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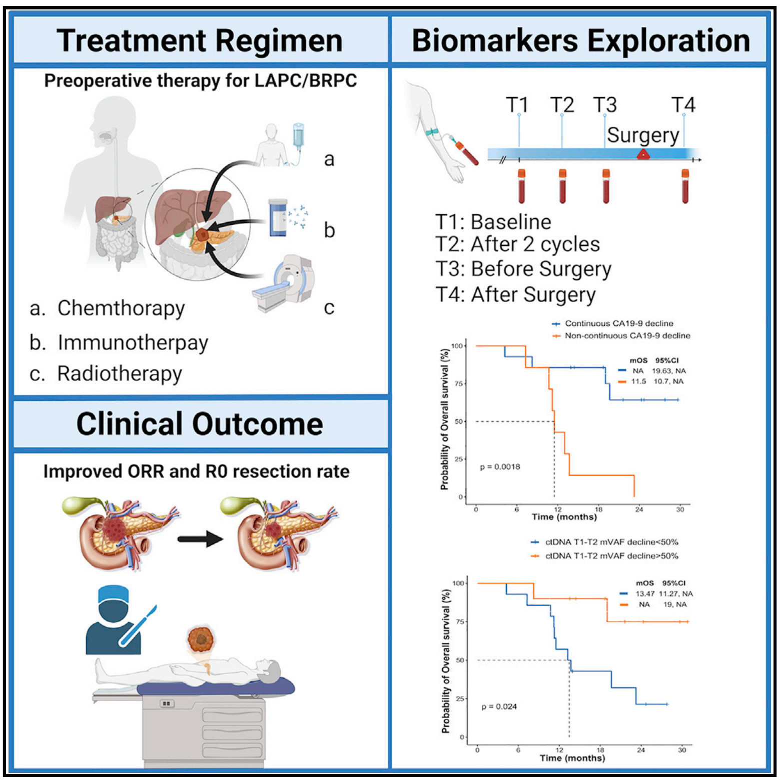
PD-1 blockade plus chemoradiotherapy as preoperative therapy for patients with BRPC/LAPC: A biomolecular exploratory, phase II trial

Graphical abstract



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In brief

Du et al. show the efficacy, safety, and predictive biomarkers of PD-1 blockade plus chemotherapy followed by concurrent SBRT with SIB as preoperative therapy for LAPC and BRPC. They report that ctDNA dynamic changes or continuous CA19-9 decline may predict tumor response and survival outcomes.

Highlights

- PD-1 blockade plus chemoradiotherapy shows promising antitumor activity for LAPC/BRPC
- PD-1 blockade plus chemoradiotherapy raises ORR and R0 resection rates for LAPC/BRPC
- Patients with a >50% ctDNA decline in maxVAF have a better survival outcome

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Article

PD-1 blockade plus chemoradiotherapy as preoperative therapy for patients with BRPC/LAPC: A biomolecular exploratory, phase II trial

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SUMMARY

This is a phase II study of PD-1 blockade plus chemoradiotherapy as preoperative therapy for patients with locally advanced or borderline resectable pancreatic cancer (LAPC or BRPC, respectively). Twenty-nine patients are enrolled in the study. The objective response rate (ORR) is 60%, and the R0 resection rate is 90% (9/10). The 12-month progression-free survival (PFS) rate and 12-month overall survival (OS) rate are 64% and 72%, respectively. Grade 3 or higher adverse events are anemia (8%), thrombocytopenia (8%), and jaundice (8%). Circulating tumor DNA analysis reveals that patients with a >50% decline in maximal somatic variant allelic frequency (maxVAF) between the first clinical evaluation and baseline have a longer survival outcome and a higher response rate and surgical rate than those who are not. PD-1 blockade plus chemoradiotherapy as preoperative therapy displays promising antitumor activity, and multiomics potential predictive biomarkers are identified and warrant further verification.

INTRODUCTION

Pancreatic ductal adenocarcinoma (PDAC) is a lethal malignant tumor with an overall 5-year survival rate of <10%.[1] Research indicates that PDAC may become the second leading cause of cancer-related death by 2030.[2] As PDAC is usually occult in onset, its diagnosis is difficult; it is resectable in only 20% of cases.[3] Even after surgery, the 5-year survival is low, and the recurrence rate is high, which are associated with surgical margin status and postoperative pathological stage.[4] Several clinical trials have demonstrated that adjuvant therapy can prolong the survival of patients after resection.[5,6] Meanwhile, R0 resection still appears relevant prognostic after pretreatment, and various studies encourage neoadjuvant and induction therapy that may increase the R0 resection rate and further improve prognosis.[7,8] The phase II LAPACT clinical trial demonstrated the efficacy and safety of gemcitabine plus nab-paclitaxel (AG) as induction therapy to enable locally advanced pancreatic cancer (LAPC) to be surgically resectable.[9] Compared with immediate surgery, neoadjuvant AG for borderline resectable pancreatic cancer (BRPC) significantly prolonged survival,[10] and a systematic review and meta-analysis concluded that neoadjuvant chemotherapy with AG was safe and effective in patients with BRPC and LAPC.[11]

Abundant chemoradiotherapy regimens have been explored in the hope of improving survival. A phase II study observed that patients with LAPC treated with stereotactic body radiotherapy (SBRT) followed by FOLFIRINOX had an unexpectedly high resectability rate compared with that in the non-SBRT group.[12] In the PREOPANC trial, compared with those in the immediate surgery group, neoadjuvant chemoradiotherapy improved the disease-free survival (DFS) and R0 resection rate of patients with BRPC.[8] Furthermore, intraoperative radiotherapy (IORT) is reportedly well tolerated without causing any serious postoperative complications.[13]

Immunotherapy is also not a routine treatment option for PDAC owing to the low tumor mutational burden (TMB) and typical characteristics of "cold" tumors, and the combination of neoadjuvant chemotherapy or chemoradiation and cancer vaccine has shown no optimistic clinical benefit.[14,15] Immune checkpoint inhibitors (ICIs) applied as monotherapies, such as PD-1 blockade, have not yielded clinical improvement.[16] However, the combination of immunotherapy and radiotherapy appeared to significantly improve treatment efficiency.[17] A patient with LAPC achieved near-pathologic complete response (pCR) after the combination of pembrolizumab and radiation therapy.[18] Moreover, the result of the CheckPAC clinical trial showed that SBRT plus nivolumab and ipilimumab was a promising therapy for patients with metastatic pancreatic cancer.[19]

Given the successful regimens and promising ongoing clinical trials, we aimed to conduct a clinical trial combining PD-1 blockade with chemoradiotherapy as preoperative therapy for patients with LAPC and BRPC for improving the resection rate and prolonging the survival outcome. Additionally, we sought to investigate some peripheral blood- and tumor-specific biomarkers for predicting the prognostic outcome and disease monitoring.

RESULTS

Study flow

Between May 2020 and October 2021, 29 patients with LAPC or BRPC were enrolled. Until the last follow-up time (November 30, 2022), 25 of them included in the intention-to-treat (ITT) analysis who completed at least two cycles of tislelizumab plus AG and underwent concurrent radiotherapy. In the ITT populations, one patient died after three cycles of treatment, which was not related to the treatment drugs. Twelve patients exhibited surgical indications after the treatment, but two patients refused surgery for personal reasons. Finally, ten patients received surgical resection, while two of them exhibited disease progression after surgery. A flowchart of the enrolled patients is shown in Figure 1A. At least two cycles of adjuvant therapy with a combination of tislelizumab and AG chemotherapy were administrated 1 month after the operation. The treatment scheme was altered for eight patients owing to disease progression (PD) during the preoperative treatment. The main inclusion and exclusion criteria are listed in Table 1, and the timeline of the treatment is shown in Figure 1B.

Characteristics of the patients

The detailed baseline demographics of the enrolled patients are presented in Table 2. The median age of patients was 62 (range: 40–75) years, comprising six women (24%). The Eastern Cooperative Oncology Group (ECOG) score of the patients was 0–1. All patients were diagnosed with BRPC (40%) or LAPC (60%). Pancreas head/uncinate (n = 13, 52%) was the most common primary tumor site, followed by body/tail (n = 8, 32%) and neck (n = 4, 16%).

Treatment response

Treatment efficacy was evaluated every two cycles of the preoperative therapy based on the investigator's assessment using RECIST 1.1. Among the patients who had completed at least one clinical response evaluation, 15 patients (60%) had a best response of partial response (PR) and 10 (40%) had stable disease (SD). The objective response rate (ORR) was 60% (95% confidence interval [CI]: 38.7%–78.9%), and the disease control rate (DCR) was 100%. The best changes compared with the baseline tumor size are shown in Figure 2A, and the overall treatment results are presented using swimmer charts in Figure 2B.

Survival and subgroup analyses

At the last follow up (November 30, 2022), six patients had died from PD, and three patients died from non-tumorous diseases not related to treatment drugs. The median follow up and progression-free survival (PFS) were 23.9 (95% CI: 18.4–27.3) and 13.7 (95% CI: 11.7–NR) months, respectively, whereas median OS was not reached. The 12-month OS and PFS rates were 72% (95% CI: 56.3%–91.9%) and 64% (95% CI: 47.6%–85.8%), respectively (Figures 2C and 2D). No significant association was observed in survival outcomes in BRPC and LAPC groups (PFS, hazard ratio [HR], 0.54; 95% CI: 0.21–1.41; p = 0.24; Figure 2E; OS, HR, 0.28; 95% CI: 0.09–0.84; p = 0.068; Figure 2F). The medium PFS (mPFS) was 28.23 months in patients with R0 resection vs. 10.62 months in patients with R1 resection or without surgery (HR, 0.38; 95% CI: 0.14–0.98; p = 0.073; Figure 2G). The mOS was not reached in patients with R0 resection vs. 13.12 months in patients with R1 resection or without surgery (HR, 0.11; 95% CI: 0.04–0.34; p = 0.011; Figure 2H).

Toxicities

Hematological and non-hematological toxicities during initial preoperative therapy are summarized in Table 3. We did not observe any grade 5 adverse events (AEs) in our study. The most common grade 3–4 hematological and non-hematological toxicities were anemia (8%), thrombocytopenia (8%), and jaundice (8%), respectively. There were no serious immune-related AEs such as autoimmune myocarditis, pneumonitis, and so on.

Conversion surgery and postoperative complications

Surgical resection

Pancreatoduodenectomy, distal pancreatectomy, and total pancreatectomy were performed in five, one, and four patients, respectively. En bloc vascular resection was required in seven patients, with venous resections in six and combined venous/arterial resections in one. The median operative time and estimated blood loss (EBL) were 528 min and 1,170 mL, respectively.

Pathologic evaluation

All tumors have been completely enclosed by pathologists. Regional lymph node metastases were identified in one patient. Negative (R0) margin resection was achieved in nine patients. Two of the ten patients achieved pCR, whereas one patient achieved major pathological response (MPR). The patient who received R1 resection was found to exhibit a poor response.

Perioperative complications

We found that postoperative complications developed in eight patients, with four patients experiencing major complications (Clavien-Dindo classification $\geq$ 3). Pancreas-specific complications included postoperative infectious complications (POICs), postoperative pancreatic fistula (POPF), and chyle leak in four, two, and two patients, respectively. We did not observe any delayed gastric emptying (DGE), postpancreatectomy hemorrhage

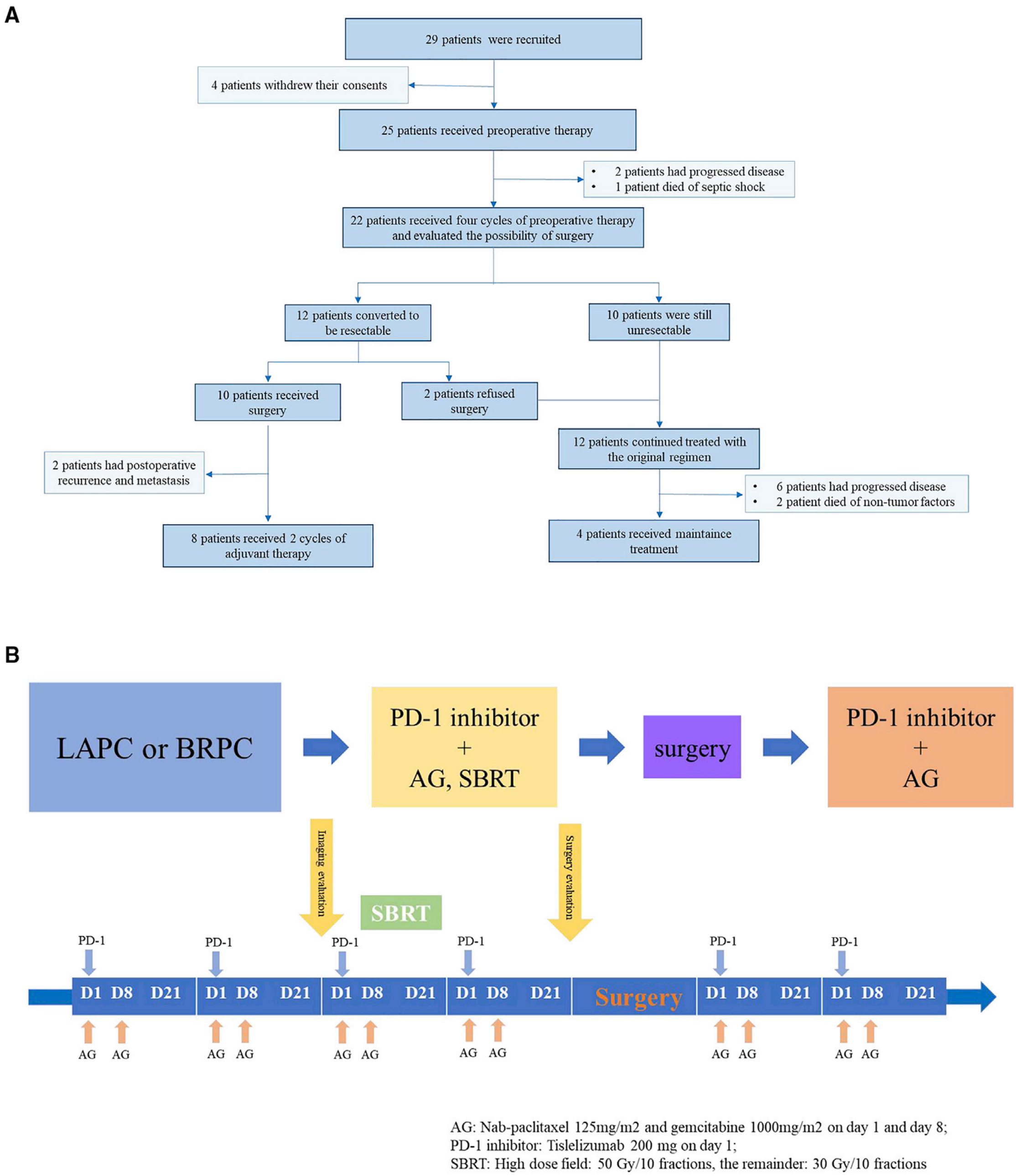

Figure 1. Study design

(A) Flowchart of the enrolled patients.

(B) Timeline of the treatment.

(PPH), and bile leakage. The median length of postoperative stay was 22.5 days, with one patient requiring subsequent 90-day re-admissions. The overall 90-day mortality rate was 0. The surgical outcomes of the patients are summarized in Table S1.

Association between peripheral blood biomarkers and tumor response

In the prespecified exploratory analysis, we first assessed the correlation between clinical response and peripheral blood biomarkers that are associated with response to immunotherapy. Reportedly, elevated peripheral blood eosinophil counts (PBECs) are associated with a better response during immunotherapy for metastatic triple-negative breast cancer.[20] Therefore, we intended to explore the association between changes in PBECs during treatment and the clinical response. Survival analysis revealed a statistically significant association between elevated PBECs during treatment and longer survival

Table 1. Inclusion and exclusion criteria of enrolled patients

Inclusion criteria	Exclusion criteria
(1) Subjects with age ≥ 18 years and ECOG score of 0–1 (2) subjects with pancreatic cancer confirmed by histology or cytology (3) The patients with potentially resectable pancreatic cancer were imaged 4) The subjects should meet the following hematological indexes: neutrophil count ≥ 1.5 * 10ˆ9/L, hemoglobin ≥ 10 g/dL, platelet count ≥ 100 * 10ˆ9/L (5) The subjects should meet the following biochemical indicators: total bilirubin ≤ 1.5* ULN; AST and ALT < 1.5* ULN; creatinine clearance rate ≥ 60 mL/min (6) Subjects of childbearing age need to take appropriate protective measures (contraceptive measures or other methods of birth control) before entering the group and during the test (7) Subjects who have signed informed consent (8) Subjects who were able to follow the protocol and follow-up procedures	(1) patients who have received systematic antitumor treatment (2) patients with previous history of other tumors, except for cervical cancer *in situ*, treated squamous cell carcinoma or bladder epithelial tumor (TA and TIS), or other malignant tumors that have received radical treatment (at least 5 years before enrollment) (3) patients with active bacterial or fungal infection (≥ level 2 of NCI-CTC, 3rd Edition) (4) patients with HIV, HCV, or HBV infection, uncontrollable coronary artery disease or asthma, uncontrollable cerebrovascular disease, or other diseases considered by researchers to be out of the group (5) patients with autoimmune diseases or immune defects who are treated with immunosuppressive drugs (6) pregnant and lactating women; pregnant women of childbearing age must test negative within 7 days before entering the group (7) patients with drug abuse or clinical or psychological or social factors that make informed consent or research implementation affected (8) patients who may be allergic to PD-1 monoclonal antibody immunotherapy drugs

outcome. The mPFS was 19 months in patients with elevated PBECs vs. 10.18 months in patients with declined PBECs (HR, 0.48; 95% CI: 0.15–1.56; p = 0.13; Figure 3A). The mOS was not reached in patients with elevated PBECs vs. 19.63 months in patients with declined PBECs (HR, 0.65; 95% CI: 0.20–2.16; p = 0.45; Figure 3B). These findings support the predictive role of elevated PBECs in immunotherapy-based preoperative therapy for pancreatic cancer.

Table 2. Baseline characteristic of patients

Characteristic	n=25
Median age, years (range)	62 (40,75)
Sex, n (%)	
Male	19 (76)
Female	6 (24)
ECOG PS score, n (%)	
0	19 (76)
1	6 (24)
Tumor location, n (%)	
Head/uncinate	13 (52)
Neck	4 (16)
Body/tail	8 (32)
Tumor type, n (%)	
BRPC	10 (40)
LAPC	15 (60)
Baseline CA19-9, n (%)	
≤27 U/mL, normal	6 (24)
≥27 U/mL, elevated	19 (76)
Vascular involvement, n (%)	
Arterial alone	6 (24)
Venous alone	10 (40)
Arterial + venous	9 (36)

ECOG PS, Eastern Cooperative Oncology Group Performance Status; BRPC, Borderline Resectable Pancreatic Cancer; LAPC, Locally Advanced Pancreatic Cancer; CA19-9, Carbohydrate Antigen 19-9.

A high neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR) in the baseline are markers of host inflammation and are associated with worse survival outcomes in immunotherapy for several tumors[21,22] but have not yet been extensively analyzed in pancreatic cancer. Thus, we investigated whether high baseline NLR or PLR was associated with clinical response in our study. We observed no significant differences in either survival outcomes or clinical response in high baseline NLR or PLR (Figures S1A–S1F). This result highlighted the heterogeneity of pancreatic cancer compared with that of other solid tumors.

Association between CA19-9 decline and tumor response

As a predictor biomarker, CA19-9 is the best-validated biomarker and an indicator of aberrant glycosylation in pancreatic cancer.[23] Normal baseline CA19-9 and declined CA-19-9 levels are associated with long-term survival in pancreatic cancer. We found that CA19-9 levels decreased after two cycles of treatment in all treated patients. Furthermore, changes in CA19-9 levels at baseline and after four treatment cycles showed a better PFS and OS trend but were not statistically significant (PFS: HR, 0.35, 95% CI: 0.06–2.26; declined vs. elevated: 14.07 vs. 6.1 months; p = 0.085; OS: HR, 0.42, 95% CI: 0.07–2.35; declined vs. elevated: 19.63 vs. 11.5 months; p = 0.18; Figures 3C and 3D). Notably, continuous CA19-9 decline during four treatment cycles was associated with improved survival outcomes and clinical response in our study. Patients with continuous CA19-9 decline did not reach mOS, whereas those without continuous CA19-9 decline reached a mOS of 11.5 months (HR, 0.18; 95% CI: 0.05–0.72; p = 0.0018), and patients with continuous CA19-9 decline reached a mPFS of 20.03 vs. 8.87 months in patients without continuous CA19-9 decline (HR, 0.28; 95% CI: 0.08–1; p = 0.0068).

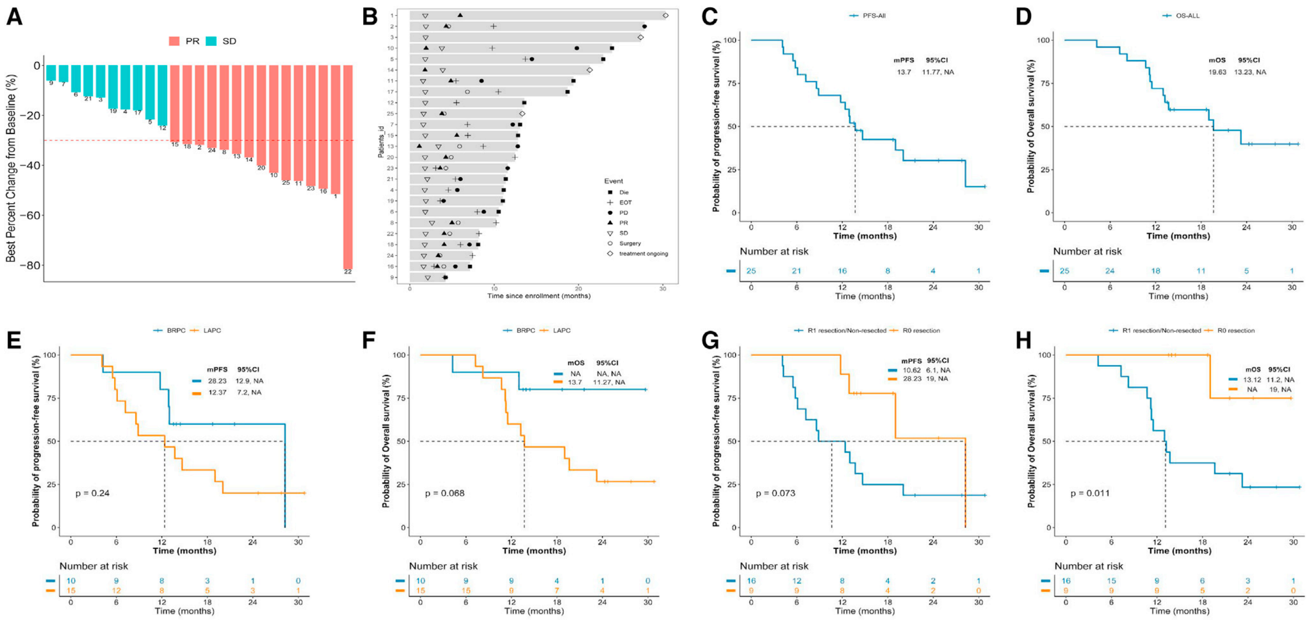

Figure 2. Treatment response and survival analysis

(A) Best percentage change from baseline on the basis of radiologic response.
(B) Duration of responses of patients in the ITT population. The length of each bar represents the duration of treatment of each patient.
(C and D) The Kaplan-Meier curves of (C) PFS and (D) OS in all enrolled patients.
(E and F) The Kaplan-Meier curves of (E) PFS and (F) OS stratified by tumor type.
(G and H) The Kaplan-Meier curves of (G) PFS and (H) OS stratified by surgical margin.
PR, partial response; SD, stable disease; PD, disease progression; EOT, end of treatment; OS, overall survival; PFS, progression-free survival; BRPC, borderline resectable pancreatic cancer; LAPC, locally advanced pancreatic cancer.

Concurrently, the ORR was 78.6% [11/14] vs. 28.6% [2/7]; p = 0.055) for these two groups. Moreover, continuous CA19-9 decline significantly improved the R0 resection rate after NAT (64.2% [9/14] vs. 0% [0/7]; p = 0.0071) (Figures 3E–3H).

Table 3. Summary of adverse events

Toxicities	Grade 1 (%)	Grade 2 (%)	Grade 3 (%)	Grade 4 (%)
Hematologic toxicities				
Anemia	8 (32)	12 (48)	2 (8)	0 (0)
Leukopenia	6 (24)	9 (36)	0 (0)	1 (4)
Neutropenia	5 (20)	5 (20)	0 (0)	0 (0)
Thrombocytopenia	8 (32)	3 (12)	1 (4)	1 (4)
Nonhematologic toxicities				
ALT↑	9 (36)	5 (20)	2 (8)	0 (0)
AST↑	5 (20)	5 (20)	2 (8)	0 (0)
Jaundice	1 (4)	1 (4)	2 (8)	0 (0)
Nausea	4 (16)	0 (0)	0 (0)	0 (0)
Anorexia	6 (24)	0 (0)	0 (0)	0 (0)
Vomiting	2 (8)	2 (8)	0 (0)	0 (0)
Diarrhea	0 (0)	1 (4)	0 (0)	0 (0)
Rash: Dermatitis	2 (8)	1 (4)	0 (0)	0 (0)
Hyperthyroidism	0 (0)	0 (0)	0 (0)	0 (0)
Pneumonia	0 (0)	0 (0)	0 (0)	0 (0)

ALT, alanine aminotransferase; AST, aspartate aminotransferase.

Association between serial ctDNA dynamic changes and tumor response

We assessed serial circulating tumor DNA (ctDNA) dynamic changes in predicting tumor responses and survival outcomes by performing next-generation sequencing testing on 539 genes. Patients with a decline in maximal somatic variant allelic frequency (maxVAF) between the first clinical evaluation (T2 [two cycles after therapy]) and baseline (T1) were assessed. We observed a better survival benefit trend without statistically significant association in the decline and non-decline groups (PFS: HR, 0.46, 95% CI: 0.16–1.33; decline vs. non-decline groups: 20.03 vs. 10.18 months, p = 0.11; OS: HR, 0.39, 95% CI: 0.13–1.22; decline vs. non-decline groups: not reached vs. 15.57 months, p = 0.11; ORR: 75% vs. 41.7%, p = 0.21) (Figures 4A–4C). Furthermore, patients with a >50% decline in maxVAF between T2 and T1 had longer survival outcomes and higher response rates than those who did not (PFS: HR, 0.33, 95% CI: 0.12–0.89; decline vs. non-decline groups: 20.03 vs. 10.32 months, p = 0.024; OS: HR, 0.21, 95% CI: 0.07–0.65; decline vs. non-decline groups: not reached vs. 13.47 months, p = 0.024; ORR: 90% vs. 35.7%, p = 0.013) (Figures 4D–4F). Moreover, maxVAF decline (T1-T2, >50%) significantly improved the surgical rate after preoperative theapy (70% vs. 21.4%; p = 0.035) (Figure 4G). Notably, no statistically significant difference was observed between the maxVAF decline (T1-T2, >50%) and MPR beneficiaries (42.9% vs. 0%; p = 0.48) (Figure 4H). To reduce the impact between extremely low or negative baseline

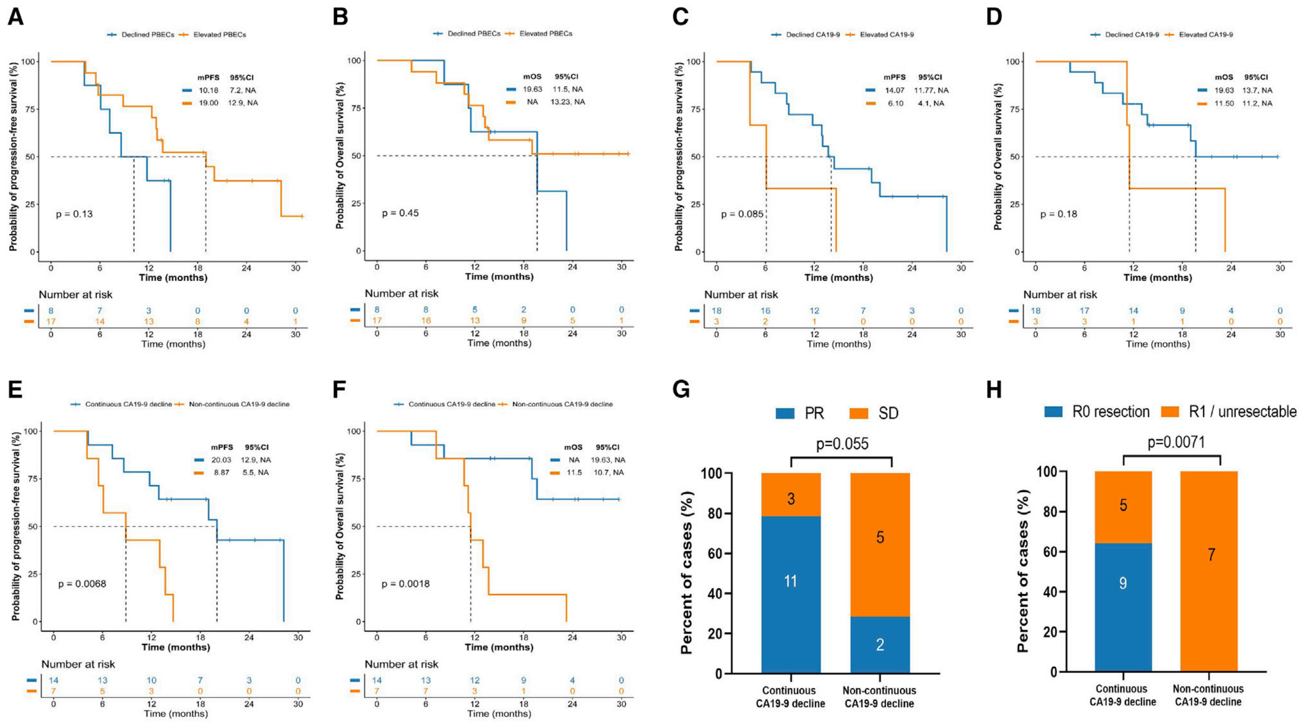

Figure 3. Association between peripheral blood biomarkers and treatment response

(A and B) The Kaplan-Meier curves of (A) PFS and (B) OS of patients stratified by PBEC (declined vs. elevated).

(C and D) The Kaplan-Meier curves of (C) PFS and (D) OS of patients stratified by CA19-9 change between baseline and after four treatment cycles (decline vs. elevated).

(E and F) The Kaplan-Meier curves of (E) PFS and (F) OS of patients stratified by CA19-9 decline from baseline, two treatment cycles, and four treatment cycles (continuous decline vs. non-continuous decline).

(G) Clinical response of patients stratified by CA19-9 decline from baseline, two treatment cycles, and four treatment cycles (continuous decline vs. non-continuous decline).

(H) Surgery margin of the patients stratified by CA19-9 decline from baseline, two treatment cycles, and four treatment cycles (continuous decline vs. non-continuous decline).

ctDNA status and maxVAF change, VAFmean was also applied to investigate the correlation between VAF changes and patient outcomes. The results showed that patients with a >50% decline in VAFmean between T2 and T1 also demonstrated a significantly longer PFS (28.2 vs. 11.7 months; HR = 0.38; p = 0.048) and a trend for longer OS (not reached vs. 13.7 months; HR = 0.27; p = 0.058) (Figures S2A–S2D). Interestingly, we found that four patients harbored ctDNA clearance after T2, and patients with ctDNA clearance demonstrated a significantly prolonged PFS and OS (28.2 vs. 12.3 months; HR = 0.18; p = 0.036) and a longer OS (not reached vs. 19 months; HR = 0.27; p = 0.049) (Figures S2E and S2F). We further investigated the changes in maxVAF between the second clinical evaluation (T3 [four cycles after therapy]) and baseline. No association was observed in the PFS or ORR in the decline and non-decline groups (Figures S3A–S3F).

The baseline genetic alterations in the cohort are depicted in Figure S4A, and the average number of genomic alterations was 1.5 mutations in each patient. *KRAS* was the most frequently altered gene, occurring in 12 (50%) patients with a missense mutation, followed by *TP53* (30%). We found that no association was observed between the TMB-high and -low groups (mPFS: HR, 1.07, 95% CI: 0.39–2.91; high vs. low: 13.70 vs. 13.83 months; p = 0.9). Similarly, the baseline mVAF (mPFS: HR, 0.49, 95% CI: 0.18–1.31; mVAF > 1% vs. < 1%: 28.23 vs. 13 months; p = 0.14) and *KRAS* mutation status in PFS (HR, 1.27, 95% CI: 0.48–3.38; mutation vs. wild type: 13.35 vs. 14.67 months; p = 0.62) did not exhibit any association (Figures S4B–S4D).

Comparison of clinical features and biomarker changes in patients with resectable vs. unresectable cancer

Owing to the poor prognosis of patients with inoperable pancreatic cancer, one of the goals of preoperative therapy is to increase the surgical rate. Thus, we compared the clinical features and biomarker changes in patients stratified by the eligibility for resection after preoperative therapy. Baseline characteristics for patients with resectable and unresectable cancer were similar for age (median values: 60.4 vs. 60.1 years; p = 0.93; Figure S5A) and the longest tumor diameter (median values: 38.8 vs. 34.6 mm; p = 0.36; Figure S5B). Compared with patients with unresectable cancer, patients with resectable cancer exhibited a greater response in decreasing the size of the longest tumor diameter from baseline to after four cycles of preoperative therapy (−40.32% vs. −14.39%; p = 0.0012; Figure S5C).

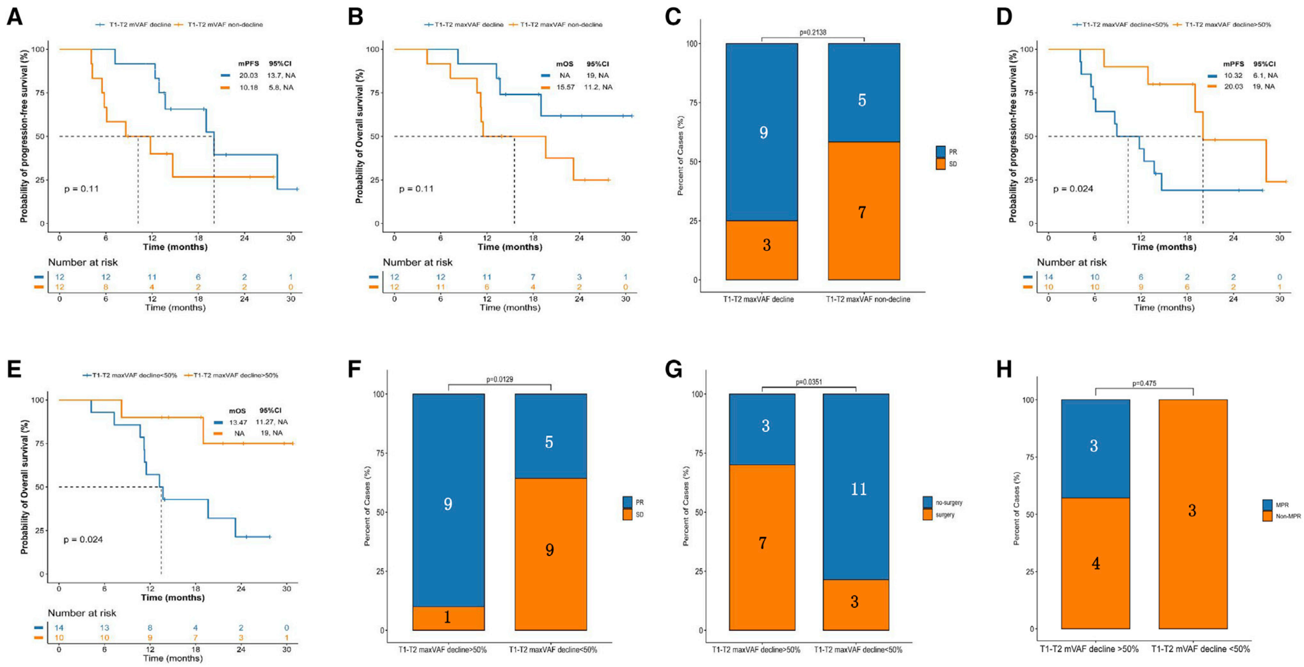

Figure 4. ctDNA dynamics and correlation with treatment response

(A and B) The Kaplan-Meier curves of (A) PFS and (B) OS of patients stratified by change of ctDNA (T1-T2 mVAF decline vs. non-decline).

(C) Treatment response of patients stratified by change of ctDNA (T1-T2 mVAF decline vs. non-decline).

(D and E) The Kaplan-Meier curves of (D) PFS and (E) OS of patients stratified by decline of ctDNA (T1-T2 mVAF > 50% vs. < 50%).

(F) Treatment response of patients stratified by decline of ctDNA (T1-T2 mVAF > 50% vs. < 50%).

(G and H) Clinical outcome (G) and postoperation pathological stage (H) of the patients stratified by decline of ctDNA (T1-T2 mVAF > 50% vs. < 50%).

ctDNA, circulating tumor DNA; T1, baseline; T2, two cycles after therapy; mVAF, maximal somatic variant allelic frequency; PR, partial response; SD, stable disease; MPR, major pathological response.

Additionally, the proportion of patients with BRPC who became resectable after preoperative therapy was higher than in those with unresectable patients (66.7% vs. 15.3%, p = 0.041; Figure S5D). Moreover, resectable participants compared with non-resectable patients had greater response in maxVAF decline (T1-T2 > 50% maxVAF decline, 63.6% vs. 16.7%, p = 0.036; Figure S5E) and CA19-9 change (continuous CA19-9 decline, 100% vs. 45.4%; p = 0.012; Figure S5F).

DISCUSSION

This single-arm, phase II trial was designed to examine the efficacy and safety of PD-1 inhibitors in combination with chemotherapy and concurrent SBRT in patients with LAPC and BRPC. Neoadjuvant and induction therapy are being increasingly applied for LAPC and BRPC, with higher resection rates and better tumor responses.[24] At the 2022 American Society of Clinical Oncology (ASCO) Annual Meeting, data of the AIO-NEONAX trial indicated improved survival and R0 resection rate in patients treated with perioperative chemotherapy compared with adjuvant therapy, respectively.[25] However, the role of neoadjuvant radiotherapy remains uncertain. Upon comparing neoadjuvant chemoradiotherapy vs. chemotherapy in patients with LAPC or BRPC, a study found that preoperative chemoradiotherapy was associated with improved treatment response and increased survival.[26] Furthermore, data of the PREOPANC trial, a multicenter randomized clinical trial, concluded that there is no significant difference in the incidence of surgical complications or mortality in patients who received preoperative chemoradiotherapy or underwent surgery immediately. Preoperative chemoradiotherapy has even been found to reduce the rate of POPF.[27]

Recently, immunotherapy is of great interest in cancer treatment. Although single-agent PD-1 inhibitor is yet to show a substantial clinical benefit in PDAC treatment, PD-1 blockade plus chemotherapy or radiotherapy provides various options for treating patients with BRPC, LAPC,[18] or metastatic PDAC.[19] In a melanoma mouse model, the combination of radiation and ICI resulted in higher response rates and improved survival.[28] Tislelizumab combined with chemotherapy as neoadjuvant therapy has shown promising efficacy in esophageal squamous cell carcinoma[29] and locally advanced gastric/gastroesophageal junction cancer.[30] Radiotherapy or chemotherapy can upregulate PD-L1 expression,[31] thereby giving rise to a mode of treatment. Our study is a prospective evaluation to demonstrate the clinical benefit and safety of preoperative therapy that combined chemoradiotherapy and PD-1 inhibitor in patients with LAPC or BRPC. Our data revealed that this regimen was potentially effective, which contributed to superior ORR and outstanding R0 resection rates without serious adverse reactions or postoperative complications. The incidence of pCR is higher in our study (20%) than in patients with LAPC or BRPC treated with neoadjuvant chemoradiation (10%),[32] which may be an effect of the immunotherapy plus a high radiation dose, and more samples are required to demonstrate the correlation.

To the best of our knowledge, no effective predictive biomarkers have been identified for pancreatic cancer therapy; neither PD-L1 expression nor TMB has been verified to predict the response to immunochemotherapy.[33,34] CA19-9 is the best-validated predictor biomarker and an indicator of aberrant glycosylation in pancreatic cancer. In our study, continuous CA19-9 decline during four treatment cycles was associated with superior survival outcomes and clinical response, providing a viable predictive biomarker. Additionally, one of the significant findings in our study was that elevated PBEC was associated with clinical benefits in survival benefits and tumor response. Eosinophils influence the function of other leukocytes by expressing major histocompatibility complex class II costimulatory molecules, releasing cytokines, and stimulating T cell proliferation.[24] Moreover, eosinophils secrete chemokines, such as CCL5, CXCL9, and CXCL10, that attract $CD8^+$ T cells into the tumor.[35,36] These are all possible reasons for the increase in eosinophil levels during treatment to be associated with better clinical benefits. Notably, a positive association between eosinophil invasion of tumor tissue or an increase in PBEC and superior response to ICIs in several types of cancer has been reported.[20,37] Although the underlying mechanism is not fully understood, there is strong evidence that eosinophils exhibit antitumor effects. Thus, eosinophils affect the immune response to diseases such as cancer, and predictive biomarkers that reflect this inflammatory response to treatment may be useful for clinical decision-making in the management of patients with cancer.

Genomic features are believed to hold great potential to predict tumor response to cancer therapy. A large sample analysis has demonstrated that ctDNA may be a feasible biomarker for various solid tumor types.[38] Moreover, ctDNA could provide longitudinal and dynamic surveillance of the tumor-specific genetic characteristics without having to repeatedly perform invasive tumor biopsies that cost more time and money.[39] In our study, serial ctDNA dynamic changes in predicting tumor responses and survival outcomes revealed that patients with a >50% decline in maxVAF between the first clinical evaluation and baseline had longer survival outcomes and higher response rates than those who did not. Additionally, maxVAF decline significantly improved the surgical rate after preoperative therapy. This rapid decline in the maxVAF of ctDNA-positive patients from baseline to postchemoradiation reflects the substantial downstaging achieved with induction treatment. Consistent with some studies, ctDNA has potential value in predicting immunotherapy efficacy in patients with non-small cell lung cancer[28] and gastric cancer[40]; it may be an accurate dynamic biomarker reflecting real-time tumor volume.

In summary, this is a prospective clinical trial that adopts a regimen of preoperative therapy for patients with BRPC or LAPC. The findings of this trial demonstrate the effectiveness and safety of the combination of PD-1 inhibitors and neoadjuvant chemoradiotherapy. It shows the potential of this treatment in improving the R0 resection rate without causing serious postoperative complications.

Limitations of the study

This study has some limitations. This is a single-arm study lacking a comparator treatment arm so that selection bias could not be ruled out. Furthermore, the small sample size and relatively short follow-up time reduced the certainty of effectiveness observed and restricted the interpretation of definite conclusions. In addition, most enrolled patients only had endoscopic ultrasound fine-needle aspiration (EUS-FNA) for diagnosis so there were not enough tissue samples for immunohistochemistry (IHC) to evaluate microsatellite instability/mismatch repair deficiency (MSI/dMMR) status and expression of PD-L1, which restricted us to analyze the association between these biomarkers and the benefits of our induction therapy. We did not conduct exploratory analyses on the resected specimens to evaluate the effect of preoperative therapy on the tumor microenvironment given the small sample size of resected patients. The study data aided in interpreting the treatment effect; nevertheless, further research on the elucidation of the underlying mechanisms is necessary.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.xcrm.2023.100972.

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AUTHOR CONTRIBUTIONS

D.J., C.L., and L.M. have contributed equally to this work. J.D., B.L., Y.Q., and L.W. were responsible for the design of the project and writing articles. D.J., C.L., and L.M. were responsible for all data sorting and writing articles. Y.Z., K.W., S.S., and X.Q. were involved in the diagnosis and the instruction of the treatment. M.T., J.H., and A.L. were responsible for imaging evaluation.

S.B., H.C., and G.L. were responsible for surgery. J.C. and Q.L. were responsible for pathological evaluation. Q.X., Q.G., D.C., C.Q., and Y.S. were responsible for sample sequencing and data analysis. All authors have agreed to the final version of the manuscript.

DECLARATION OF INTERESTS

D.C., Y.S., and C.Q. were employed by Jiangsu Simcere Diagnostics Co., Ltd.

INCLUSION AND DIVERSITY

We support inclusive, diverse, and equitable conduct of research.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Biological samples		
Blood samples for analysis were collected from 29 patients recruited in the trial	This manuscript	N/A
Chemicals, peptides, and recombinant proteins		
Gemcitabine	Hengrui, Jiangsu, China	https://www.hengrui.com/
Nab-paclitaxel	Lilly Pharmaceutical, IN, USA	https://www.lilly.com.cn/
Tislelizumab	BeiGene, Beijing, China	https://www.beigene.com.cn/
Software and algorithms		
SAS software version 9.4	SAS Institute, Cary, NC, USA	https://support.sas.com/software/94
Prism version 8.0.2	GraphPad Software	https://www.graphpad.com/scientific-software/prism/

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Baorui Liu (baoruiliu@nju.edu.cn).

Materials availability

This study did not generate new, unique reagents.

Data and code availability

- The raw sequencing data are available under restricted access due to data privacy laws. Data are available on request sharing by sending requests to the corresponding author Baorui Liu (baoruiliu@nju.edu.cn), which will need the approval of the institutional ethical committees. Clinical data were not publicly available due to involving patient privacy, but can be accessed from the corresponding author, upon request for 3 years; individual de-identified patient data will be shared for clinical study analyses. The remaining data are available in the manuscript, supplemental information, or Source Data file. The study protocol is provided in the supplemental information file.
- This paper does not report original code.
- Any additional information required to reanalyze the data reported in this work paper is available from the lead contact upon request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Human subject

Chinese adults with histologically confirmed locally advanced pancreatic cancer or borderline resectable pancreatic cancer were enrolled in the study. Demographic information along with the key inclusion criteria and exclusion criteria were provided. To determine the sample size for this clinical trial, ORR improvement with standard of care chemotherapy was assumed and estimated. In this study, 29 treatment-naive patients were enrolled and 25 of them were analyzed, comprising six female (24.0%). The Eastern Cooperative Oncology Group (ECOG) score of the patients was 0 (76%) and 1 (24%). All patients provided written informed consent prior to enrollment. The study was performed per the Declaration of Helsinki and was reviewed and approved by the Medical Ethics Committee of Drum Tower Hospital Affiliated to Nanjing University Medical School (2020-088-01).

METHOD DETAILS

Study design and patients

This is a phase II, single-arm, prospective study of PD-1 inhibitor plus chemotherapy and concurrent SBRT as preoperative therapy for patients with LAPC or BRPC between May 2020 and October 2021 at the Nanjing Drum Tower Hospital, the Affiliated Hospital of

Nanjing University Medical School. The patients were screened within one week before the initial treatment, and all the patients involved in this study provided written informed consent. The protocol of the study has already been published.[41]

Treatment

Patients who met the inclusion criteria received two treatment cycles of PD-1 inhibitor plus chemotherapy firstly. Briefly, each cycle lasted for 3 weeks, including gemcitabine (1000 mg/m^2) and nab-paclitaxel (125 mg/m^2) administered intravenously (IV) on days 1 and 8, with tislelizumab (200 mg) IV on day 1. After two cycles of treatment, an imaging examination was performed to assess the prognosis, and patients without PD received SBRT with SIB (high dose field: 50 Gy/10 fractions; the remainder: 30 Gy/10 fractions) during the third cycle. On completion of four cycles of treatment and radiotherapy, multiple disciplinary team (MDT), comprising medical oncologists, pancreatic surgeons, gastroenterologists, radiologists, pathologists et al., would reassess the surgical possibility according to NCCN Version 2.2021[42] for resection following neoadjuvant therapy, including imaging checkups, positron emission computed tomography (PET-CT), changes in tumor markers, and the health status of patients. Patients whose CA19-9 was stable or decreased and radiographic findings didn't demonstrate clear progression, or the standardized uptake value (SUV) max parameter of the lesion decreased in PET-CT, were eligible for resection. Adjuvant therapy including a combination of tislelizumab and AG was administered in at most four cycles after resection according to the patient's physical condition. Patients who were not suitable for surgery continued the treatment until investigator-assessed PD, unacceptable toxicity, withdrawal of consent, investigator decision, or study completion.

Endpoints and assessments

The primary endpoint was the R0 resection rate and ORR. Resection status (R0, R1, or R2) is graded according to institutional guidelines. ORR in this trial refers to the best overall response during four courses of the preoperative therapy which was calculated as complete response (CR) rate plus partial response (PR) rate under CT according to RECIST 1.1.[43] CR was defined as total tumor regression, while PR was defined as greater than 30% reduction. And DCR was defined as the proportion of patients with CR, PR and SD The secondary objectives included safety, median overall survival (mOS), median progression-free survival (mPFS), and postoperative pathological stage. OS is defined as the time from the date of enrollment to the date of death owing to any cause. PFS is defined as the time from initial treatment to the first evidence of PD based on RECIST 1.1 or disease-related death. Two individual pathologists blinded to the clinical outcome assessed the tumor response. MPR defined as 90%–99% tumor necrosis in resected tissue and pCR defined as no residual cancer cells in the resected tissue. Adverse events (AEs) were evaluated according to Adverse Events (CTCAE) version5. Clavien–Dindo classification was applied for postoperative complications, with major complications defined as grade $\geq$ III.[44] CR-POPF (Grade B/C), biliary leakage (BL), chylous fistula, delayed gastric emptying (DGE), and post-pancreatectomy hemorrhage (PPH) were diagnosed according to the International Study Group of Pancreatic Surgery (ISGPS).[45–47] Wound infection, intra-abdominal infection, bacteremia, pneumonia and urinary tract infection were all included. Multiomics biomarkers associated with clinical response were assessed as exploratory objectives.

Peripheral blood biomarker collection and evaluation

According to the protocol designs, peripheral blood biomarkers were measured at baseline and before each cycle of treatment. Peripheral neutrophil, lymphocyte, thrombocyte, and PBECs and carbohydrate antigen 19-9 (CA19-9) levels were measured. NLR was calculated by division of absolute neutrophil and lymphocyte counts, while PLR was calculated by division of thrombocyte and lymphocyte counts. Patients with normal baseline CA19-9 levels (<27 U/mL) were excluded from the CA19-9 response evaluation because they were less likely to exhibit a significant decline in CA19-9 levels. In addition, if the CA19-9 value was greater than 27 U/mL at any time point, the patient was included in the analysis.

ctDNA sequencing and bioinformatics analysis

Plasma samples were collected at the following time points: before preoperative therapy (baseline), two cycles after the initiation of preoperative therapy, 4 cycles after preoperative therapy or before surgery (preop), and within 1 month after surgery (postop) or at time of progression. For each sample, 10 mL of peripheral (intravenous) blood was collected and stored in a 10 mL BD EDTA-K2 anticoagulation tube. Double centrifugation was applied to eliminate leukocyte contamination. Plasma was isolated within 2 h by centrifugation (1200g, 15 min), and then the collected plasma was removed into a 1.5 mL low-adsorption centrifuge tube (Eppendorf DNA Lobind tube, 12000g, 10 min).

A total of 86 dynamic plasma samples and 25 leukocyte germline control samples were collected and subjected to panel sequencing of 539 cancer-related genes. The 539-gene panel includes genes associated with targeted medicines approved by Food and Drug Administration (FDA) or recommended by the NCCN guideline, genes involved in the major signaling pathways regulating cancer cell survival and proliferation, and potential cancer driver genes. Clonal hematopoiesis of indeterminate potential (CHIP) interference variants could be distinguished and excluded using the paired whole-blood control first. ctDNA positive is defined as detectable somatic mutations, we tracked the dynamic change of the mutation with the highest variant allele frequency (maxVAF) at baseline and predefined points in each patient. maxVAF change is defined as the change in maxVAF during the treatment, calculated by subtracting maxVAF at baseline from maxVAF at different points. Also, VAFmean is defined as the mean of the VAF(s) of somatic mutated genes in each patient. VAFmean change is defined as the change in VAFmean during the treatment, calculated by subtracting VAFmean at baseline from VAFmean at different points. ctDNA clearance is defined as lack of detectable mutation from this panel covering 539 cancer-related genes at predefined points, with an average sequencing depth of 15000× and 0.2% detection limit.

DNA library and corresponding cDNA library were standardized using the library homogenization method, purified by magnetic beads, and sequenced using the Illumina NextSeq 550Dx platform. Before library normalization, the next-generation sequencing libraries enriched by hybridization capture were quantified using the Qubit dsDNA HS Assay Kit. The fastp tool (V.2.20.0) was used for adapter pruning and to filter low-quality sequencing reads.[48] Cleaned reads were alignd to the human reference genome (hg19) using the BWA-mem algorithm.[49] Somatic mutations including point mutations, small insertions, and deletions were identified and annotated using VarDict and Inter-Var, respectively.[50,51] We screened for germline variations using the internal database. Copy number variation involved amplification and deletion were identified by CNVkit.[52] bTMB was defined as the number of somatic SNVS and indels in examined coding region. All SNVs and indels in the coding region of targeted genes, including missense, silent, stop gain, stop loss, in-frame and frameshift mutations, are initially considered. Known germline SNVs, defined as population frequency more than 0.015, in dbSNP, 1000 genome, and ESP6500 were filtered. Variants with allele frequencies more than 30%, which are more likely germline mutations, were not counted. TMB high was defined as the median value in this study.

QUANTIFICATION AND STATISTICAL ANALYSIS

Historical data showed that the ORR rate after neoadjuvant chemotherapy (LAPACT) in patients with LAPC was 33.6%. We estimated that a sample size of 26 patients would detect approximately 26% improvement (60%) in ORR rate with a power of 80%, using a one-sided alpha of 0.025. Assuming a 10% drop-out rate, a total of 29 treatment-naive patients with LAPC/BRPC were planned to accrue in our study.

Statistical analysis was conducted using SAS statistical software (V.9.4,SAS Institute). Efficacy analyses were performed in patients who underwent one or more post-treatment scans in the intention-to-treat populations. Safety outcomes were analyzed in patients who received at least one of the aforementioned doses of the study regimen. Categorical variables, as the proportions of patients with an objective response or adverse events were summarized by descriptive statistics with 95% confidence interval Wilson score (CIs). Continuous variables were expressed as median (range). Response differences (ORR) and other binary outcomes among clinical subgroups were assessed with the Fisher's exact test. Furthermore, we provided Kaplan-Meier plots for PFS and OS, the log rank test was used to compare the survival functions among different subgroups. For all analyzes, p value < 0.05 was considered to be statistically significant.

ADDITIONAL RESOURCES

This study has been registered on https://www.chictr.org.cn/, ID: ChiCTR2000032955.

Cell Reports Medicine, Volume *4*

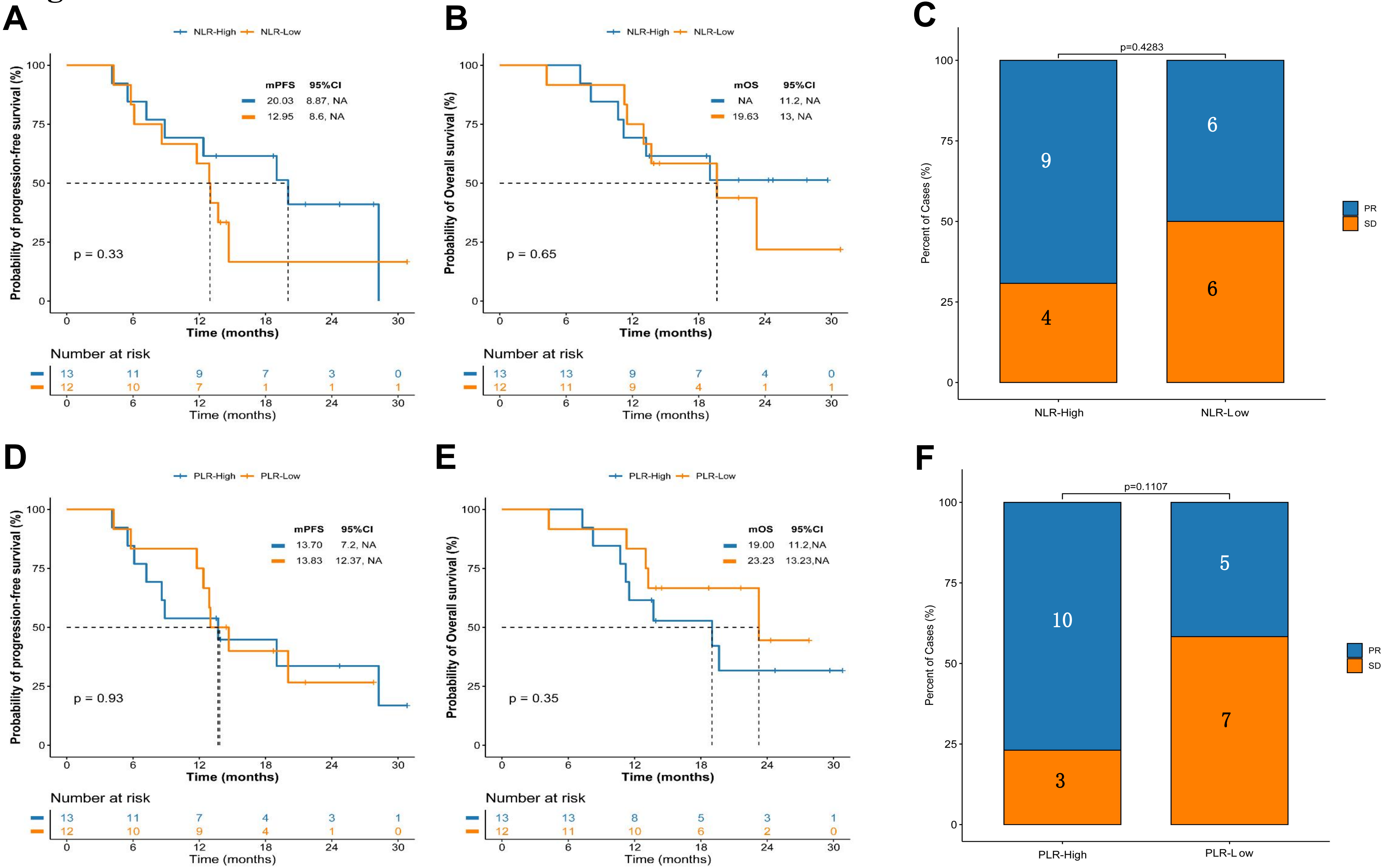
Supplemental information

PD-1 blockade plus chemoradiotherapy as preoperative therapy for patients with BRPC/LAPC: A biomolecular exploratory, phase II trial

Juan Du, Changchang Lu, Liang Mao, Yahui Zhu, Weiwei Kong, Shanshan Shen, Min Tang, Shanhua Bao, Hao Cheng, Gang Li, Jun Chen, Qi Li, Jian He, Aimei Li, Xin Qiu, Qing Gu, Dongsheng Chen, Chuang Qi, Yunjie Song, Xiaoping Qian, Lei Wang, Yudong Qiu, and Baorui Liu

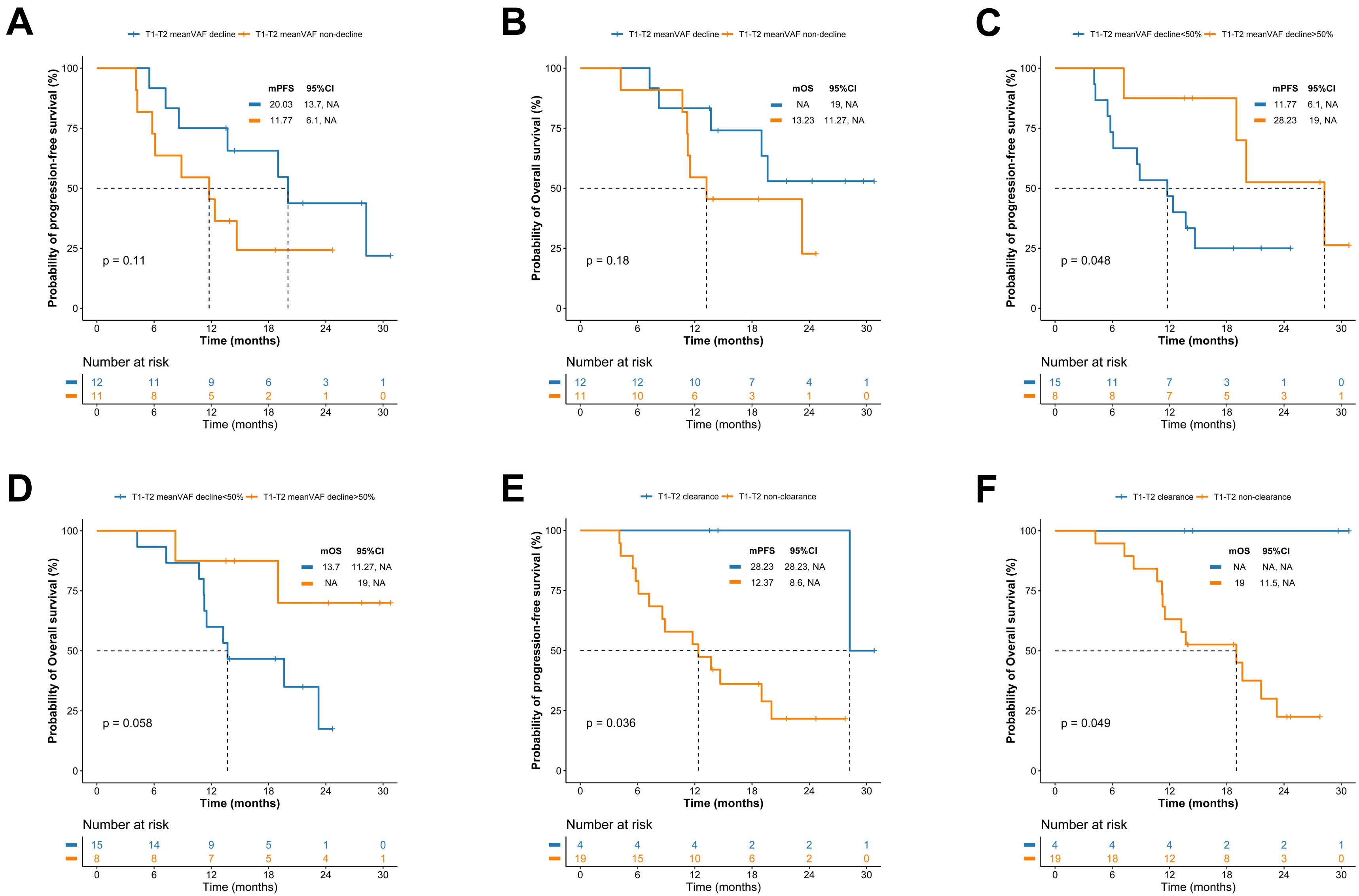
Contents

sFigure 1



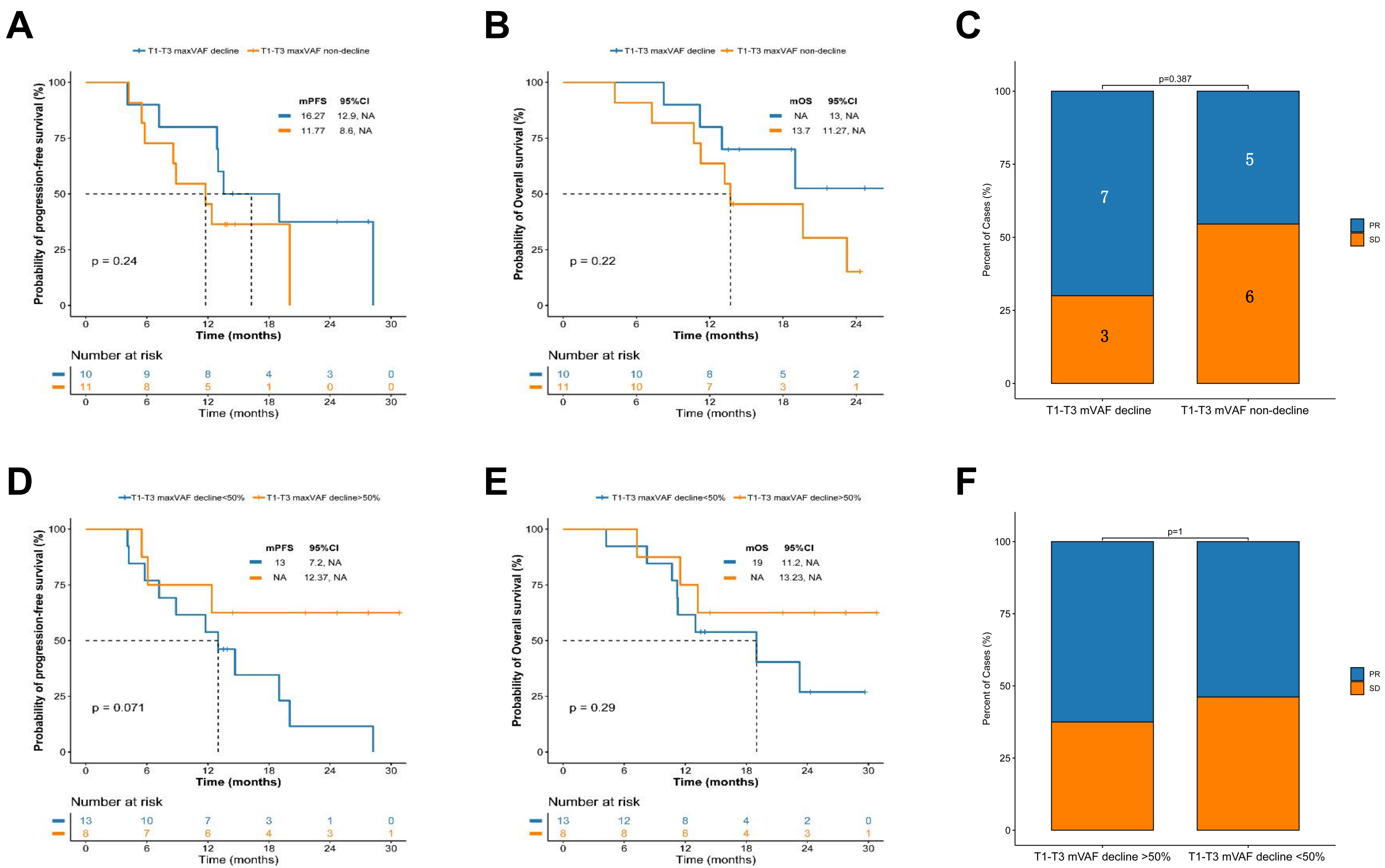
Supplementary figure1: The Kaplan-Meier curves of (A) PFS and (B) OS of patients stratified by NLR (high v. low); (C) Treatment response of patients stratified by NLR (high v. low); The Kaplan-Meier curves of (D) PFS and (E) OS of patients stratified by PLR (high v. low); (D) Treatment response of patients stratified by PLR (high v. low). NLR, neutrophil to lymphocyte ratio; PLR, platelet to lymphocyte ratio.

sFigure 2



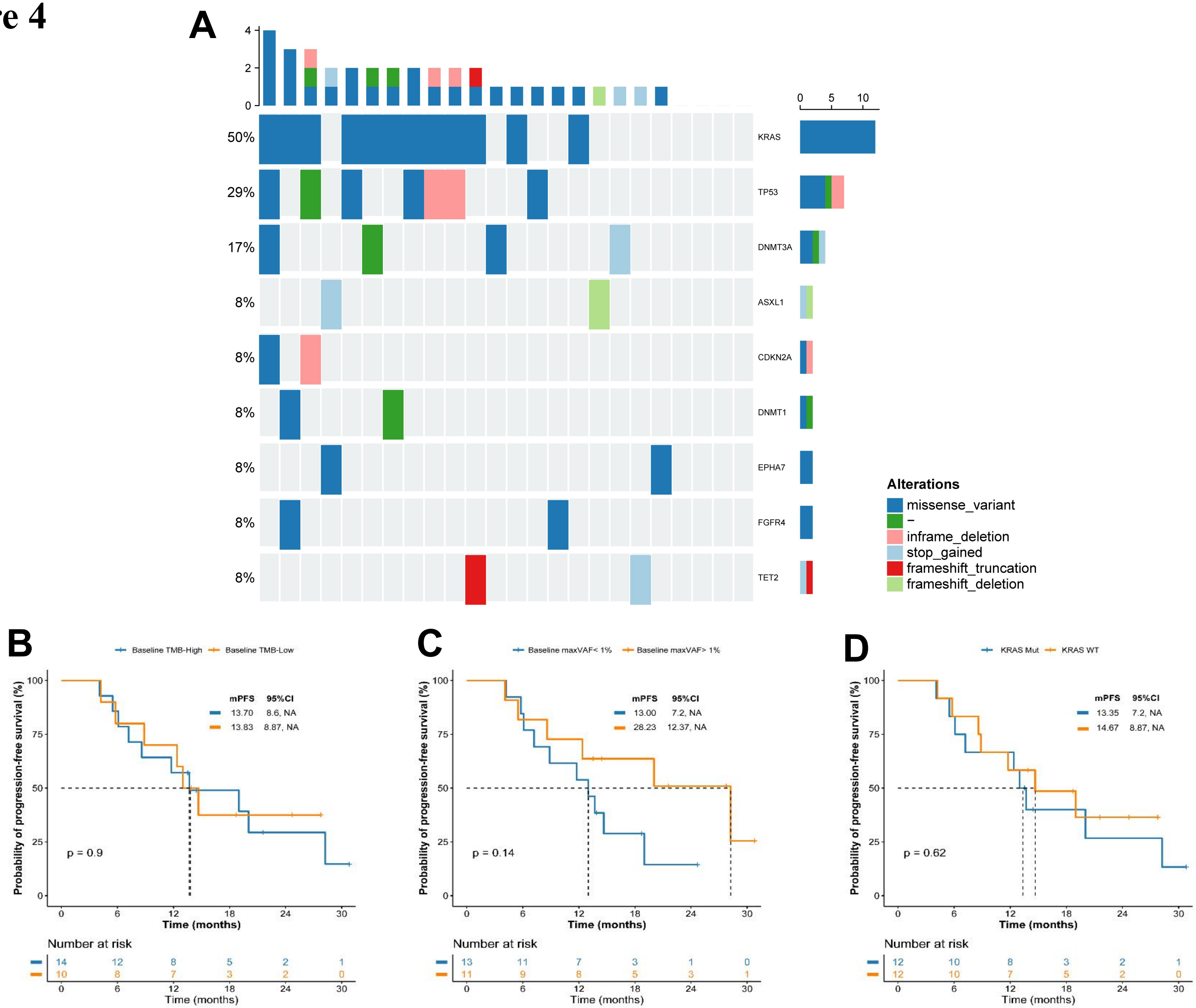
Supplement figure2: ctDNA dynamics and correlation with treatment response. (A, B), The Kaplan-Meier curves of (A) PFS and (B) OS of patients stratified by change of ctDNA (T1-T2 meanVAF decline v non-decline). The Kaplan-Meier curves of (C) PFS and (D) OS of patients stratified by decline of ctDNA (T1-T2 meanVAF >50% v <50%). The Kaplan-Meier curves of (E) PFS and (F) OS of patients stratified by clearance of ctDNA (T1-T2 clearance v non-clearance). ctDNA, circulating tumor DNA; T1, baseline; T2, two cycles after therapy, meanVAF, mean somatic variant allelic frequency.

sFigure 3



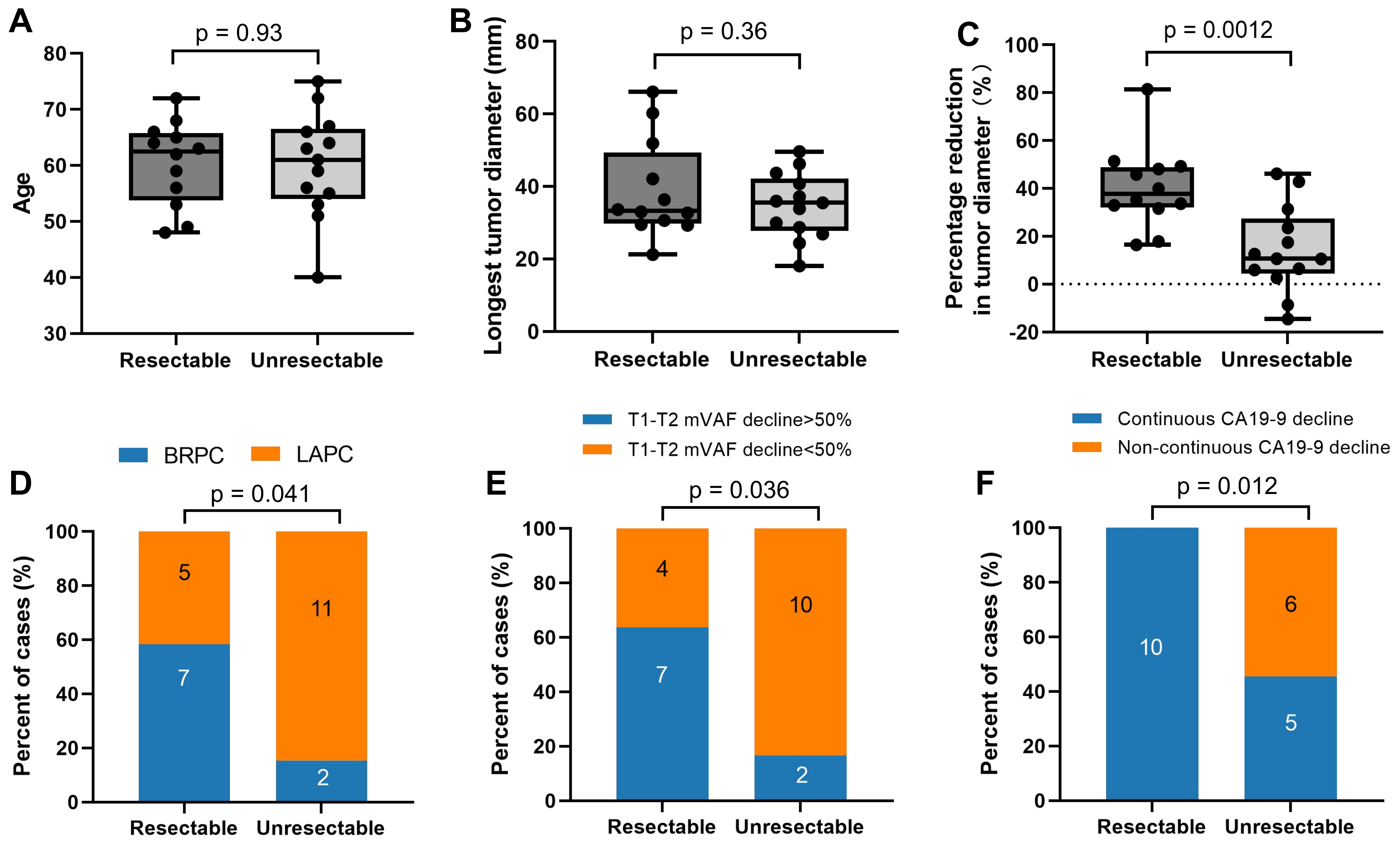
Supplementary figure3: ctDNA dynamics and correlation with treatment response. (A, B), The Kaplan-Meier curves of (A) PFS and (B) OS of patients stratified by change of ctDNA (T1-T3 mVAF decline v non-decline). (C) Treatment response of patients stratified by change of ctDNA (T1-T3 mVAF decline v non-decline). (D, E) The Kaplan-Meier curves of (D) PFS and (E) OS of patients stratified by decline of ctDNA (T1-T3 mVAF >50% v <50%). (F) Treatment response of patients stratified by decline of ctDNA (T1-T3 mVAF >50% v <50%). ctDNA, circulating tumor DNA; T1, baseline; T3, four cycles after therapy, mVAF, maximal somatic variant allelic frequency, TMB, tumor mutational burden.

sFigure 4



Supplement Figure4: (A) Mutation analysis of the corhort, a waterfall map of the genetic mutations in the study. (B)The Kaplan-Meier curves of PFS of patients stratified by baseline TMB, mVAF (C) and KRAS mutation (D).

sFigure 5



Supplement Figure5: Comparison of clinical features and biomarker changes in patients with resectable versus unresectable cancer. The comparison of age (A), longest tumor diameter (B) and reduction of tumor diameter (C) in patients with resectable versus unresectable. (D) Tumor status of patients with resectable versus unresectable. ctDNA decline (E) and CA19-9 decline (F) of patients with resectable versus unresectable. BRPC, borderline resectable pancreatic cancer; LAPC, locally advanced pancreatic cancer; CA19-9, carbohydrate antigen 19-9.

sTable 1: Summary of patients who underwent surgery

Patient	Resectability (Baseline)	Resectability (Preoperative)	Surgery method	Resection margin	Vessel Reconstruction	Tumor Regression	TNM stage	Postoperative complications (Clavien–Dindo classification and type of complication)
Patient 2	BRPC	BRPC	TPD	R0	PV	80%	IIA (T3N0M0)	3a, CL
Patient 8	LAPC	LAPC	PD	R0	SMV	80%	IA (T1N0M0)	0
Patient 13	BRPC	BRPC	PD	R0	SMV	70%	IA (T1cN0M0)	2, POIC
Patient 16	LAPC	LAPC	TP-CAR	R1	PV	70%	IIB (T2N1M0)	0
Patient 17	LAPC	LAPC	TP-CAR	R0	PV+HA	>95%	IB (T2N0M0)	4, AKI
Patient 20	BRPC	Resectable	RAMPS	R0	No	70%	IB (T2N0M0)	3a, POPF
Patient 22	BRPC	BRPC	PPPD	R0	SMV	100%	0 (T0N0M0)	2, POIC
Patient 23	BRPC	BRPC	TPD	R0	SMV	70%	IB (T2N0M0)	2, POIC
Patient 24	BRPC	Resectable	PPPD	R0	No	80%	IA (T1cN0M0)	2, POIC and CL
Patient 25	BRPC	BRPC	PPPD	R0	No	100%	0 (T0N0M0)	3a, POPF

BRPC: borderline resectable pancreatic cancer; LAPC: locally advanced pancreatic cancer; TPD: total pancreatoduodenectomy; PD: pancreatoduodenectomy; TP-CAR: total pancreatoduodenectomy with en-bloc celiac axis resection; RAMPS: radical antegrade modular pancreatosplenectomy; PPPD: pylorus preserving pancreaticoduodenectomy; PV: hepatic portal vein; SMV: superior mesenteric vein; HA: hepatic artery; TNM: tumor node metastasis classification; CL: chyle leak; POIC: postoperative infectious complications; AKI: acute kidney injury; POPF: postoperative pancreatic fistula

Study Title: Study protocol for a prospective, open-label, single-arm, phase II clinical trial on the combination of tislelizumab, nab-paclitaxel, gemcitabine and concurrent radiotherapy as the preoperative therapy for patients with locally advanced and borderline resectable pancreatic cancer

Primary sponsor： Nanjing Drum Tower Hospital，The Affiliated Hospital of Nanjing University Medical School

Study leader： Baorui Liu, Juan Du

Protocol Version: V 1.0

Date: March 1st, 2020

1. BACKGROUND

Pancreatic ductal adenocarcinoma (PDAC) is a highly fatal disease with increasing incidence rates. It is predicted to become the third leading cause of cancer-related death in the United States by 2030 (1). The major reasons include limited effective therapeutic options and severe mortality rates. The standard treatment is surgery followed by adjuvant chemotherapy (2). However, only approximately 15-20% of all patients are deemed resectable and many of whom are potentially resectable (30-40%) including those with locally advanced pancreatic cancer (LAPC) and borderline resectable pancreatic cancer (BRPC). Further, approximately 50% of patients are diagnosed with distant metastasis (3-5). In addition, the 5-year overall survival (OS) rate remains in the single digits despite advances in medicine and surgical techniques (6) (7).

The purpose of adjuvant therapy is to reduce postsurgical recurrence and prolong survival (2). However, the main aim of neoadjuvant therapy is to improve the patient selection for surgical intervention and increase the potential R0 resection (8). A meta-analysis of FOLFIRINOX as a first-line treatment for LAPC analyzed 13 studies and revealed that the median OS is 24.2 months and the median progression free survival (PFS) is 15 months (9). Another meta-analysis of 14 studies involving 365 patients with LAPC concluded that the median OS is 8.9-25.0 months and the resection rate is 28% (10). In addition, the median OS of patients with BRPC who received neoadjuvant therapy such as FOLFIRINOX and gemcitabine-based chemoradiotherapy was approximately 17-22.2 months and the R0 resection rate of patients in the immediate surgery group was almost half that of patients treated with neoadjuvant therapy (20-40% vs 50-80%) (11) (12) (13). A study demonstrated that the median OS for patients with BRPC and LAPC who underwent resection after neoadjuvant therapy was 37.7 months (14). The currently used neoadjuvant regimens for PDAC are FOLFIRINOX (5-fluorouracil, oxaliplatin, irinotecan and leucovorin) and gemcitabine and/or nab-paclitaxel (15). Besides chemotherapy, radiotherapy (RT) is included in neoadjuvant therapy, especially stereotactic body radiotherapy (SBRT), which has been proved to be safe and effective (16).

Recently, immune checkpoint inhibitor (ICI) with PD-1/PD-L1 antibodies has displayed remarkable efficacy in several cancers, particularly lung cancer, melanoma and renal cancer (17). Nevertheless, the treatment of PDAC with a single PD-1 inhibitor was not effective since most patients had a low tumor mutational burden (TMB-L) (18). Some reports have shown that the anti-PD-1 antibodies applied to neoadjuvant chemoimmunotherapy exhibited promising effects for the treatment of gastric cancer (19), esophageal squamous cell carcinoma (20), and non-small cell lung cancer (21). We conducted a phase II study enrolling 50 patients with LAPC or BRPC to evaluate the safety and efficacy of this regimen (tislelizumab plus gemcitabine and nab-paclitaxel (AG) and sequential SBRT).

Circulating tumor DNA (ctDNA) containing tumor-specific DNA mutations can be detected in the cell-free component of peripheral blood in most patients with PDAC (22). For resectable pancreatic cancer, post-surgical ctDNA detection was an independent negative predictor of decreased recurrence-free survival and OS (23). As for localized pancreatic cancer, detectable ctDNA post-operatively appeared higher risk of recurrence on gemcitabine-based adjuvant therapy (24). For early cancer detection, ctDNA assays hold substantial potential as a cancer screening test (25). However, whether the genomic features and the serial ctDNA status can predict patients' outcomes in the context of chemoimmunotherapy is still under investigation. In this study, we aim to investigate the role of genomic mutation features and serial ctDNA dynamic change in predicting tumor response and outcomes in patients with LAPC and BRPC. This trial is expected to demonstrate the feasibility of the combined therapeutic approach as the neoadjuvant treatment in patients with LAPC and BRPC and provide evidence for further research on the same.

2. STUDY OBJECTIVES

2.1 Primary Objective

To evaluate the efficacy of tislelizumab plus nab-paclitaxel, gemcitabine and concurrent radiotherapy as preoperative therapy in patients with locally advanced and borderline resectable pancreatic cancer.

2.2 Secondary Objective

To evaluate the safety and feasibility of tislelizumab plus nab-paclitaxel, gemcitabine and concurrent radiotherapy as preoperative therapy in patients with locally advanced and borderline resectable pancreatic cancer.

2.3 Exploratory Study Objectives

To explore the biomarkers related to the efficacy of tislelizumab plus nab-paclitaxel, gemcitabine and concurrent radiotherapy as preoperative therapy in patients with locally advanced and borderline resectable pancreatic cancer.

3. STUDY ENDPOINT

3.1 Primary Endpoint

Objective response rate (ORR) and R0 resection rate.

3.2 Secondary Endpoints

Overall survival (OS), progression-free survival (PFS), disease control rate (DCR), pathological grade of tumor tissue after therapy and adverse reaction.

3.3 Exploratory Endpoint

Correlation between biomarkers and therapeutic response to therapy.

4. STUDY POPULATION

Historical data showed that the ORR rate after neoadjuvant chemotherapy (LAPACT) in patients with locally advanced PC was 33.6%. We estimated that a sample size of 26 patients would detect approximately 26 % improvement (60%) in ORR rate with a power of 80%, using a one-sided alpha of 0.025. Assuming a 10% drop-out rate, a total of 29 treatment-naive patients with locally advanced and borderline resectable pancreatic cancer were planned to accrue in our study.

4.1 Inclusion Criteria

a. Subjects with age >= 18 years and ECOG score of 0-1;

b. Subjects with pancreatic cancer confirmed by histology or cytology;
c. The patients with potentially resectable pancreatic cancer were imaged;
d. The subjects should meet the following hematological indexes: Neutrophil count >= 1.5 * 10^9/L, Hemoglobin >= 10g/dl Platelet count >= 100 * 10^9/L;
e. The subjects should meet the following biochemical indicators: Total bilirubin <= 1.5* ULN; AST and ALT < 1.5* ULN; Creatinine clearance rate >= 60ml / min;
f. Subjects of childbearing age need to take appropriate protective measures (contraceptive measures or other methods of birth control) before entering the group and during the test.
g. Subjects who have signed informed consent;
h. Subjects who were able to follow the protocol and follow-up procedures.

4.2 Exclusion Criteria

a. Patients who have received systematic anti-tumor treatment.
b. Patients with previous history of other tumors, except for cervical cancer in situ, treated squamous cell carcinoma or bladder epithelial tumor (TA and TIS) or other malignant tumors that have received radical treatment (at least 5 years before enrollment).
c. Patients with active bacterial or fungal infection (>= level 2 of NCI-CTC, 3rd Edition).
d. Patients with HIV, HCV, HBV infection, uncontrollable coronary artery disease or asthma, uncontrollable cerebrovascular disease or other diseases considered by researchers to be out of the group.
e. Patients with autoimmune diseases or immune defects who are treated with immunosuppressive drugs.
f. Pregnant and lactating women. Pregnant women of childbearing age must be tested negative within 7 days before entering the group.
g. Patients with drug abuse, clinical or psychological or social factors make informed consent or research implementation affected.
h. Patients who may be allergic to PD-1 monoclonal antibody immunotherapy drugs.

5. STUDY DESIGN AND PLAN

5.1 Discussion of Study Design

This is a single-arm clinical study aimed to evaluate the clinical efficacy and safety of tislelizumab plus nab-paclitaxel, gemcitabine and concurrent radiotherapy as preoperative therapy in patients with locally advanced and borderline resectable pancreatic cancer. Treatment-naive Patients who pathologically and imaging confirmed locally advanced and borderline resectable pancreatic cancer will be enrolled in this study after sign the ICF.

5.2 Drugs and Treatments Administered

5.2.1 Drugs

Anti-PD-1 inhibitor (Tislelizumab)**：** Tislelizumab is administered by intravenous infusion at a recommended dose of 200 mg once every three weeks until disease progression, intolerable toxicity,.

Chemotherapy： Gemcitabine 1000mg/m2 and nab-paclitaxel 125mg/m2 are administered by intravenous infusion on day 1 and day 8.

Radiotherapy: SBRT every day with a total dose of 30Gy/10f at PTV and 50Gy/10f at PGTV simultaneously with the third cycle of chemotherapy and immunotherapy.

Surgery and adjuvant therapy: Surgery is evaluated after 4 cycles of the treatment. Patients with resection will receive at least 2 cycles of adjuvant therapy whose regimen is the same as preoperative therapy.

The combination regimen was continued until disease progression, intolerable toxicity, or complete surgery and adjuvant therapy.

5.2.2 Adverse Drug Reactions and Dose adjustment

Anti-PD-1 inhibitor : Tislelizumab dose adjustment is not permitted throughout the study, and the principles for dose interruption and permanent discontinuation of tislelizumab are shown in the table below.

Tislelizumab - related AEs	Severity	Dose adjustment
Pneumonia	Grade 2 pneumonia	Dose interruption[a]
	Recurrent grade 2 pneumonia, grade 3 or 4 pneumonia	Permanent discontinuation
Diarrhea/colitis	Grade 2 or 3 diarrhea or colitis	Dose interruption[a]
	Grade 4 diarrhea or colitis	Permanent discontinuation
Hepatitis	Grade 2 AST, ALT, or TBIL elevation for subjects with normal AST, ALT, or TBIL at baseline; AST, ALT, or TBIL elevation by ≥ 50% (meeting the criteria for grade 2) lasting for < 7 days for subjects with AST, ALT, or TBIL > ULN at baseline	Dose interruption[a]
	Grade 3 or 4 AST, ALT, or TBIL elevation for subjects with normal AST, ALT or TBIL at baseline; AST, ALT, or TBIL elevation of ≥ 50% (reaching the requirements of grade 3 or 4) for ≥ 7 days for subjects with AST, ALT, or TBIL > ULN at baseline	Permanent discontinuation
Skin adverse reactions	Grade 3	Dose interruption[a]
	Grade 4 Stevens-Johnson syndrome (SJS) or toxic epidermal necrolysis (TEN)	Permanent discontinuation
Hypophysitis	Grade 2 or 3 hypophysitis	Dose interruption[b]
	Grade 4 hypophysitis	Permanent discontinuation
Adrenocortical insufficiency	Grade 2 adrenocortical insufficiency	Dose interruption[b]
	Grade 3 or 4 adrenocortical insufficiency	Permanent discontinuation
Thyroid function abnormal	Symptomatic grade 2 or 3 hypothyroidism, or grade 2 or 3 hyperthyroidism	Dose interruption[b] Permanent discontinuation

Tislelizumab - related AEs	Severity	Dose adjustment
	Grade 4 hypothyroidism, or grade 4 hyperthyroidism	Permanent discontinuation
Diabetes/hyperglycemia	Grade 3 hyperglycemia	Dose interruption[b]
	Grade 4 hyperglycemia	Permanent discontinuation
Nephritis	Grade 2 or 3 increased blood creatinine	Dose interruption[a]
	Grade 4 increased blood creatinine	Permanent discontinuation
Thrombocytopenia	Grade 3 thrombocytopenia	Dose interruption[a]
	Grade 4 thrombocytopenia	Permanent discontinuation
Other AEs	Grade 3 or 4 hyperamylasemia or lipase increased Grade 2 or 3 pancreatitis Grade 2 myocarditis* Grade 2 or 3 other AEs (first occurrence)	Dose interruption[a]
	Grade 4 pancreatitis or relapsed pancreatitis of all grades Grade 3 or 4 myocarditis Grade 3 or 4 encephalitis Grade 4 other AEs (first occurrence)	Permanent discontinuation [c]

a: Resume administration of the study drug after symptoms improve to grade 0–1 or baseline levels.

* The safety of resuming treatment with tislelizumab after myocarditis returns to Grade 0–1 in severity is yet unclear

b: Resume the administration if hypophysitis, adrenocortical insufficiency, thyroid function insufficiency/hypothyroidism, or type I diabetes mellitus is adequately controlled and only physiological hormone replacement therapy is required.

c: For grade 4 laboratory abnormalities, whether to terminate the treatment shall be determined based on clinical signs/symptoms and the clinical judgment of the investigator.

Permanently discontinue tislelizumab if treatment-related adverse reactions do not return to Grade 0–1 or the baseline level within 12 weeks after the last dose, except：

1) If corticosteroids are used for the treatment of immune-related adverse reactions, the maximum discontinuation of tislelizumab due to corticosteroid reduction should not exceed 12 weeks. In these cases, a comprehensive investigator evaluation is required to determine whether tislelizumab can be continued. Imaging tests to efficacy assessment were performed as planned and were not affected by the drug suspension.

2) Tislelizumab was suspended for more than 12 weeks due to treatment of AE unrelated to itself. In these cases, a comprehensive investigator evaluation is required to determine whether tislelizumab can be continued. Imaging tests to efficacy assessment were performed as planned and were not affected by the drug suspension.

5.3 The principle of Tislelizumab Resume Administration

Resume administration of Tislelizumab after symptoms improve to grade 0–1 or baseline levels and ECOG PS 0-1.

5.4 Management of tislelizumab infusion-related reactions

Tislelizumab may lead to severe or life-threatening infusion-related reactions, including severe hypersensitivity or allergic reactions. Signs and symptoms usually occur during or after the drug infusion and usually resolve within 24 h after the infusion completion. The guidelines for management of tislelizumab infusion-related reactions are shown in the table below.

Table 3. Guidelines for management of tislelizumab infusion-related reactions

NCI CTCAE Grades	Treatment	Premedications for subsequent infusions
Grade 1 Mild reaction; infusion interruption not indicated; intervention not indicated	According to patient's medical indications, monitor the vital signs closely until the subject is stable as determined by the investigator.	None
Grade 2 Treatment or infusion interruption required, but responds promptly to timely symptomatic treatment (e.g. antihistamines, non-steroidal anti-inflammatory drugs [NSAIDS], anesthetics, intravenous fluids replacement); prophylactic medications indicated for ≤ 24 h	Stop the infusion and monitor symptoms. Other appropriate treatments include but are not limited to: IV infusion Antihistamines NSAIDS Acetaminophen anesthetics According to patient's medical indications, monitor the vital signs closely until the subject is stable as determined by the investigator. If symptoms resolve within 1 h after the interruption of the infusion, then the infusion can be resumed at 50% of the original infusion rate (e.g. from 100 mL/h to 50 mL/h). Otherwise, interrupt the treatment until symptoms resolve. Premedications should be given for subsequent infusions. If grade 2 toxicities occur despite of adequate premedications, the study drugs should be permanently discontinued.	The following premedications are recommended within 1.5 h (± 30 min) prior to tislelizumab infusion: Diphenhydramine 50 mg PO (or equivalent antihistamines). Acetaminophen 500–1000 mg PO (or equivalent antipyretics).
Grade 3 or 4 Grade 3: Prolonged (i.e. not rapidly responsive to symptomatic medication and/or brief	Discontinue the infusion. Other appropriate treatments include but are not limited to: Epinephrine**	Not applicable

NCI CTCAE Grades	Treatment	Premedications for subsequent infusions
interruption of infusion); recurrence of symptoms following initial improvement; hospitalization indicated for other clinical sequelae (e.g. renal impairment, pulmonary infiltration) Grade 4: Life threatening; pressors or ventilatory support indicated	IV infusion Antihistamines NSAIDS Acetaminophen anesthetics Oxygen Pressors Corticosteroids According to patient's medical indications, monitor the vital signs closely until the subject is stable as determined by the investigator. Hospitalization may be indicated. **Epinephrine should be used immediately for allergic reactions. The study drugs should be permanently discontinued.	
Appropriate first-aid equipment should be provided in the ward and physicians should be available at all times during the administration. For more information, refer to "Common Terminology Criteria for Adverse Events" (CTCAE) V5.0 (http://ctep.cancer.gov)		

6. EFFICACY EVALUATION AND SAFETY EVALUATION

6.1 Efficacy Evaluation

RECISTv1.1(see appendix 1) will be mainly used for this study, and tumor response will be assessed every 6 weeks. Meanwhile, subject will follow up with accepted examination of blood routine, liver and kidney functional, electrolytes, coagulation function, tumor indicators, immune-related indicators, etc. Exploration for resection is considered according to National Comprehensive Cancer Network (NCCN) Guideline. The postsurgical pathology of patients will be graded according to the American Joint Committee on Cancer (AJCC).

6.2 Safety Evaluation

The following safety and tolerance of treatment will be evaluated in this study

- AE： in this study， AEs will be reported by the subject (or a caregiver, surrogate, or legal representative of the subject).
- ECOG PS score（see appendix 2）
- Clinical laboratory tests
- Vital signs (pulse, temperature, blood pressure, respiratory rate)

➢ Physical examination

Any clinically significant abnormalities that persist at the end of the study will be closely followed until the problem is resolved or the patient reaches a clinically stable endpoint. Safety analysis is the analysis of the incidence of AEs. AEs will be reported from the time subjects signed the ICF until 30 days after the last dose, and AEs were assessed at each follow-up cycle according to the National Cancer Institute Common Naming Standard for Adverse Events (NCI CTCAE) version 5.0.

6.3 Monitoring Indicators

(1) Patient's vital signs: temperature, pulse, respiration, blood pressure

(2) Blood routine, urine routine, fecal routine (including at least the beginning and end of each cycle)

(3) Liver and kidney function, electrolytes, coagulation function, amylase, blood lipids, creatine kinase, thyroid function, antinuclear antibodies, lymphocyte subsets analysis, tumor indicators (at least including AFP, PVIKA, CEA, CA199)

(4) Circulating tumor DNA (ctDNA) (including baseline, the first and the second clinical evaluation)

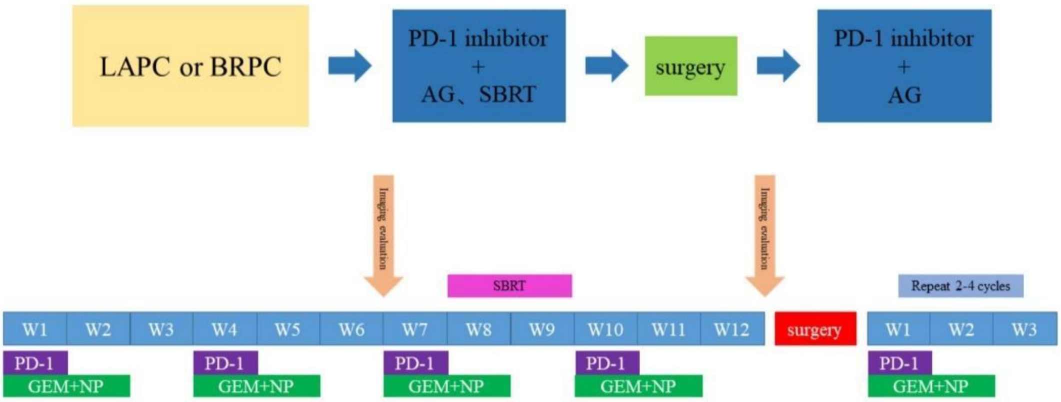
(5) PET-CT(consistent with baseline)

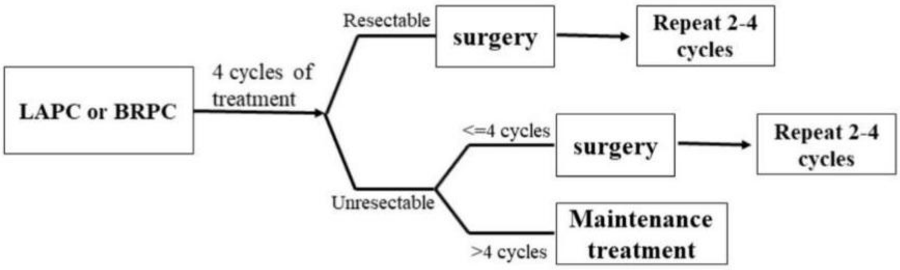
(6) CT of pelvic cavity and lung (if necessary)

(7) bone scanning (if necessary)

(8) NGS gene test (if patients agree)

7. STUDY PROCEDURES





8 DRUG MANAGEMENT

8.1 Management of Tislelizumab

The study drug of this study is tislelizumab. All experimental drugs are transported to each site in cold chain, and should be kept and distributed by special personnel.

The investigational drugs should be stored in a refrigerator only accessible to the authorized personnel. After receiving the study drugs, the investigator should ensure that the temperature during transport is maintained within the specified range, sign for receipt upon verification, and store the drugs at the specified temperature. If abnormalities of the storage temperature during either the transportation or storage at the study site arise, the drugs should be moved to an environment in the specified temperature as soon as possible and should not be administered to the subjects at the moment. Innovent should be timely notified and the advice of Innovent should be followed.

All the study drugs provided by the sponsor should only be used for this clinical trial. Any usage of the study drug other than those specified in the protocol are prohibited. The investigator must agree not to provide the investigational drugs to anyone unrelated to this trial.

8.2 Drug Return and Destruction

The used containers for tislelizumab injections can be destroyed on-site according to the appropriate guidelines and operating procedures established by study sites and local authorities. Upon the completion or discontinuation of the study, all unused or expired study drugs must be returned for destruction.

8.3 Study Drug-related Records

The designated personnel of the Site shall make timely records of the receipt, distribution, use, inventory, destruction, recovery and destruction of the drugs in accordance with the requirements of relevant regulations and guidelines.

9. ADVERSE EVENT REPORTING

9.1 Definitions of Adverse Event

An adverse event (AE) is defined as any adverse unexpected medical event within the period from the signing of the informed consent form, regardless of whether or not considered as related to the study drug. AEs include but are not limited to the following:

- Worsening of pre-existing (before enrollment) medical conditions/diseases (including symptoms, signs, and laboratory test abnormalities);
- Any new adverse medical conditions (including symptoms, signs, and newly diagnosed diseases);
- Clinically significant laboratory test abnormalities.

AEs include SAEs and non-SAEs.

9.2 Definitions of Serious Adverse Event

According to ICH and EU pharmacovigilance guidelines for medical products for human use,
a serious adverse event (SAE) is an unexpected medical event that occurs at any drug dose and meets any one of the following criteria:

Leading to death;

Life-threatening (the subject is under threat of death at the time of the event, excluding events that may

theoretically lead to death if the situation becomes more severe);

Leading to hospitalization or prolonged hospitalization, excluding the following cases:

Rehabilitation facility

sanatorium

Routine emergency room admission

Same-day surgery (e.g. outpatient/same-day/ambulatory surgery)

Hospitalization or prolonged length of stay that is not associated with SAE. For example, there were no new adverse events or exacerbations of pre-existing conditions (e.g., to check for laboratory abnormalities that persisted prior to the trial); Hospital admissions for management reasons (e.g., annual routine medical examinations); Hospitalization during the clinical trial as specified by the protocol (e.g., operation as required by the protocol); Elective hospitalizations not associated with worsening AE (e.g., elective surgery); Scheduled treatments or surgical procedures should be documented throughout the protocol and/or in the baseline data of the individual subject; Hospital admission solely for blood use.

Leading to a permanent or significant disability/function loss;

Causing deformities/birth defect;

Being suspected to transmit any source of infection through the study product;

Significant medical events*.

*The decision to adopt rapid reporting process in situations other than those listed above shall be made based on medical and scientific judgment. For example, significant medical events may not be immediately life-threatening or lead to death or hospitalization, but may harm the patient or may require therapeutic intervention to prevent occurrence of the above situations. They are usually considered SAEs.

9.3 Assessment of Adverse Events

The investigator will evaluate all AEs according to the NCI "Common Terminology Criteria for Adverse Events" (CTCAE) V5.0. AEs with altered CTCAE grade will be documented in the AE case report form (CRF)/worksheet. All AEs, regardless of the CTCAE grade, must be assessed for whether they are SAEs or not.

9.4 AE Documentation

The investigator should document AEs and SAEs using medical terms and concepts. Colloquialisms/abbreviations should be avoided. All AEs (including SAEs) shall be documented on the AE forms in the CRFs.

9.5 Adverse Event Collection and Times

The investigator should learn about AEs by asking the subjects non-leading questions. After signing the informed consent form but before starting the study treatment, only the SAEs caused by the interventions procedures specified in the study protocol (for example, invasive procedures such as biopsy) should be reported.

All AEs, including SAEs, that occur from the initiation of the treatment with the study drug to 30 days after the last dose or patient starts a new treatment （Whichever comes first）shall be collected, regardless of whether they are related to the study treatment and whether they are observed by the investigator or self-reported by the subject. Thereafter, the investigator shall report any SAEs that are considered related to the study drugs or procedures in 30 days after the last dose.

9.6 Follow-up of AEs

The AE should be followed until the events return to the baseline values or grade 0–1, or until the investigator believes that no further follow-up is required for reasonable reasons (if the event cannot be resolved or has already been improved). If the event cannot be resolved, a reasonable explanation should be documented in the CRF. The outcome of an AE/SAE and the date should be documented in the CRF and medical record, regardless of whether the event is related to the study drugs.

9.7 Contents of AE Documentation

The investigator should document the complete information of any AE, including diagnosis (in the absence of diagnosis, symptoms and signs including laboratory test abnormalities should be documented), time and date of occurrence (if applicable), CTCAE severity grade and alteration (for events ≥ grade 3), whether it is an SAE, measures taken for the study drugs, treatment for the AE, outcome of the event, and causality between the event and study drugs.

For an SAE, the investigator shall also provide the date when the AE meets the criteria for an SAE, the date when the investigator is informed of the SAE, the reason of being an SAE, date of hospitalization, date of discharge, possible cause of death, date of death, whether an autopsy has been performed, causality assessment of the study procedures, causality assessment of other drugs, and other possible causes of the SAE. The investigator shall provide the rationales of the causality and a description of the SAE. In the SAE description, the following shall also be included: number, age, gender, height, and weight of the subject; indication for and the stage when receiving the investigational drug, and overall condition; clinical disease course including occurrence, development, outcome, and result of the SAE; laboratory results related to the SAE (the time of the examination, units, and normal ranges must be provided); medical history, onset and duration of concurrent diseases related to the SAE; medication history and initiation, duration, and dosage of concomitant medications related to the SAE; initiation, duration, and dosage of the study drug.

Descriptions of the AE are as follows:

- Diagnosis, signs, and symptoms

The diagnosis, if there is one, should be documented in the CRF rather than individual signs and symptoms (e.g. hepatic failure rather than jaundice, transaminases increased, and flapping tremor). Signs and symptoms should be reported as separate AEs/SAEs if unable to be attributed to the diagnosis. If it is determined that the signs and symptoms are caused by the diagnosis, then only the diagnosis which includes the signs and symptoms shall be reported. The record of signs and symptoms shall then be deleted for AE. An updated follow-up report shall be submitted for in the case of SAE.

- AEs secondary to other events

Generally, AEs secondary to other events (such as result of another event or clinical sequelae) should be documented as the primary event, unless the event is severe or is an SAE. However, clinically significant secondary events should be recorded as independent AEs in the eCRFs if they occur at different time points from the primary event. If the relationship between events is unclear, document them as separate events in the CRFs.

- Persistent or recurrent AEs

A persistent AE refers to an event that does not resolve and is ongoing between two assessment time points. These AEs should only be documented once in the CRFs. The initial severity level should be documented, and the information should be updated if the event exacerbates to record the most severe level of the event.

Recurrent AEs refer to AEs that have resolved between the two time points of assessment but subsequently occur again. These events should be independently documented in the eCRFs.

- Laboratory test abnormalities

All clinically significant laboratory test abnormalities are reported as AEs. The investigators have responsibilities to review all the laboratory test abnormalities and determine whether the abnormalities should be reported as AEs.

- Death

During the entire course of the study, all deaths should be documented in the Mortality Report Form in the CRFs, regardless of the causal relationship with the study drug. If the investigator judges the death that occurs during the AE reporting period specified in the study protocol as separate disease progression, the death should only be recorded on the CRF page of study completion/early termination. All other deaths in the study, regardless of the relationship with the study drugs, must be recorded as AE and reported to relevant authorities.

- Pre-existing medical conditions

Symptoms/signs presenting during the screening period will be recorded and reported as AEs only if their severity level, frequency, or property becomes aggravated (except for worsening of the disease under study). The relative change from previous condition should be expressed, such as "increased frequency of headaches".

- Disease progression

Disease progression is defined as the worsening of subject condition, the appearance of new lesions, or the progression of the primary lesion, caused by the primary tumor that the investigational drug is targeting. Expected disease progression should not be reported as an AE. Any deaths, life threatening events, hospitalization or prolonged hospitalization, permanent or significant disability/incapacity, congenital anomaly/birth defects, or other important medical events resulted from symptoms and signs of the expected disease progression should not be reported as an SAE.

- New anti-tumor therapy

If the subject initiates new anti-tumor therapy within 30 days after the last dose, then only SAEs considered to be related to the study drugs are required to be documented and reported.

9.8 SAE and Pregnancy Rapid reporting

SAE report

The SAE reporting period is for serious adverse events occurring within 30 days (inclusive) from the signing of informed consent to the date of the last dose. All SAEs as determined by the investigator shall be recorded in a "Serious Adverse Event Report Form" within 24 h after the investigator is informed, and then reported to Innovent (drugsafety@innoventbio.com) and to the national regulatory authorities and ethics committees in accordance with Chinese regulatory requirements. SAEs that occurred outside of this period but are considered to be related to the study drug should also be reported.

Pregnancy

Safety risk of embryotoxicity exists in the similar kind of drugs. All subjects with childbearing potential participating in the clinical trial must take effective contraceptive measures.

During the study, if a female subject exposed to the study drugs becomes pregnant, the subject must be withdrawn from the study. The investigator must report to Innovent within 24 h of learning the pregnancy and fill in the "Innovent Clinical Study Pregnancy Report/Follow-Up Form". During the study, if the female partner of a male subject exposed to the study drugs becomes pregnant, the subject will continue the study. The investigator must report to Innovent within 24 h of learning the pregnancy and fill in the "Innovent Clinical Study Pregnancy Report/Follow-Up Form".

The investigator must continuously monitor and follow up on the outcome of the pregnancy until 8 weeks after the delivery. The outcome shall be reported to Innovent.

If the outcome of the pregnancy is stillbirth, spontaneous abortion, fetal malformation (any congenital anomaly/birth defects), or induced abortion for medical reasons, it should be considered as an SAE, and the event is required to be reported in accordance with SAE procedures and time limits.

If the subject also experiences an SAE during pregnancy, the event should be reported according to SAE procedures.

9.9 Immune-related AEs

Given that the mechanism of tislelizumab is to induce T-cell activation and proliferation, it is possible that immune-related adverse events (irAE) were observed during this study. Subjects should be monitored for signs and symptoms of irAE. If there is no clear alternative cause (e.g., infection), the signs or symptoms of the disease occurring in the subject should be considered to be immune system related.

The guidelines for tislelizumab dose adjustment and management of adverse events are described in Sections 5.4 of protocol.

10. QUALITY ASSURANCE AND QUALITY CONTROL

According to the GCP guidelines, it is the sponsor's responsibility to implement and maintain a quality assurance and quality control system in accordance with the corresponding standard operating procedures to ensure that the implementation of clinical trials as well as the data collection, recording, and reporting comply with the requirements of the protocol, GCP, and local laws and regulations.

11. ETHICS

11.1 Ethics Committee

The sponsor or designee will prepare the relevant documents including the trial protocol, ICF, Investigator's Brochure, subject recruitment materials or advertising, and other documents required by regulations, which are to be submitted to the corresponding Ethics Committee (EC) in of the study site for approval. The written approval from the EC must specify the name, number, version number of the study protocol and other documents (such as ICF), and date of approval. The investigator shall notify the sponsor of written comment on the delay, suspension, or reapprove from EC.

The study site must follow the requirements of the EC in the study site. This may include revisions on protocol, ICF, and recruitment materials should be submitted to the EC for approval. Local safety reports should be made and updated regularly in accordance with the regulations from the EC, and the final report should be submitted. All the above documents and EC approvals must be provided to the sponsor or designee.

11.2 Ethics of this Study

The implementation of this study and the access to Informed consent form shall comply with the requirements of the ICH, GCP, and local laws and regulations.

The GCP is an international ethical and scientific specification for designing, conducting, recording and reporting clinical trials that involve the participation of human subjects. This study will be conducted in accordance with the GCP and relevant national regulations and in accordance with the relevant ethical principles of "the Declaration of Helsinki" to protect the rights, safety, and health of the subjects.

The investigator is required to follow the procedures specified in this protocol and must not change the procedures without the permission from the sponsor. Protocol deviations, if any, must be reported to the EC, sponsor, or regulatory authorities.

11.3 Subject Information and Informed Consent Form

Before the start of any study procedure, the informed consent form (ICF) is used to explain the risks and benefits of this study to potential participants. The language used on the informed consent form should be straightforward. It should be clarified in the ICF statement that the ICF is voluntarily signed, and the risks and benefits of participating in this study should be clearly outlined. It should also be pointed out that subjects are free to withdraw from the study at any time. The investigator may only enroll a subject after fully explaining the details of the study, answering questions to the subject's satisfaction, giving the subject sufficient time for consideration, and obtaining written consent from the subject or the subject's legal representative. All signed ICFs must be kept in the investigator files or in the subject folders.

The investigator is responsible for explaining the contents of the ICF and obtaining the ICF signed and dated by the subject or the subject's legal representative prior to the study. After that, the investigator should provide the subject with a copy of the signed ICF. The investigator must record the process of informed consent in the source documents of the trial.

11.4 Data Protection

An ICF shall include (or in some cases, use separate files together) information on data and privacy protection.

Take precautions to ensure the confidentiality of the documents and prevent the disclosure of information that can identify a subject. However, under special circumstances, some personnel may have access to the genetic data and individual identification number of a subject. For example, in the event of a medical emergency, the sponsor, designated physician, or investigator will have access to the subject identification code and the subject's genetic data. In addition, relevant regulatory authorities may require access to relevant documents.

12. STUDY MANAGEMENT

12.1 Data Handling and Records Retention

The investigator is responsible for retaining and managing all study documents in accordance with GCP requirements, including but not limited to the protocol, eCRF, and signed ICF. These documents should be kept on file at the site until 5 years after the end of the study.

Study documents should be properly preserved for future access or data traceability. Safety and environmental risks should be considered in records retention.

12.2 Raw Data/File Access

The investigator agrees that the sponsor, CRO, and relevant authorized regulatory authorities have direct access to all study-related documents, including subjects' medical records.

12.3 Changes to the Protocol

Any change to the protocol during the study must be approved by the sponsor and investigators before implementation.

All amendments of the protocol shall be maintained as supplements. Any change to the protocol shall be submitted to the Ethics Committee for approval, and amendments shall be approved or filing in accordance with ethics Committee regulations.

12.4 Responsibilities of Investigator

The investigator will conduct the study in strict accordance with the protocol, ICH, GCP, and local laws and regulations. The relevant detailed responsibilities of the investigators are listed in Chapter 5 (Responsibilities of the Investigators) of the Chinese GCP (Office Order No. 3).

12.5 Publication of Results

All data generated in this study are confidential. The sponsor has the right to publish the research results. Information on publication policies between each sponsor and investigator will be described in the clinical trial agreement

All information about this study (not limited to the following documents: study protocol, investigator's brochure) must be strictly confidential. The investigator must recognize that the scientific or medical information derived from the study may be of commercial value to the sponsor. Therefore, the information and data relevant to this study should be kept confidential by the investigator. Manuscripts, abstracts, or other modes of presentation arising from the results of the study must be reviewed and approved in writing by the sponsor in advance. In order to protecting the sponsor's proprietary, the sponsor may request that the investigator refrain from publishing information about the study until the product is approved for marketing.

The sponsor has the right to publish information or data related to the study, or to report it to the drug administration. The sponsor must obtain the consent of the investigator for the use of the investigator's name in publication or advertising.

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Appendix 1

Response Evaluation Criteria in Solid Tumors RECIST Version 1.1 (Excerpt)

Measurability of tumour at baseline

Definitions

At baseline, tumour lesions/lymph nodes will be categorized measurable or non-measurable as follows:

3.1.1. Measurable

Tumour lesions: Must be accurately measured in at least one dimension (longest diameter in the plane of measurement is to be recorded) with a minimum size of:

• 10mm by CT scan (CT scan slice thickness no greater than 5 mm; see Appendix II on imaging guidance).

• 10mm caliper measurement by clinical exam (lesions which cannot be accurately measured with calipers should be recorded as non-measurable).

• 20mm by chest X-ray.

Malignant lymph nodes: To be considered pathologically enlarged and measurable, a lymph node must be P15mm in short axis when assessed by CT scan (CT scan slice thickness recommended to be no greater than 5 mm). At baseline and in follow-up, only the short axis will be measured and followed (see Schwartz et al. in this Special Issue15). See also notes below on 'Baseline documentation of target and non-target lesions' for information on lymph node measurement.

3.1.2. Non-measurable

All other lesions, including small lesions (longest diameter <10mm or pathological lymph nodes with P10 to <15mm short axis) as well as truly non-measurable lesions. Lesions considered truly non-measurable include: leptomeningeal disease, ascites, pleural or pericardial effusion, inflammatory breast disease, lymphangitic involvement of skin or lung, abdominal masses/abdominal organomegaly identified by physical exam that is not measurable by reproducible imaging techniques.

3.1.3. Special considerations regarding lesion measurability

Bone lesions, cystic lesions, and lesions previously treated

with local therapy require particular comment:

Bone lesions:

• Bone scan, PET scan or plain films are not considered adequate imaging techniques to measure bone lesions. However, these techniques can be used to confirm the presence or disappearance of bone lesions.

• Lytic bone lesions or mixed lytic-blastic lesions, with identifiable soft tissue components, that can be evaluated by cross sectional imaging techniques such as CT or MRI can be considered as measurable lesions if the soft tissue component meets the definition of measurability described above.

• Blastic bone lesions are non-measurable.

Cystic lesions:

• Lesions that meet the criteria for radiographically defined simple cysts should not be considered as malignant lesions (neither measurable nor non-measurable) since they are, by definition, simple cysts.

• 'Cystic lesions' thought to represent cystic metastases can be considered as measurable lesions, if they meet the definition of measurability described above. However, if noncystic lesions are present in the same patient, these are preferred for selection as target lesions.

Lesions with prior local treatment:

• Tumour lesions situated in a previously irradiated area, or in an area subjected to other loco-regional therapy, are usually not considered measurable unless there has been demonstrated progression in the lesion. Study protocols should detail the conditions under which such lesions would be considered measurable.

3.2. Specifications by methods of measurements

3.2.1. Measurement of lesions

All measurements should be recorded in metric notation, using calipers if clinically assessed. All baseline evaluations should be performed as close as possible to the treatment start and never more than 4 weeks before the beginning of the treatment.

3.2.2. Method of assessment

The same method of assessment and the same technique should be used to characterise each identified and reported lesion at baseline and during follow-up. Imaging based evaluation should always be done rather than clinical examination unless the lesion(s) being followed cannot be imaged but are assessable by clinical exam.

Clinical lesions: Clinical lesions will only be considered measurable when they are superficial and P10mm diameter as assessed using calipers (e.g. skin nodules). For the case of skin lesions, documentation by colour photography including a ruler to estimate the size of the lesion is suggested. As noted above, when lesions can be evaluated by both clinical exam and imaging, imaging evaluation should be undertaken since it is more objective and may also be reviewed at the end of the study.

Chest X-ray: Chest CT is preferred over chest X-ray, particularly when progression is an important endpoint, since CT is more sensitive than X-ray, particularly in identifying new lesions. However, lesions on chest X-ray may be considered measurable if they are clearly defined and surrounded by aerated lung. See Appendix II for more details.

CT, MRI: CT is the best currently available and reproducible method to measure lesions selected for response assessment. This guideline has defined measurability of lesions on CT scan based on the assumption that CT slice thickness is 5mm or less. As is described in Appendix II, when CT scans have slice thickness greater than 5 mm, the minimum size for a measurable lesion should be twice the slice thickness. MRI is also acceptable in certain situations (e.g. for body scans). More details concerning the use of both CT and MRI for assessment of objective tumour response evaluation are provided in Appendix II.

Ultrasound: Ultrasound is not useful in assessment of lesion size and should not be used as a method of measurement. Ultrasound examinations cannot be reproduced in their entirety for independent review at a later date and, because they are operator dependent, it cannot be guaranteed that the same technique and measurements will be taken from one assessment to the next (described in greater detail in Appendix II). If new lesions are identified by ultrasound in the course of the study, confirmation by CT or MRI is advised. If there is concern about radiation exposure at CT, MRI may be used instead of CT in selected instances.

Endoscopy, laparoscopy: The utilisation of these techniques for objective tumour evaluation is not advised. However, they can be useful to confirm complete pathological response when biopsies are obtained or to determine relapse in trials where recurrence following complete response or surgical resection is an endpoint.

4. Tumour response evaluation

4.3.1. Evaluation of target lesions

Complete Response (CR): Disappearance of all target lesions. Any pathological lymph nodes (whether target or non-target) must have reduction in short axis to <10 mm.

Partial Response (PR): At least a 30% decrease in the sum of diameters of target lesions, taking as reference the baseline sum diameters.

Progressive Disease (PD): At least a 20% increase in the sum of diameters of target lesions, taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest on study). In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm. (Note: the appearance of one or more new lesions is also considered progression).

Stable Disease (SD): Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum diameters while on study.

4.3.2. Special notes on the assessment of target lesions

Lymph nodes. Lymph nodes identified as target lesions should always have the actual short axis measurement recorded (measured in the same anatomical plane as the baseline examination), even if the nodes regress to below 10mm on study. This means that when lymph nodes are included as target lesions, the ‘sum’ of lesions may not be zero even if complete response criteria are met, since a normal lymph node is defined as having a short axis of <10mm. Case report forms or other data collection methods may therefore be designed to have target nodal lesions recorded in a separate section where, in order to qualify for CR, each node must achieve a short axis <10mm. For PR, SD and PD, the actual short axis measurement of the nodes is to be included in the sum of target lesions. Target lesions that become ‘too small to measure’. While on study, all lesions (nodal and non-nodal) recorded at baseline should have their actual measurements recorded at each subsequent evaluation, even when very small (e.g. 2mm). However, sometimes lesions or lymph nodes which are recorded as target lesions at baseline become so faint on CT scan that the radiologist may not feel comfortable assigning an exact measure and may report them as being ‘too small to measure’. When this occurs it is important that a value be recorded on the case report form. If it is the opinion of the radiologist that the lesion has likely disappeared, the measurement should be recorded as 0mm. If the lesion is believed to be present and is faintly seen but too small to measure, a default value of 5mm should be assigned (Note: It is less likely that this rule will be used for lymph nodes since they usually have a definable size when normal and are frequently surrounded by fat such as in the retroperitoneum; however, if a lymph node is believed to be present and is faintly seen but too small to measure, a default value of 5mmshould be assigned in this circumstance as well). This default value is derived from the 5mm CT slice thickness (but should not be changed with varying CT slice thickness). The measurement of these lesions is potentially non-reproducible, therefore providing this default value will prevent false responses or progressions based upon measurement error. To reiterate, however, if the radiologist is able to provide an actual measure, that should be recorded, even if it is below 5mm. Lesions that split or coalesce on treatment. As noted in Appendix II, when non-nodal lesions ‘fragment’, the longest diameters of the fragmented portions should be added together to calculate the target lesion sum. Similarly, as lesions coalesce, a plane between them may be maintained that would aid in obtaining maximal diameter measurements of each individual lesion. If the lesions have truly coalesced such that they are no longer separable, the vector of the longest diameter in this instance should be the maximal longest diameter for the ‘coalesced lesion’.

4.3.3. Evaluation of non-target lesions

This section provides the definitions of the criteria used to determine the tumour response for the group of non-target lesions.

While some non-target lesions may actually be measurable, need not be measured and instead should be assessed only qualitatively at the time points specified in the protocol. Complete Response (CR): Disappearance of all non-target lesions and normal is ation of tumour marker level. All lymph nodes must be non-pathological in size (<10mm short axis). Non-CR/Non-PD: Persistence of one or more non-target lesion(s) and/or maintenance of tumour marker level above the normal limits.

Progressive Disease (PD): Unequivocal progression (see comments below) of existing non-target lesions. (Note: the appearance of one or more new lesions is also considered progression).

4.3.4. Special notes on assessment of progression of non-target disease

The concept of progression of non-target disease requires additional explanation as follows:

When the patient also has measurable disease. In this setting, to achieve 'unequivocal progression' on the basis of the non-target disease, there must be an overall level of substantial worsening in non-target disease such that, even in presence of SD or PR in target disease, the overall tumour burden has increased sufficiently to merit discontinuation of therapy (see examples in Appendix II and further details below). A modest 'increase' in the size of one or more non-target lesions is usually not sufficient to quality for unequivocal progression status. The designation of overall progression solely on the basis of change in non-target disease in the face of SD or PR of target disease will therefore be extremely rare.

When the patient has only non-measurable disease. This circumstance arises in some phase III trials when it is not a criterion of study entry to have measurable disease. The same general concepts apply here as noted above, however, in this instance there is no measurable disease assessment to factor into the interpretation of an increase in non-measurable disease burden. Because worsening in non-target disease cannot be easily quantified (by definition: if all lesions are truly non-measurable) a useful test that can be applied when assessing patients for unequivocal progression is to consider if the increase in overall disease burden based on the change in non-measurable disease is comparable in magnitude to the increase that would be required to declare PD for measurable disease: i.e.an increase in tumour burden representing an additional 73% increase in ‘volume’ (which is equivalent to a 20% increase diameter in a measurable lesion). Examples include an increase in a pleural effusion from ‘trace’ to ‘large’, an increase in lymphangitic disease from localised to widespread, or may be described in protocols as ‘sufficient to require a change in therapy’. Some illustrative examples are shown in Figs. 5 and 6 in Appendix II. If ‘unequivocal progression’ is seen, the patient should be considered to have had overall PD at that point. While it would be ideal to have objective criteria to apply to non-measurable disease, the very nature of that disease makes it impossible to do so, therefore the increase must be substantial.

4.3.5. New lesions

The appearance of new malignant lesions denotes disease progression; therefore, some comments on detection of new lesions are important. There are no specific criteria for the identification of new radiographic lesions; however, the finding of a new lesion should be unequivocal: i.e. not attributable to differences in scanning technique, change in imaging modality or findings thought to represent something other than tumour (for example, some ‘new’ bone lesions may be simply healing or flare of pre-existing lesions). This is particularly important when the patient’s baseline lesions show partial or complete response. For example, necrosis of a liver lesion may be reported on a CT scan report as a ‘new’ cystic lesion, which it is not.

A lesion identified on a follow-up study in an anatomical location that was not scanned at baseline is considered a new lesion and will indicate disease progression. An example of this is the patient who has visceral disease at baseline and while on study has a CT or MRI brain ordered which reveals metastases. The patient’s brain metastases are considered to be evidence of PD even if he/she did not have brain imaging at baseline.

If a new lesion is equivocal, for example because of its small size, continued therapy and follow-up evaluation will clarify if it represents truly new disease. If repeat scans confirm there is definitely a new lesion, then progression should be declared using the date of the initial scan.

While FDG-PET response assessments need additional study, it is sometimes reasonable to incorporate the use of FDG-PET scanning to complement CT scanning in assessment of progression (particularly possible 'new' disease). New lesions on the basis of FDG-PET imaging can be identified according to the following algorithm:

a. Negative FDG-PET at baseline, with a positive l FDG-PET at follow-up is a sign of PD based on a new lesion.

b. No FDG-PET at baseline and a positive FDG-PET at follow-up:

If the positive FDG-PET at follow-up corresponds to a new site of disease confirmed by CT, this is PD.

If the positive FDG-PET at follow-up is not confirmed as a new site of disease on CT, additional follow-up CT scans are needed to determine if there is truly progression occurring at that site (if so, the date of PD will be the date of the initial abnormal FDG-PET scan).

If the positive FDG-PET at follow-up corresponds to a pre-existing site of disease on CT that is not progressing on the basis of the anatomic images, this is not PD.

4.4.2. Missing assessments and inevaluable designation

When no imaging/measurement is done at all at a particular time point, the patient is not evaluable (NE) at that time point. If only a subset of lesion measurements are made at an assessment, usually the case is also considered NE at that time point, unless a convincing argument can be made that the contribution of the individual missing lesion(s) would not change the assigned time point response. This would be most likely to happen in the case of PD. For example, if a patient had a baseline sum of 50mm with three measured lesions and at follow-up only two lesions were assessed, but those gave a sum of 80 mm, the patient will have achieved PD status, regardless of the contribution of the missing lesion.

4.4.4. Special notes on response assessment

When nodal disease is included in the sum of target lesions and the nodes decrease to 'normal' size (<10 mm), they may still have a measurement reported on scans. This measurement should be recorded even though the nodes are normal in order not to overstate progression should it be based on increase in size of the nodes. As noted earlier, this means that patients with CR may not have a total sum of ‘zero’ on the case report form (CRF).

In trials where confirmation of response is required, repeated ‘NE’ time point assessments may complicate best response determination. The analysis plan for the trial must address how missing data/assessments will be addressed in determination of response and progression. For example, in most trials it is reasonable to consider a patient with time point responses of PR-NE-PR as a confirmed response.

Patients with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be reported as ‘symptomatic deterioration’. Every effort should be made to document objective progression even after discontinuation of treatment. Symptomatic deterioration is not a descriptor of an objective response: it is a reason for stopping study therapy. The objective response status of such patients is to be determined by evaluation of target and non-target disease as shown in Tables 1–3.

Conditions that define ‘early progression, early death and inevaluability’ are study specific and should be clearly described in each protocol (depending on treatment duration, treatment periodicity).

In some circumstances it may be difficult to distinguish residual disease from normal tissue. When the evaluation of complete response depends upon this determination, it is recommended that the residual lesion be investigated (fine needle aspirate/biopsy) before assigning a status of complete response. FDG-PET may be used to upgrade a response to a CR in a manner similar to a biopsy in cases where a residual radiographic abnormality is thought to represent fibrosis or scarring. The use of FDG-PET in this circumstance should be prospectively described in the protocol and supported by disease specific medical literature for the indication. However, it must be acknowledged that both approaches may lead to false positive CR due to limitations of FDG-PET and biopsy resolution/sensitivity.

For equivocal findings of progression (e.g. very small and uncertain new lesions; cystic changes or necrosis in existing lesions), treatment may continue until the next scheduled assessment. If at the next scheduled assessment, progression is confirmed, the date of progression should be the earlier date when progression was suspected.

Table 1 – Time point response: patients with target (+/– non-target) disease.

Target lesions	Non-target lesions	New lesions	Overall response
CR	CR	No	CR
CR	Non-CR/non-PD	No	PR
CR	Not evaluated	No	PR
PR	Non-PD or not all evaluated	No	PR
SD	Non-PD or not all evaluated	No	SD
Not all evaluated	Non-PD	No	NE
PD	Any	Yes or No	PD
Any	PD	Yes or No	PD
Any	Any	Yes	PD

CR = complete response, PR = partial response, SD = stable disease, PD = progressive disease, and NE = inevaluable.

Table 2 – Time point response: patients with non-target disease only.

Non-target lesions	New lesions	Overall response
CR	No	CR
Non-CR/non-PD	No	Non-CR/non-PD[a]
Not all evaluated	No	NE
Unequivocal PD	Yes or No	PD
Any	Yes	PD

CR = complete response, PD = progressive disease, and NE = inevaluable.

a 'Non-CR/non-PD' is preferred over 'stable disease' for non-target disease since SD is increasingly used as endpoint for assessment of efficacy in some trials so to assign this category when no lesions can be measured is not advised.

Table 3 – Best overall response when confirmation of CR and PR required.

Overall response First time point	Overall response Subsequent time point	BEST overall response
CR	CR	CR
CR	PR	SD, PD or PR[a]
CR	SD	SD provided minimum criteria for SD duration met, otherwise, PD
CR	PD	SD provided minimum criteria for SD duration met, otherwise, PD
CR	NE	SD provided minimum criteria for SD duration met, otherwise NE
PR	CR	PR
PR	PR	PR
PR	SD	SD
PR	PD	SD provided minimum criteria for SD duration met, otherwise, PD
PR	NE	SD provided minimum criteria for SD duration met, otherwise NE
NE	NE	NE

CR = complete response, PR = partial response, SD = stable disease, PD = progressive disease, and NE = inevaluable.

a If a CR is truly met at first time point, then any disease seen at a subsequent time point, even disease meeting PR criteria relative to baseline, makes the disease PD at that point (since disease must have reappeared after CR). Best response would depend on whether minimum duration for SD was met. However, sometimes 'CR' may be claimed when subsequent scans suggest small lesions were likely still present and in fact the patient had PR, not CR at the first time point. Under these circumstances, the original CR should be changed to PR and the best response is PR.

4.6. Confirmatory measurement/duration of response

4.6.1. Confirmation

In non-randomised trials where response is the primary endpoint, confirmation of PR and CR is required to ensure responses

identified are not the result of measurement error. This will also permit appropriate interpretation of results in the context of historical data where response has traditionally required confirmation in such trials (see the paper by Bogaerts et al. in this Special Issue10). However, in all other circumstances i.e. in randomised trials (phase II or III) or studies where stable disease or progression are the primary endpoints, confirmation of response is not required since it will not add value to the interpretation of trial results. However, elimination of the requirement for response confirmation may increase the importance of central review to protect against bias, in particular in studies which are not blinded.

In the case of SD, measurements must have met the SD criteria at least once after study entry at a minimum interval (in general not less than 6–8 weeks) that is defined in the study protocol.

4.6.2. Duration of overall response

The duration of overall response is measured from the time measurement criteria are first met for CR/PR (whichever is first recorded) until the first date that recurrent or progressive disease is objectively documented (taking as reference for progressive disease the smallest measurements recorded on study). The duration of overall complete response is measured from the time measurement criteria are first met for CR until the first date that recurrent disease is objectively documented.

4.6.3. Duration of stable disease

Stable disease is measured from the start of the treatment (in randomised trials, from date of randomisation) until the criteria for progression are met, taking as reference the smallest sum on study (if the baseline sum is the smallest, this is the reference for calculation of PD).

The clinical relevance of the duration of stable disease varies in different studies and diseases. If the proportion of patients achieving stable disease for a minimum period of time is an endpoint of importance in a particular trial, the protocol should specify the minimal time interval required between two measurements for determination of stable disease.

Note: The duration of response and stable disease as well as the progression-free survival are influenced by the frequency of follow-up after baseline evaluation. It is not in the scope of this guideline to define a standard follow-up frequency. The frequency should take into account many parameters including disease types and stages, treatment periodicity and standard practice. However, these limitations of the precision of the measured endpoint should be taken into account if comparisons between trials are to be made.

Appendix 2: Eastern Cooperative Oncology Group Performance Status (ECOG-PS)

Scale	Performance status
0	Fully active, able to carry on all predisease activities without restriction
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature (e.g., light housework, office work).
2	Ambulatory and capable of all self-care but unable to carry out any work activities. Up and about more than 50% of waking hours.
3	Capable of only limited self-care, confined to bed or chair 50% or more of waking hours.
4	Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.
5	Death.

Statistical Analysis Plan

A prospective, open-label, single-arm, phase II clinical trial on the combination of tislelizumab, nab-paclitaxel, gemcitabine and concurrent radiotherapy as the preoperative therapy for patients with locally advanced and borderline resectable pancreatic cancer

Protocol Version and Date:	Version 1.0 March 1st,2020
Phase:	Phase 2
Methodology:	Single-Arm Study
Sponsor:	Nanjing Drum Tower Hospital，The Affiliated Hospital of Nanjing University Medical School
Analysis Plan Date:	March 1st, 2020
Analysis Plan Version:	Version 1.0

TABLE OF CONTENTS

1. INTRODUCTION

This document outlines the statistical methods to be implemented during the analyses of the study phase data collected within the scope of Protocol (Clinical research on tislelizumab plus nab-paclitaxel, gemcitabine and concurrent radiotherapy as the preoperative therapy for patients with locally advanced and borderline resectable pancreatic cancer). The purpose of this plan is to provide specific guidelines from which the analyses will proceed. Any deviations from these guidelines will be documented in the clinical study report (CSR). The scope of this plan includes the detailed specifications of the statistical analyses for the study only. The analyses described in this plan are considered a priori, in that they have been defined prior to database lock of study. Post hoc analyses will be labeled as such on the outputs and identified in the CSR. Further details about study design and procedures can be found in the protocol.

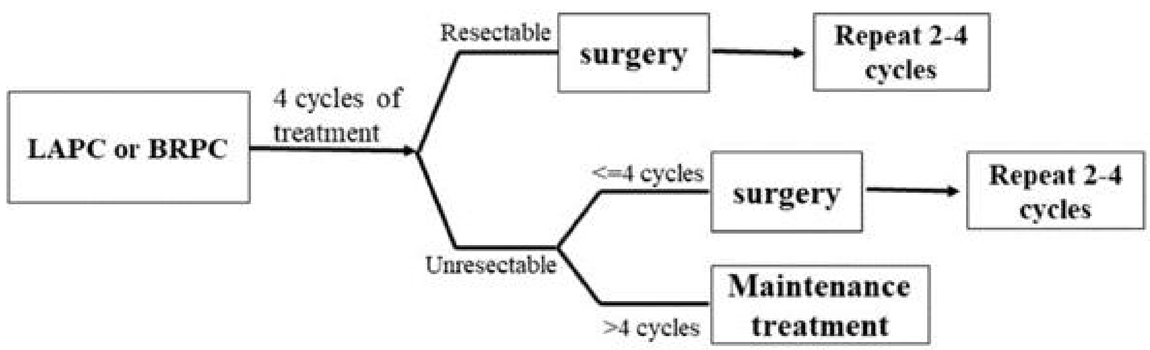
2. STUDY OBJECTIVES

The primary objective is to evaluate the efficacy of tislelizumab plus nab-paclitaxel, gemcitabine and concurrent radiotherapy as the preoperative therapy for patients with locally advanced and borderline resectable pancreatic cancer. The secondary objectives are to evaluate the safety and feasibility of tislelizumab plus nab-paclitaxel, gemcitabine and concurrent radiotherapy as the preoperative therapy for patients with locally advanced and borderline resectable pancreatic cancer.

3. STUDY DESIGN AND PLAN

This is a single-arm clinical study aimed to evaluate the clinical efficacy and safety of tislelizumab plus nab-paclitaxel, gemcitabine and concurrent radiotherapy as the preoperative therapy for patients with locally advanced and borderline resectable pancreatic cancer. The trial design is shown in Figure 1.25 treatment-naive Patients who pathologically and imaging confirmed locally advanced and borderline resectable pancreatic cancer, provide written informed consent and meet all of the inclusion and none of the exclusion criteria will be enrolled in this study. For the first 6-8 cycles after enrollment, the subject's dose will be 1000 mg/m^2 gemcitabine and 125 mg/m^2 nab-paclitaxel on days 1 and 8, respectively, per 21-day cycle, as well as 200 mg tislelizumab intravenously on day 1 of each 21-day cycle. SBRT every day with a total dose of 30Gy/10f at PTV and 50Gy/10f at PGTV simultaneously with the third cycle of chemotherapy and immunotherapy. This combination regimen was sustained until disease progression, intolerable toxicity, or completion of surgery.

Subjects will have a screening period of up to 30 days prior to Day 0. Subjects will accept examination every 3 weeks, including blood routine, coagulation function, liver and kidney functional electrolytes, tumor indicators, immune-related indicators and so on. Tumor imaging evaluations will be assessed every 6 weeks. AEs will be reported from the time subjects first dose until 30 days after the last dose.



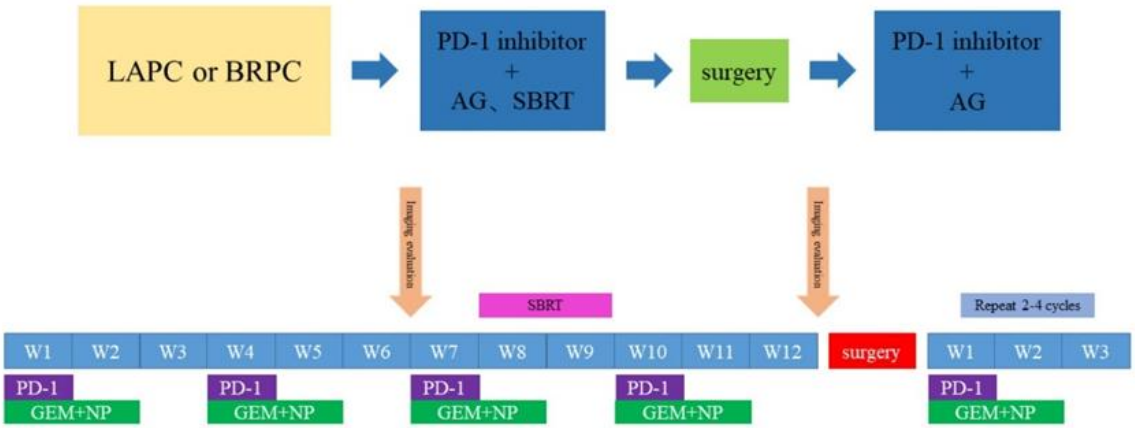


Figure 1: Study Design Procedures

4. DETERMINATION OF SAMPLE SIZE

Historical data showed that the ORR rate after neoadjuvant chemotherapy (LAPACT) in patients with locally advanced PC was 33.6%. We estimated that a sample size of 26 patients would detect approximately 26 % improvement (60%) in ORR rate with a power of 80%, using a one-sided alpha of 0.025. Assuming a 10% drop-out rate, a total of 29 treatment-naive patients with locally advanced and borderline resectable pancreatic cancer were planned to accrue in our study.

5. STUDY ENDPOINTS

5.1. Primary Endpoints

Objective response rate (ORR) and R0 resection rate.

5.2. Secondary Endpoints

Overall survival (OS), progression-free survival (PFS), disease control rate (DCR), pathological grade of tumor tissue after therapy and adverse reaction.

5.3. Exploratory Endpoints

Correlation between biomarkers and therapeutic response to therapy.

6. GENERAL ANALYSIS CONSIDERATIONS

6.1. Data Reporting

The statistical analyses will be reported using summary tables, figures, and data listings. Individual subject data obtained from the case report forms (CRFs), external laboratory data, and any derived data (such as change from Baseline and percent change from Baseline) will be presented in data listings by subject. Data from all assessments, whether scheduled or unscheduled, will be listed by subject and visit. Unscheduled visits and visits occurring more than one day outside protocol defined window will not be included in the summaries.

All statistical analyses were performed using SAS (V9.2 or higher).

6.2. Data Analysis and Summaries

Unless otherwise specified, variable data will be described using the mean ± standard deviation or median (maximum and minimum), and attributes data will be described using frequency and percentage. 95% confidence intervals were calculated if necessary.

6.3. Data Handling

6.3.1. Baseline Characteristics

For baseline characteristics, parameters are defined as the most recent no missing values prior to administration of investigational product on Day 1. No missing value estimation.

6.3.2. Partial Dates

If only a partial date is available and is required for a calculation, the following standards will be applied:

- Date (If the date record is incomplete and does not affect logic)
  - For missing day only: Day will be imputed as the 15_{th} day of the month if does not contradict another date.
  - For missing day, month and year: no missing value estimation.
- Efficacy
  - All missing of primary efficacy Measurements due to withdrawal were included in the analysis as "not evaluable”.
  - When calculating the time variables (e.g., OS, PFS), subjects with missing tumor assessment after treatment will be checked on a case-by-case basis to determine the deletion time during data audit.
- Safety
  - No missing value estimation.

6.3.3. Standard Calculations

Variables requiring calculation will be derived using the following formulas:

- **Days**: A duration expressed in days between one date (*date1*) and another later date (*date2*) will be calculated using the following formulas:

  duration (days) = date2 –date 1 + 1

- **Months**: A duration expressed in months is calculated as the number of days divided by 365.25 / 12.
- **Years**—A duration expressed in years between one date (*date1*) and another date (*date2*) is calculated using the following formulas:

  duration (years) = (date2 - date1+ 1) / 365.25

- **Age** – Age is calculated as the number of years from the date of birth (*DOB*) to the date of informed consent (*DOIC*). The following formula is used:

  age (years) = year of DOIC - year of DOB +1.

7. ANALYSIS POPULATIONS

The analysis population includes intent to treat (ITT) analysis set, full analysis set (FAS), and safety set (SS).

7.1. Intent-to-Treat (ITT) Population

All subjects who receive any amount of investigational product will be included in the ITT population. The ITT population will be used for the analysis of all efficacy data.

7.2. Full Analysis Set (FAS)

Subset of the ITT analysis set, including the patients who had measurable lesions at baseline, and who have received at least one dose of the study drug. This dataset will be used as the primary analysis data set for the efficacy assessment of ORR.

7.3. Safety Set (SS)

The Safety population will include all subjects who receive any amount of investigational product. Treatment assignment will be based on the treatment actually received. The Safety population will be used for the analysis of all safety data.

8. STUDY POPULATION

8.1. Subject Disposition

Subject disposition information will be summarized and listed for all subjects. The number and percentage of subjects enrolled, completed or early terminated will be summarized.

8.2. Protocol Deviations

Protocol deviations for missed visits, missed assessments, out of window visits or assessments, and violations of inclusion/exclusion criteria will be determined based on available data. All other protocol deviations will be collected.

8.3. Demographic and Baseline Characteristics

Demographic variables will include the following:

- Age at informed consent
- Sex

Other Baseline characteristics will include the following:

- History of cancer

  Disease term, tumor location, tumor type, vascular involvement etc.,

- Baseline height and weight (BMI, body surface area)
- Baseline vital signs: systolic blood pressure, diastolic blood pressure, pulse, temperature, respiration, ECOG PS score
- Virus detection and hepatitis B 5 items, HBV DNA, HCV RNA

Demographic and Baseline characteristics will be summarized for the ITT populations. The patients' demographic characteristics (gender, age), tumor diagnosis information (pathological diagnosis, clinical staging), and other baseline information (height, and weight [body mass index]), vital signs, ECOG PS, and laboratory tests will be analyzed using descriptive statistics.

8.4. Prior and New Concomitant Medications

Verbatim terms on CRFs will be mapped to ATC class and preferred term using the World Health Organization Drug Dictionary Enhanced (WHO-DDE June 2014).

Pretreatment medications: medications with start and stop dates prior to the first dose of investigational product.

Prior concomitant medications: medications that started prior to, and continued after, the first dose of investigational product.

New concomitant medications: medications that were started after the first dose of investigational product.

If it cannot be determined whether the medication was a new concomitant medication due to a partial start or stop date or if the medication is taken on the same date as the first dose in, then it will be counted as a new concomitant medication.

Pretreatment medications will be presented in listings only. Prior and new concomitant medications will be summarized by World Health Organization ATC class and preferred term using the ITT Population. New concomitant medications will be summarized separately. These summaries will present the number and percentage of subjects using each medication. Prior and new concomitant medications will be presented a data listing.

9. EFFICACY ANALYSE

The primary and secondary efficacy analyses will be based on the ITT Population.

9.1. Primary Efficacy Analyses

The primary efficacy endpoints are Objective response rate (ORR) and R0 resection rate on the ITT population. ORR assessed by the investigator: According to RECIST V1.1, the proportion of patients who achieve CR or PR assessed in the analysis population.

9.2. Secondary Efficacy Analyses

Secondary efficacy are Overall survival (OS), progression-free survival （PFS）， disease control rate (DCR)， pathological grade of tumor tissue after therapy and adverse reaction..

9.2.1. OS

Overall survival (OS): Time from first dose to death recorded for any cause. Patients who are still alive at the time of analysis are censored at the last contacted date. The inter-group comparison of OS will be performed using the stratified log-rank test, the median OS and corresponding 95% CI will be estimated using the Kaplan-Meier method, and the survival curves will be plotted.

9.2.2. PFS

PFS assessed by the investigator: According to RECIST V1.1, the time from first dose to the first recorded imaging disease progression or death caused by any reason as assessed by the investigator, whichever occurs first. Patients who are still alive with no disease progression record at the time of analysis are censored at the last imaging evaluation date. Patients who are still alive with no imaging evaluation record after baseline are censored at the first dose date.

9.2.3. DCR

DCR assessed by the investigator: According to RECIST V1.1, the proportion of patients who achieve CR, PR, or SD assessed by the investigator in the analysis population.

9.2.4. Pathological grade of tumor tissue after therapy

The postsurgical pathology of patients will be graded according to the American Joint Committee on Cancer (AJCC).

9.3. Exploratory Analyses

Correlation between biomarkers and therapeutic response to therapy. Peripheral blood samples of patients are collected to detect circulating tumor DNA (ctDNA) in different periods including baseline, the first and the second clinical evaluation.

10. SAFETY ANALYSES

All safety analyses will be based on the SS, with safety parameters in including AEs, laboratory tests, vital signs, 12-lead ECG, etc.

10.1. Drug Exposure

The drug exposure and duration of treatment (number of treatment cycles) will be summarized. The duration of investigational product exposure will be calculated as Follows:

- Exposure to investigational product (week= {[(Date of last investigational product dose-Date of 1st investigational product dose) + 1] - Total duration of temporary investigational product discontinuation}

10.2. Adverse Events (AEs)

All AEs will be coded and classified using MedDRA, and graded as per CTCAE V5.0.

All AE summaries will be restricted to treatment-emergent adverse events (TEAEs), which are defined as any AEs that newly appear, increase in frequency, or worsen in severity following initiation of study medication. The incidences (frequency) of all TEAEs, TEAEs at grade 3 and above, TEAEs related to the study drugs, irAEs, SAEs, SAEs related to study drugs, TEAEs leading to discontinued study medication, and TEAEs leading to study termination will be summarized, and the above-mentioned AEs will be summarized based on SOCs and PTs in MedDRA coding. In addition, the severity levels of TEAEs and relationship with the study drug were also summarized by SOC and PT.

The incidence of pre-treatment AEs and pre-treatment SAEs occurring after ICF signoff and before the first dosing of investigational product (OCA or placebo) will be tabulated in the same manner as above for all subjects participating in the washout period.

The following listings will be presented by subject:

- All AEs
- Serious AEs (subset of the AEs where serious is marked as "Yes")
- Death information will be provided in a separate listing, should any deaths occur
- Severe AEs (subset of AEs where severity is marked as "Severe" or severity is missing)
- Related AEs (subset of AEs where relationship to study medication is marked as "Definite", "Possible" or "Probable")
- AE's leading to withdrawal of investigational product (subset of AEs where action taken with study medication is marked as "Drug Withdrawn")
- AE's leading to Study Discontinuation (subset of AEs where subject discontinued from study is checked)

10.3. Clinical Laboratory Evaluations

A listing of available laboratory reference/normal ranges for each laboratory parameter will be provided including age, sex, values with units. For laboratory tests, the observed values and changes from the baseline will be analyzed using descriptive statistics. The baseline results and the worst results during the trial were presented in a crosstab. Laboratory test abnormalities will be graded and summarized according to CTCATE V5.0.

10.4. Vital Signs, physical examinations, and other safety-related examinations

Measured values and changes from baseline for vital signs, physical examinations, and other safety-related examination values will be analyzed using descriptive statistics. The baseline results and the worst results during the trial were presented in a crosstab.

ECOG and PS will be analyzed and summarized using descriptive statistics.

11. COMPLIANCE ANALYSIS

The proportion and frequency of patients violating the expected administration regimen, the proportion of patients in whom the doses of study drugs account for between 80–120% of the those prescribed by the protocol, the proportion of patients completing the study, and the proportion of patients completing different treatment cycles will be summarized.

12. INTERIM ANALYSIS

The interim analysis will not be carried out in this study