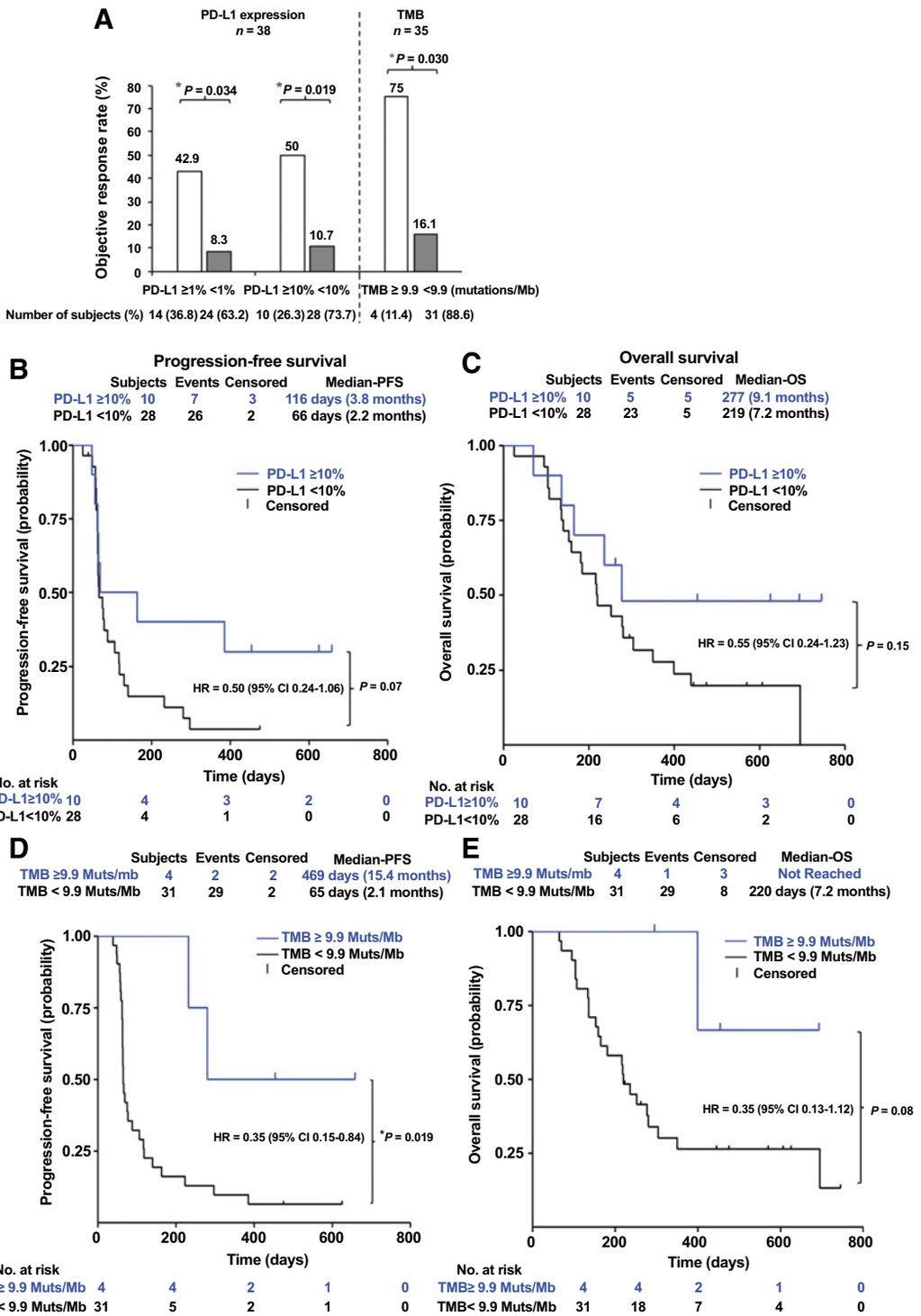

Figure 2.

Clinical response in relation to tumor PD-L1 expression and TMB. **A,** PD-L1–positive status was defined as the presence of membrane staining of any intensity in ≥1% of tumor cells or interstitial cells by SP142 IHC staining. The TMB was calculated by total somatic mutations within the coding regions by whole-exome sequencing. Top 10% TMB value of 9.9 mutations per Mb was selected as a cutoff. **B,** PFS per RECIST v1.1 of PD-L1≥10% vs. PD-L1<10% patients. **C,** Overall survival of PD-L1≥10% vs. PD-L1<10% patients. **D,** Progression-free survival per RECIST v1.1 of TMB ≥ 9.9 Muts/Mb vs. TMB <9.9 Muts/Mb patients. **E,** Overall survival of TMB≥9.9 Muts/Mb vs. TMB <9.9 Muts/Mb patients. Percentages of survival patients are shown at indicated time points. Censored patients are marked with "|" in the graph. Numbers of patients at risk at indicated time points are shown below the *x*-axis. NR, not reached.

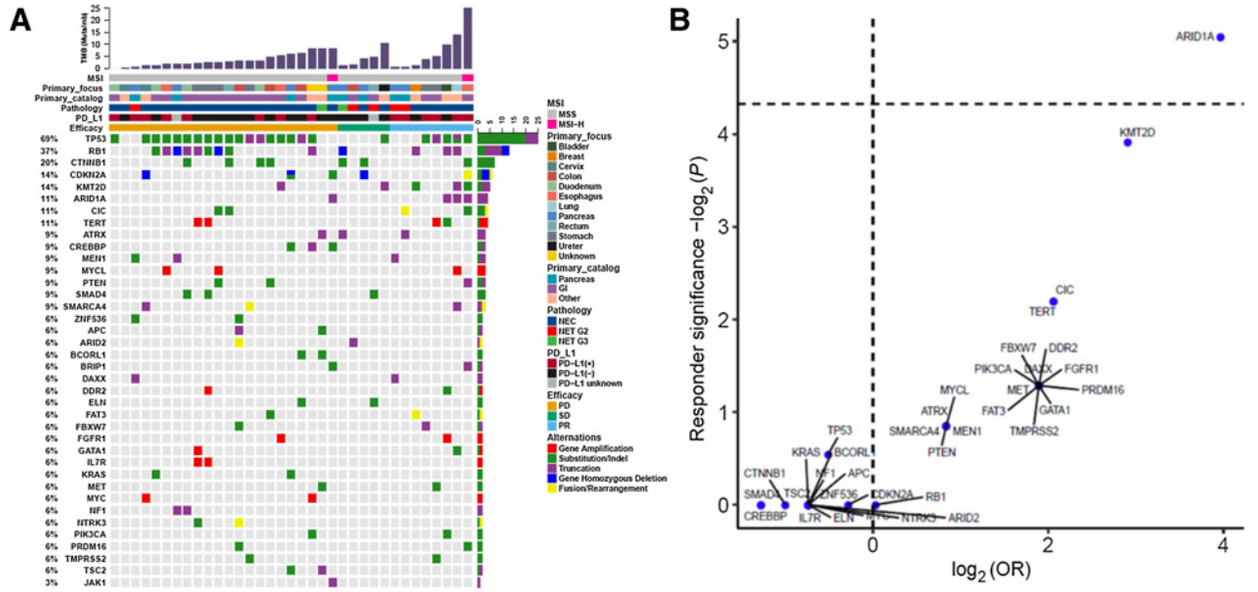

Figure 3.

Genetic alterations and frequencies identified by whole-exome sequencing (WES) from 35 available patients. **A,** Patients were grouped by PD-L1 expression status, tumor primary origin, and clinical responses. **B,** Correlation of genetic alterations with objective clinical response. PD, progressive disease; PR, partial response; SD, stable disease.

preferentially benefit from the treatment. The genomic mutation of ARID1A and high genomic rearrangements might be correlated with clinical benefit to toripalimab. In recent years, combination strategy of tyrosine kinase inhibitors (TKI) with PD-1 checkpoint inhibitors have shown promising clinical benefit in patients with various solid tumors, including metastatic renal cell carcinoma (30), endometrial cancer (31), and mucosal melanoma (32). Combination of toripalimab with VEGFR/FGFR small-molecule inhibitor sulfatinib, is currently being explored in metastatic NENs for safety and clinical efficacy (NCT03879057).

Disclosure of Potential Conflicts of Interest

W. Wang is an employee of OrigiMed. H. Feng is an employee of and holds ownership interest (including patents) in Shanghai Junshi Biosciences Co., LTD. S. Yao is an employee of TopAlliance Biosciences, Inc. No potential conflicts of interest were disclosed by the other authors.

Authors' Contributions

Conception and design: M. Lu, P. Zhang, L. Shen

Development of methodology: M. Lu, H. Wu, L. Shen

Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): M. Lu, Y. Zhang, Z. Li, J. Gong, Jian Li, Y. Li, X. Zhang, Z. Lu, X. Wang, J. Zhou, Z. Peng, L. Shen

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): M. Lu, P. Zhang, Y. Zhang, J. Gong, Jie Li, Z. Peng, W. Wang, S. Yao

Writing, review, and/or revision of the manuscript: M. Lu, P. Zhang, H. Feng, S. Yao, L. Shen

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): M. Lu, P. Zhang, Y. Zhang, Z. Li, Jie Li, Jian Li, Y. Li, Z. Lu, J. Zhou, H. Feng, S. Yao

Study supervision: M. Lu, L. Shen

Acknowledgments

The study was supported by Shanghai Junshi Biosciences Co., LTD, Shanghai, China, the National Key Research and Development Program of China (no. 2017YFC1308900, 2017YFC0908400), also supported by National Major Science & Technology Major Project (no. 2015ZX09102017, 2017ZX09302009). The authors thank the patients who participated in this study and their families.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received December 10, 2019; revised January 12, 2020; accepted February 17, 2020; published first February 21, 2020.

References

1. Niederle MB, Hackl M, Kaserer K, Niederle B. Gastroenteropancreatic neuroendocrine tumours: the current incidence and staging based on the WHO and European Neuroendocrine Tumour Society classification: an analysis based on prospectively collected parameters. Endocr Relat Cancer 2010;17:909–18.
2. Hentic O, Hammel P, Couvelard A, Rebours V, Zappa M, Palazzo M, et al. FOLFIRI regimen: an effective second-line chemotherapy after failure of etoposide–platinum combination in patients with neuroendocrine carcinomas grade 3. Endocr Relat Cancer 2012;19:751–7.
3. Hadoux J, Malka D, Planchard D, Scoazec J, Caramella C, Guigay J, et al. Post-first-line FOLFOX chemotherapy for grade 3 neuroendocrine carcinoma. Endocr Relat Cancer 2015;22:289–98.
4. Olsen IH, Sørensen JB, Federspiel B, Kjaer A, Hansen CP, Knigge U, et al. Temozolomide as second or third line treatment of patients with neuroendocrine carcinomas. ScientificWorldJournal 2012;2012:170496.
5. Sorbye H, Strosberg J, Baudin E, Klimstra DS, Yao JC. Gastroenteropancreatic highgrade neuroendocrine carcinoma. Cancer 2014;120:2814–23.

6. Raymond E, Dahan L, Raoul JL, Bang YJ, Borbath I, Lombard-Bohas C, et al. Sunitinib malate for the treatment of pancreatic neuroendocrine tumors. N Engl J Med 2011;364:501–13.
7. Yao JC, Shah MH, Ito T, Bohas CL, Wolin EM, Van Cutsem E, et al. Everolimus for advanced pancreatic neuroendocrine tumors. N Engl J Med 2011;364:514–23.
8. Yao JC, Fazio N, Singh S, Buzzoni R, Carnaghi C, Wolin E, et al. Everolimus for the treatment of advanced, non-functional neuroendocrine tumours of the lung or gastrointestinal tract (RADIANT-4): a randomised, placebo-controlled, phase 3 study. Lancet 2016;387:968–77.
9. Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. Nat Rev Cancer 2012;12:252–64.
10. Blank C, Brown I, Peterson AC, Spiotto M, Iwai Y, Honjo T, et al. PD-L1/B7H-1 inhibits the effector phase of tumor rejection by T cell receptor (TCR) transgenic CD8+ T cells. Cancer Res 2004;64:1140–5.
11. Okazaki T, Honjo T. PD-1 and PD-1 ligands: from discovery to clinical application. Int Immunol 2007;19:813–24.
12. da Silva A, Bowden M, Zhang S, Masugi Y, Thorner AR, Herbert ZT, et al. Characterization of the neuroendocrine tumor immune microenvironment. Pancreas 2018;47:1123–9.
13. Kim ST, Ha SY, Lee S, Ahn S, Lee J, Park SH, et al. The impact of PD-L1 expression in patients with metastatic GEP-NETs. J Cancer 2016;7:484–9.
14. Cavalcanti E, Armentano R, Valentini AM, Chieppa M, Caruso ML. Role of PD-L1 expression as a biomarker for GEP neuroendocrine neoplasm grading. Cell Death Dis 2017;8:e3004.
15. Ferrata M, Schad A, Zimmer S, Musholt TJ, Bahr K, Kuenzel J, et al. PD-L1 expression and immune cell infiltration in gastroenteropancreatic (GEP) and non-GEP neuroendocrine neoplasms with high proliferative activity. Front Oncol 2019;9:343.
16. Tang B, Yan X, Sheng X, Si L, Cui C, Kong Y, et al. Safety and clinical activity with an anti-PD-1 antibody JS001 in advanced melanoma or urologic cancer patients. J Hematol Oncol 2019;12:7.
17. Chi Z, Tang B, Sheng X, Si L, Cui C, Kong Y, et al. A phase II study of JS001, a humanized PD-1 mAb, in patients with advanced melanoma in China. J Clin Oncol 2018;36:9539
18. Watanabe H, Okada M, Kaji Y, Satouchi M, Sato Y, Yamabe Y, et al. [New response evaluation criteria in solid tumours-revised RECIST guideline (version 1.1)]. Gan To Kagaku Ryoho 2009;36:2495–501.
19. Wolchok JD, Hoos A, O'Day S, Weber JS, Hamid O, Lebbe C, et al. Guidelines for the evaluation of immune therapy activity in solid tumors: immune-related response criteria. Clin Cancer Res 2009;15:7412–20.
20. Thompson ED, Zahurak M, Murphy A, Cornish T, Cuka N, Abdelfatah E, et al. Patterns of PD-L1 expression and CD8 T cell infiltration in gastric adenocarcinomas and associated immune stroma. Gut 2017;66:794–801.
21. Samstein RM, Lee CH, Shoushtari AN, Hellmann MD, Shen R, Janjigian YY, et al. Tumor mutational load predicts survival after immunotherapy across multiple cancer types. Nat Genet 2019;51:202–6.
22. Ready N, Farago AF, de Braud F, Atmaca A, Hellmann MD, Schneider JG, et al. Third-line nivolumab monotherapy in recurrent SCLC: CheckMate 032. J Thorac Oncol 2019;14:237–44.
23. Horn L, Mansfield AS, Szczesna A, Havel L, Krzakowski M, Hochmair MJ, et al. First-line atezolizumab plus chemotherapy in extensive-stage small-cell lung cancer. N Engl J Med 2018;379:2220–9.
24. Mehnert JM, Rugo HS, O'Neil BH, Santoro A, Schellens JHM, Cohen RB, et al. 4270Pembrolizumab for patients with PD-L1–positive advanced carcinoid or pancreatic neuroendocrine tumors: results from the KEYNOTE-028 study. Ann Oncol 2017;28(suppl_5):v142-57.
25. Vanderwalde A, Spetzler D, Xiao N, Gatalica Z, Marshall J. Microsatellite instability status determined by nextgeneration sequencing and compared with PDL1 and tumor mutational burden in 11,348 patients. Cancer Med 2018;7:746–56.
26. Ott PA, Elez E, Hiret S, Kim DW, Morosky A, Saraf S, et al. Pembrolizumab in patients with extensive-stage small-cell lung cancer: results from the phase Ib KEYNOTE-028 study. J Clin Oncol 2017;35:3823–9.
27. Le DT, Durham JN, Smith KN, Wang H, Bartlett BR, Aulakh LK, et al. Mismatch repair deficiency predicts response of solid tumors to PD-1 blockade. Science 2017;357:409–13.
28. Goodman AM, Kato S, Bazhenova L, Patel SP, Frampton GM, Miller V, et al. Tumor mutational burden as an independent predictor of response to immunotherapy in diverse cancers. Mol Cancer Ther 2017;16:2598–608.
29. Wang F, Wei XL, Wang FH, Xu N, Shen L, Dai GH, et al. Safety, efficacy and tumor mutational burden as a biomarker of overall survival benefit in chemo-refractory gastric cancer treated with toripalimab, a PD1 antibody in phase Ib/II clinical trial NCT02915432. Ann Oncol 2019;30:1479–86.
30. Atkins MB, Plimack ER, Puzanov I, Fishman MN, McDermott DF, Cho DC, et al. Axitinib in combination with pembrolizumab in patients with advanced renal cell cancer: a non-randomised, open-label, dose-finding, and dose-expansion phase 1b trial. Lancet Oncol 2018;19:405–15.
31. Makker V, Rasco D, Vogelzang NJ, Brose MS, Cohn AL, Mier J, et al. Lenvatinib plus pembrolizumab in patients with advanced endometrial cancer: an interim analysis of a multicentre, open-label, single-arm, phase 2 trial. Lancet Oncol 2019;20:711–8.
32. Sheng X, Yan X, Chi Z, Si L, Cui C, Tang B, et al. Axitinib in combination with toripalimab, a humanized immunoglobulin G4 monoclonal antibody against programmed cell death-1, in patients with metastatic mucosal melanoma: an open-label phase IB trial. J Clin Oncol 2019;37:2987–99.