Anlotinib Plus Toripalimab and Nab-Paclitaxel in Patients with Locally Advanced or Metastatic Pancreatic Cancer: Study Protocol for An Open-Label, Non-Randomized, Phase II Study (ATNPA)

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Study protocol

Keywords: Locally advanced pancreatic cancer, Metastatic pancreatic cancer, Anlotinib, Toripalimab, Nab-paclitaxel, Study protocol

Posted Date: December 3rd, 2021

DOI: https://doi.org/10.21203/rs.3.rs-1076724/v1

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Abstract

Background

There have not been standard second-line or maintenance regimens with definite survival benefits so far for patients with pancreatic carcinoma who have lost the opportunity of curable resections or failed first-line chemotherapy. Anlotinib, a potent small-molecule tyrosine kinase inhibitor, exhibits anti-angiogenic and anti-tumour effects by specifically binding to multiple targets such as VEGFR, FGFR, PDGFR, c-Kit and Ret. Toripalimab, a novel anti-PD-1 mAb, has been proved to significantly prolong progression-free survival (PFS) and overall survival (OS) in various solid tumours with manageable toxicities when combining with cytotoxic chemotherapy. We design this study to assess the combination of anlotinib, toripalimab and nab-paclitaxel as a second-line or maintenance therapy for locally advanced pancreatic cancer (LAPC) or metastatic pancreatic cancer (MPC).

Patients and Methods:

This is an open-label, non-randomized, single-arm phase III study, aimed at evaluating the efficacy and safety profile of the above-mentioned combination strategy in first-line therapy-failed LAPC or MPC. Totally 53 patients are to be enrolled and receive anlotinib (12 mg, po. qd.) plus toripalimab (240 mg, ivgtt. q3w.) and nab-paclitaxel (125 mg/m², ivgtt, d1, d8) every 3 weeks as a cycle until disease progression or intolerable adverse events. The primary endpoint is PFS. Secondary end points include OS, disease control rate (DCR), object response rate (ORR), quality of life (QoL) and safety. Enrollment started in April 2021, and follow-up will be finished in April 2023.

Discussion and Significance:

Combination of anlotinib, toripalimab and nab-paclitaxel may promote vessel normalization and drug delivery, and activate the immune response, thus exerting synergistic anti-tumour effects and counteracting the immunosuppressive microenvironment of pancreatic cancer. As the first intending to assess this combination in pancreatic cancer, this study will provide comprehensive evidence for second-line or maintenance therapy of LAPC and MPC.

Trial registration:


Contributions To The Literature
This study protocol has detailed the process and criteria for evaluating the efficacy and safety of chemotherapy combined with anti-angiogenic therapy and immunotherapy, and could provide scientific guidance for the implementation of clinical studies in the field of oncology.

ATNPA could provide a practical and promising second-line or maintenance regimen for patients with pancreatic cancer who have lost the chance of radical resection or failed first-line chemotherapy.

We have discussed the compelling advantages of the triple combination regimen from the perspective of tumour microenvironment. Anlotinib plus toripalimab and nab-paclitaxel may counteract the immunosuppressive microenvironment of pancreatic cancer by exerting synergistic anti-tumour effects.

Background

Based on the American Cancer Society, there has been about 56,000 newly diagnosed pancreatic cancer in the United States in 2019, becoming the third leading cause of cancer-related death.\(^1\) Approximately 34,500 men and 23,200 women died of pancreatic cancer each year in China, more than in the USA.\(^2,3\) As a highly malignant gastrointestinal disease, the 5-year survival rate of pancreatic cancer is just over 5%, with a median survival time of 6 months\(^4\). R0 resection remains the only possible chance of a complete cure for pancreatic cancer. Unfortunately, 80-85% of patients are diagnosed with unresectable advanced disease at the first visit\(^5\). Furthermore, even after radical resection, it is almost inevitable for most patients to relapse.

For patients with advanced pancreatic cancer, systemic chemotherapy is the exclusive treatment to prolong survival time and improve life quality. Gemcitabine was defined as the standard first-line chemotherapy for advanced pancreatic cancer in 1997\(^6\), but the clinical efficacy is limited.\(^7\) The FOLFIRINOX regimen (comprising fluorouracil, irinotecan, leucovorin and oxaliplatin) increased the median overall survival (OS) of metastatic pancreatic cancer (MPC) from 6.8 to 11.1 months compared to gemcitabine.\(^8\) Besides, nab-paclitaxel is widely used in combination with gemcitabine or S-1 to treat advanced pancreatic cancer due to its small particle diameter and prominent anti-tumour activity.\(^9,10\) Although these chemotherapeutic regimens have achieved certain effects on treating pancreatic cancers, the overall efficacy is still unsatisfactory, only increasing the median survival by 2-4 months, and related to considerable toxicity.\(^8,11\)

The definite benefits of second-line therapy and targeted therapy such as anti-angiogenic therapy and tumour immunotherapy for patients with locally advanced pancreatic cancer (LAPC) and MPC have not been fully elucidated and validated. In according with Folkman's theory that tumour growth is dependent on angiogenesis,\(^12\) pancreatic cancer is characterized by high microvascular density, impaired microvascular integrity, and poor perfusion,\(^13,14\) which are associated with early recurrence, metastasis and short survival after tumour resection.\(^15\) Yapp et al. have demonstrated that metronomic chemotherapy, targeting activated endothelial cells of tumour, exerted an effect on both tumour blood
vessels and pancreatic cancer cells,\textsuperscript{16} suggesting that drug delivery and efficacy could be improved by inducing vascular normalization in LAPC or MPC\textsuperscript{17}.

Multi-targeted tyrosine kinase inhibitors (TKIs) that exhibit anti-angiogenic effects have been highlighted as promising agents for patients who have failed to respond to gemcitabine-based chemotherapy,\textsuperscript{18} since sunitinib shows a prolonged progression-free survival (PFS) and OS in well-differentiated pancreatic neuroendocrine tumours.\textsuperscript{19} Wegner et al. observed that sunitinib treatment induced changes in the tumour microenvironment in pancreatic ductal adenocarcinoma (PDAC).\textsuperscript{20,21} Besides, Awpari et al. found that nintedanib could inhibit the proliferation and migration of pancreatic cancer associated cells, enhance gemcitabine anti-tumour response and inhibit local tumour growth by blocking PI3K/MAPK signaling pathway in mouse PDAC xenograft model.\textsuperscript{22}

As a novel oral, small-molecule and multi-receptor TKI, anlotinib has been proved to achieve a stronger anti-angiogenic activity than sunitinib and nintedanib,\textsuperscript{23} by suppressing the activity of kinases such as vascular endothelial growth factor receptor (VEGFR), platelet-derived growth factor receptor (PDGFR), fibroblast growth factor receptor (FGFR) and c-Kit.\textsuperscript{24,25} It was approved in China for the treatment of patients with locally advanced or metastatic non-small cell lung cancer (NSCLC) in 2018.\textsuperscript{26} Preclinical studies have revealed that anlotinib can significantly inhibit VEGF/PDGF-BB/FGF-2-induced angiogenesis, suppress the proliferation of human pancreatic cancer cell lines by inducing G2/M phase arrest and triggering apoptosis, and decrease the tumour size in transplantation models.\textsuperscript{24,25,27}

These multi-targeted anti-angiogenic drugs show significant preclinical effects, and several studies are ongoing to explore the efficacy and safety of TKIs in combination with cytotoxic chemotherapy in advanced pancreatic cancer (sunitinib: NCT00397787, NCT00967603, NCT00462553; nintedanib: NCT02902484; Anlotinib: NCT03457844, NCT04718701).

In addition to anti-angiogenic treatment, immunotherapy has recently been a hotspot of tumour-targeted therapy, especially programmed cell death protein-1 or programmed death receptor ligand-1 (PD-1/PD-L1), which mediates the immune escape of tumour cells.\textsuperscript{28} By binding to PD-L1 on tumour cells, PD-1 protein downregulates T cell activity and thereby causing immune suppression.\textsuperscript{28} It has been well validated that the expression of PD-L1 in the tumour microenvironment (TME) of pancreatic cancer is associated with OS.\textsuperscript{29} Despite the efficacy of single PD1/PD-L1 blockade has been proved disappointing in PDAC,\textsuperscript{29} the combination of PD-1 monoclonal antibodies with other chemotherapeutic agents is predicted to have anti-tumour synergistic effect. A phase I b study of pembrolizumab including 11 MPC patients has demonstrated that pembrolizumab could be safely combined with gemcitabine or nab-paclitaxel,\textsuperscript{30} and moreover, similar studies suggested that pembrolizumab treatment was positively associated with prolonged PFS and OS.\textsuperscript{31}

As a novel recombinant humanized anti-PD-1 mAb, toripalimab was approved by the China National Medical Products Administration (NMPA) to treat second-line metastatic melanoma in 2018.\textsuperscript{32} A clinical
trial (NCT02915432) reported that toripalimab showed controlled safety and anti-tumour activity in patients with advanced gastric cancer (AGC), especially in combination with XELOX regimen. A case has reported that a patient with MPC has a long-term partial response and good tolerance to toripalimab plus gemcitabine and nab-paclitaxel. Besides, a phase study found significant improved PFS and OS among patients with metastatic non-squamous NSCLC attributed to the addition of atezolizumab to bevacizumab, carboplatin and paclitaxel, which provide compelling evidence that the cancer-cell-killing effects of PD-1 blockade could be enhanced by immunoregulation mediated by cytotoxic chemotherapy and anti-angiogenesis. Thus, combining toripalimab with cytotoxic nab-paclitaxel and small-molecule multi-receptor TKI may become a potential therapeutic strategy for LAPC or MPC patients.

Here we design this exploratory phase clinical study to assess the efficacy and safety profile of anlotinib plus toripalimab and nab-paclitaxel as a second-line treatment or maintenance treatment in LAPC or MPC, intending to provide scientific evidence for optimizing treatment strategies, prolonging the survival and improving the quality of life of patients.

**Methods**

**Study design and eligibility criteria**

This is a prospective, open-label, non-randomized, single-arm phase II study (ClinicalTrials.gov: NCT04718701) and it would be carried out in the First Affiliated Hospital of Nanjing Medical University. The checklists of SPIRIT guidelines have shown in Additional file. Eligible patients (eligibility criteria are detailed in Table 1) who failed first-line therapy with locally advanced or metastatic pancreatic cancer confirmed by histopathology will be assigned to receive anlotinib plus toripalimab and nab-paclitaxel as second-line or maintenance treatment (Fig. 1).

**Table 1. Eligibility criteria.**
<table>
<thead>
<tr>
<th>Inclusion criteria</th>
<th>Exclusion criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Males or females aged 18-75.</td>
<td>1. Previous treatment with anlotinib, toripalimab or nab-paclitaxel.</td>
</tr>
<tr>
<td>2. Locally advanced / metastatic pancreatic cancer confirmed by histopathology.</td>
<td>2. Coexistence with intestinal perforation, massive intestinal gas, acute intestinal obstruction, severe infection and so on.</td>
</tr>
<tr>
<td>3. Life expectancy ≥ 3 months.</td>
<td>3. Multiple factors affecting oral medication such as inability to swallow, chronic diarrhea and intestinal obstruction.</td>
</tr>
<tr>
<td>4. Treatment failure* after first-line monotherapy or combination chemotherapy (at least one cycle), or relapse within 6 months after adjuvant / neoadjuvant therapy.</td>
<td>4. Active bleeding of primary lesions within 2 months.</td>
</tr>
<tr>
<td>5. At least one measurable tumour lesion without local treatment such as radiotherapy according to the Response Evaluation Criteria in Solid Tumours, version 1.1 (RECIST 1.1); or tumour lesions in target area of previous radiotherapy confirmed progress.</td>
<td>5. History of congenital/acquired immunodeficiency or organ transplantation.</td>
</tr>
<tr>
<td>6. Eastern Cooperative Oncology Group (ECOG) performance status (PS) of 0–1.</td>
<td>6. Symptomatic brain metastases or meningeal metastases.</td>
</tr>
<tr>
<td>7. Adequate organ and bone marrow function.</td>
<td>7. Existence with anyone of the following conditions: severe cardiovascular diseases, liver diseases, psychiatric disorders, poorly-controlled diabetes (fasting blood glucose ≥ 10 mmol/L) or hypertension (systolic blood pressure ≥ 150 mmHg and/ or diastolic blood pressure ≥ 100 mmHg), active or uncontrolled severe infections, long-term use of hormones or active autoimmune diseases, significant bleeding tendencies or under thrombolytic or anticoagulant treatment; occurrence of arterial and venous thrombotic events or clinically significant cardiovascular events within 6 months before enrollment; routine urinalysis showing urine protein ≥ ++ and confirmed 24-hour urine protein quantification &gt; 1.0 g.</td>
</tr>
<tr>
<td>8. Female of childbearing age must have a negative pregnancy test (serum or urine) within 7 days prior to enrolment. Males and females voluntarily use appropriate methods of contraception during study.</td>
<td>8. Known to be allergic to the test drug.</td>
</tr>
<tr>
<td>9. Voluntary provision of informed consents.</td>
<td>9. Pregnant or breastfeeding female patients</td>
</tr>
<tr>
<td>10. Other serious hazards to the safety of patients or complications that affect the study according to the judgment of the researchers.</td>
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</tbody>
</table>
*Definition of failure: intolerable toxicity or disease progression during treatment or recurrence after treatment;

#Definition of intolerance: haematological toxicity reached grade IV (thrombocytopenia reached grade III or above) or non-haematological toxicity reached grade III or above.

Unstained specimens are to be prepared using anti-slip slides. A combined positive score (CPS) of LAPC or MPC patients will be detected by performing PD-L1 immunohistochemistry on 18-20 serial sections of obtained puncture tissue.

**Treatment administration**

Anlotinib Hydrochloride Capsules (12 mg orally, once daily before breakfast; CTTQ, Lianyungang, Jiangsu, China), Toripalimab (240 mg intravenously, once every 3 weeks; Shanghai Junshi Biosciences Co., Ltd., Shanghai, China) and Nab-paclitaxel (125 mg/m² intravenously, twice every 3 weeks; CSPC, Shijiazhuang, Hebei, China) are to be administered as a second-line therapy at most 6 cycles (concrete study treatments are detailed in Table 2).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose</th>
<th>Frequency</th>
<th>Route of administration</th>
<th>Dosing time of each 3-week cycle</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Second-line therapy:</strong></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Anlotinib</td>
<td>12 mg*</td>
<td>Q3W</td>
<td>Oral</td>
<td>QD Days 1-14 of cycles 1-6</td>
</tr>
<tr>
<td>Toripalimab</td>
<td>240 mg</td>
<td>Q3W</td>
<td>IV infusion</td>
<td>Day 1 of cycles 1-6</td>
</tr>
<tr>
<td>Nab-paclitaxel</td>
<td>125 mg/m²</td>
<td>Q3W</td>
<td>IV infusion</td>
<td>Day 1 and Day 8 of cycles 1-6</td>
</tr>
<tr>
<td><strong>Maintenance therapy:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anlotinib</td>
<td>12 mg</td>
<td>Q3W</td>
<td>Oral</td>
<td>QD Days 1-14</td>
</tr>
<tr>
<td>Toripalimab</td>
<td>240 mg</td>
<td>Q3W</td>
<td>IV infusion</td>
<td>Day 1</td>
</tr>
</tbody>
</table>

*A treatment cycle is defined as 21 days of one daily dose of anlotinib.

*Q3W: every 3 weeks; QD: once daily.

Patients assessing as CR/PR/SD continue to receive a maintenance treatment up to two years until disease progression or intolerable toxicity.

Predefined dose modification of anlotinib and nab-paclitaxel is based on treatment-related adverse events. When patients experience grade 3 or above haematological toxicities or grade 2 or above non-haematological toxicities, dose suspension and reduction of anlotinib are considered. Treatment interruption is permitted to perform for no more than 14 days (either continuously or cumulatively) or no
more than two times in a defined treatment cycle to ensure adequate drugs concentration. Dose adjustments of anlotinib are allowed at most two times, and it is not be allowed to be adjusted back up to the previous level after a dose reduction (Supplementary Table 1). The dose of toripalimab is not allowed to modify and can only be suspended or discontinued.

**Staging assessments and follow-up**

Observations and assessments will be conducted before treatment, on day 7, 21 of cycle 1, on day 21 of cycle 2, every 2 cycles (42 days) during following cycles and after treatment. Baseline recording of tumour lesions and brain metastases are required to be performed during screening (within 14 days before allocation) according to RECIST 1.1 and RANO-BM criteria. Besides, imaging assessments (including chest, abdomen, pelvic cavity and brain CT or MRI) during therapy period will be administered under the same condition as the baseline. ECOG performance status and serum tumour markers (CEA, CA199, CA724) will be examined at the same time as imaging assessment. Safety assessment, adverse event recording will continue to be conducted for all patients up to the 21st day after the last administration.

For patients withdrawing from the study for reasons other than disease progression, imaging assessments are required (if there are no imaging examinations within 7 days before discontinuation). Follow-up for survival status and subsequent antineoplastic therapy data collecting will be performed by telephone interview or face-to-face every 6 weeks after treatment until disease progression, death or end of the study (whichever occurs first).

**Study end points and adverse events**

The efficacy of the combined therapy is evaluated by PFS, which is the primary end point and defined as the time from allocation to the first time of disease progression confirmed by imaging modalities or all cause death, whichever occurs first. Secondary end points include OS, object response rate (ORR), disease control rate (DCR), quality of life (QoL) and safety. OS is defined as the time from allocation to any cause death or last follow-up. ORR and DCR are evaluated among patients having measurable tumour lesions at baseline according to the RECIST 1.1, respectively contain the incidence of complete response (CR), partial response (PR) and the incidence of CR, PR and stable disease (SD). QoL refers to EORTC QLQ-C30 (version 3, Chinese version), and will be recorded in case report form (CRF) by observing clinical symptoms and scoring related examination before and after study treatment.

Severe adverse events (SAEs) and adverse events (AEs) will be graded and recorded by the investigators according to the National Cancer Institute Common Toxicity Criteria (NCI-CTC, version 4.0) to evaluate the safety of the combined treatment. AEs are defined as any adverse medical events that occur in subjects from initiation of allocation up to 30 days after the last administration, whether or not causally related to the combined therapy. SAEs are defined as all adverse medical events at any drug dose that result in a fatal, life-threatening event or prolonged hospital stay, permanent disability or other important medical conditions. The occurrence time (start time) and the recovery time (end time) of AEs and SAEs, and the possible relevance with study treatment will also be assessed and documented in the CRF.
Sample size calculation and study period

According to previous studies, the median PFS of the second-line therapy of pancreatic cancer was 2.9 months (CONKO-003 study). Using 2.9 months served as a control, the expected median PFS of patients treated with anlotinib plus toripalimab and nab-paclitaxel in this study is prespecified as 4.5 months. Significance level $\alpha = 0.05$ (two-tailed) and test power $(1 - \beta) = 0.80$ are used for sample size calculation by PASS (Power Analysis and Sample Size) software. Taking a 20% drop-out rate into consideration, the sample size is approximately 53 patients. An interim analysis will be performed using a Lan–DeMets Pocock type boundary to assess the futility and efficacy when approximately half of the predetermined sample size is allocated. If the interim analysis results indicate that the study is of futility and the benefit/risk ratio is significantly worse, the trial will be terminated by the investigators. Otherwise, the trial will continue until the full sample size of 53 has been accumulated. The estimated recruitment time from enrollment of the first patient to the last patient’s enrollment is approximately 12 months (from April 2021 to April 2022), and the estimated follow-up time is about 12 months (from April 2022 to April 2023).

Statistical analysis

The statistical analysis plan is developed by biostatistics professionals based on the study protocol and refinements will be made for the modification of important research indicators or corresponding statistical analysis methods after the initiation of the study. The final version will be determined before the database is locked. All statistical analyses will be accomplished using the SAS software (version 9.4 or above).

The expression level of PD-L1 (CPS $\geq 1$ vs. CPS $<1$) will be regarded as a factor of stratified analysis. Efficacy is to be analyzed in the full analysis set (FAS), the response evaluable set (RES) and per-protocol set (PPS). Safety is to be analyzed in safety set (SS) including all assigned patients who receive at least one dose of study combined therapy and have safety records of medication. Statistical descriptions of subject distribution, demographic data and baseline characteristics will be performed. For study endpoints, the Kaplan–Meier method is to be applied for the PFS and OS curve with estimation for median PFS, median OS and 95% CI. ORR = (CR+PR) / sample size $\times 100\%$; DCR = (CR+PR+SD) / sample size $\times 100\%$. The 95% CI of the ORR and DCR are to be calculated by exact binomial method based on the F distribution. ECOG PS scores and QoL scores will be described at each visit time. For safety analysis, only treatment emergent adverse event (TEAE) will be included and analyzed in this experiment, which is defined as AEs that are post-dose or heavier than the baseline. Medical Dictionary for Regulatory Activities (MedDRA), system organ class (SOC), preferred terms (PT) and NCI CTCAE 5.0 will be used to standardize and classify all adverse events and summarize the incidence of AEs and association with treatments. Vital signs, ECG, and laboratory evaluation results will be compared and analyzed before and after treatment.

Discussion
As a malignant gastrointestinal tumour with high rates of recurrence, metastasis and mortality, pancreatic carcinoma is predicted to be the second leading cause of cancer-related death by 2030. Chemotherapy remains the predominant treatment for LAPC and MPC, and there are alternative and available first-line chemotherapy regimens primarily based on gemcitabine, 5-FU, nab-paclitaxel etc. for patients whether in good or poor performance status. However, there is no standard second-line or maintenance regimen with definite survival benefits. Studies of different second-line regimens for those patients who have lost the opportunity of radical resection and failed first-line monotherapy or combination chemotherapy are ever-increasing in recent years, such as S-1 monotherapy (median OS: 4.5 months in the daily group, 4.4 months in the alternate-day group) and attenuated FOLFIRINOX regimen (median PFS: 3.8 months [95% CI: 1.5-6.0 months], median OS: 8.5 months [95% CI 5.6-11.4 months]), whereas most published results of efficacy and tolerability did not reach the predetermined level of statistical significance or determined clinical value.

Pancreatic cancer TME is characterized by abundant stromal desmoplasia and extracellular matrix (ECM) deposition, which induces vascular collapse, making it difficult for traditional chemotherapy drugs to enter into. Nab-paclitaxel enhances the intracellular delivery of paclitaxel for its relatively small particle diameter and excellent tissue penetration, thus heightening the ability to kill pancreatic cancer cells with an acceptable toxicity. It has been indicated that cytotoxic nab-paclitaxel is synergized and influenced by anti-angiogenic treatment and PD-1 blockade. Moreover, multi-targeted anti-angiogenic drugs could further enhance the efficacy of nab-paclitaxel by inducing vascular normalization, improving tumour blood perfusion and reducing hypoxia in pancreatic cancer.

In previous phase III clinical trials, bevacizumab in combination with gemcitabine did not improve median OS in patients with advanced pancreatic cancer. The reason seems to be that pancreatic tumour angiogenesis is attributed to the activation and interaction of multiple complex signaling pathways, and it is far more likely that inhibiting VEGF/VEGFR signaling by bevacizumab may cause activation of compensatory pathways such as upregulation of PDGFR or FGFR, which promotes VEGFR-TKI resistance. Impotently, small-molecule multi-targeted TKI anlotinib could avoid intrinsic or acquired resistance to anti-VEGF therapy alone by simultaneously targeting VEGFR, PDGFR, FGFR, c-Kit and Ret, and maintain an acceptable safety and tolerability profile, thus becoming the optimum choice for combination with nab-paclitaxel to effectively control tumour angiogenesis, growth and metastasis.

Preclinical studies have reported that anlotinib can ameliorate immunosuppressive TME of pancreatic cancer by down-regulating PD-L1 expression on vascular endothelial cells (VECs). Meanwhile, anlotinib could increase the infiltration of innate immune cells with anti-tumour effects, including natural killer (NK) cells, CD8+ T cells, dendritic cells (DCs) and M1-like tumour-associated macrophages (M1 TAMs) whereas inhibit the infiltration of immuno-suppressive cells such as M2 TAMs and regulatory T cells (Tregs) (Fig. 2). This provides a strong rationale for anlotinib to enhance immunotherapy via the immunomodulatory effect on pancreatic cancer. Notably, the clinical evidence involved in PD-1/PD-L1 blockade plus anti-angiogenic treatment has been well elucidated in various solid tumours. A phase I b
A clinical trial reported the DCR of unresectable hepatocellular carcinoma in patients using multiple-target TKI lenvatinib plus pembrolizumab is up to 93.3% with manageable toxicities. A phase III study found significant improved PFS and OS among patients with metastatic non-squamous NSCLC when combining atezolizumab, bevacizumab, carboplatin and paclitaxel. This provides compelling evidence that combining PD-1 blockade with anlotinib and nab-paclitaxel may be a potential therapeutic strategy for patients with advanced pancreatic cancer.

As the first PD-1 monoclonal antibody independently developed in China and approved by NMPA, toripalimab shows a satisfactory efficacy in various solid tumours including melanoma, NSCLC, AGC etc. Toripalimab specifically binds to PD-1 on the surface of T lymphocytes, reducing PD-1 expression, interrupting the immunosuppressive effects mediated by PD-1/PD-L1 signaling pathway, and then activating anti-tumour immune response. Furthermore, inflammatory factors interferon-γ (IFN-γ) derived from activated immune response will promote vessel normalization and regression. Certainly, toripalimab and VEGFR-TKI such as anlotinib can present synergistic therapeutic benefits based on the interaction between vessel normalization and immune microenvironment reprogramming.

Theoretically, combination of anlotinib, toripalimab and nab-paclitaxel would promote vessel normalization, immune cell infiltration and drug delivery to tumour, thus counteracting immunosuppressive TME of pancreatic cancer with less adverse event. Nevertheless, combination therapy-related adverse events are still worth paying close attention to. In IMpower150 study, no increase of AEs incidence was observed in combination of chemotherapy, anti-angiogenic therapy and immunotherapy, whereas for individuals, multiple treatments lead to more complex AEs and toxicity profile, such as concurrent PD-1 blockade-related and anti-angiogenic drug-related toxicities.

As the first study intending to assess anlotinib plus toripalimab and nab-paclitaxel as a second-line or maintenance therapy, this innovative design will make a positive contribution to comprehensive information concerning the treatment of anti-angiogenic therapy combined with immunotherapy and cytotoxic chemotherapy. If this regimen proves to reach clinically significant efficacy with an acceptable safety and tolerability profile and meet the primary endpoint, it would provide therapeutic strategy options for LAPC and MPC. Importantly, given that this is an open-label, single-arm and single-center trial, our study would be limited to small sample size, external control and enrollment of patients with all subtypes of pancreatic cancers. Thus, validation in larger multi-center studies that enroll more subjects would be needed.

**Abbreviations**

OS: overall survival; MPC: metastatic pancreatic cancer; LAPC: locally advanced pancreatic cancer; TKIs: tyrosine kinase inhibitors; PFS: progression-free survival; MRI: magnetic resonance imaging; PDAC: pancreatic ductal adenocarcinoma; VEGFR: vascular endothelial growth factor receptor; PDGFR: platelet-derived growth factor receptor; FGFR: fibroblast growth factor receptor; NSCLC: non-small cell lung
cancer; HUVECs: human umbilical vein endothelial cells; ROS: reactive oxygen species; PD-1/PD-L1: programmed cell death protein-1 or programmed death receptor ligand-1; TME: tumour microenvironment; NMPA: China National Medical Products Administration; AGC: advanced gastric cancer; RECIST 1.1: response evaluation criteria in solid tumours, version 1.1; ECOG: Eastern Cooperative Oncology Group; PS: performance status; CTCAE: Common Terminology Criteria for Adverse Events; ORR: object response rate; DCR: disease control rate; QoL: quality of life; CR: complete response; PR: partial response; SD: stable disease; CRF: case report form; SAEs: Severe adverse events; AEs: adverse events; NCI-CTC: National Cancer Institute Common Toxicity Criteria; FAS: full analysis set; RES: response evaluable set; PPS: per-protocol set; SS: safety set; TEAE: treatment emergent adverse event; MedDRA: Medical Dictionary for Regulatory Activities; SOC: system organ class; PT: preferred terms; ECM: extracellular matrix; VECs: vascular endothelial cells; NK: natural killer; DCs: dendritic cells; M1 TAMs: M1-like tumour-associated macrophages; Tregs: regulatory T cells; IFN-γ: interferon-γ

Declarations

Ethics approval and consent to participate

This trial was approved by the Institutional Review Board and Ethics Committees of the First Affiliated Hospital of Nanjing Medical University (ethic approval ID: 2020-SR-496). All patients will be required to sign informed consents before their enrollment into the trial.

Consent for publication

All authors give consent for the publication of manuscript.

Availability of data and materials

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Funding

This study was supported by National Natural Science Foundation of China (81703307) and the Natural Science Foundation of Jiangsu Province (BK20181371) and the Priority Academic Program Development of Jiangsu Higher Education Institutions (Public Health and Preventive Medicine).

Authors' contributions

Chen, J. was the major contributor in writing the manuscript. Liu,Y. drafted the eligibility criteria and was responsible for patient admission. Shu,Y. and Liu,L. determined the specific dose and period of drug treatment and study endpoints. Zhu,Y. and Cui,S developed the details of the safety and efficacy
assessment of the regimen. Sun, C. revised and polished the manuscript. Bai, J. calculated the sample size and guided the statistical analysis of the study protocol. All authors read and approved the final manuscript.

Acknowledgments

Not applicable.

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References


**Figures**

![Fig 1 Diagram](image)

**Major inclusion criteria:**
- Males or females aged 18-75
- ECOG PS 0-1
- LAPC/MPC confirmed by histopathology
- First-line standard treatment failure
- At least one measurable tumor lesions

**Second-line therapy:**
- Anlotinib, 12mg + Toripalimab, 240mg + Nab-paclitaxel, 125mg/m² (d1, d8)

**N=53**

**At most 6 cycles**

**Maintenance therapy:**
- Anlotinib, 12mg + Toripalimab, 240mg

**Primary endpoint**
- PFS

**Secondary endpoints**
- ORR, DCR, OS, Safety, QoL

**Stratification factors**
- Expression level of PD-L1 (CPS ≥1 vs. CPS <1)

**Figure 1**

Eligible patients (eligibility criteria are detailed in Table 1) who failed first-line therapy with locally advanced or metastatic pancreatic cancer confirmed by histopathology will be assigned to receive anlotinib plus toripalimab and nab-paclitaxel as second-line or maintenance treatment (Fig. 1).
Meanwhile, anlotinib could increase the infiltration of innate immune cells with anti-tumour effects, including natural killer (NK) cells, CD8+ T cells, dendritic cells (DCs) and M1-like tumour-associated macrophages (M1 TAMs) whereas inhibit the infiltration of immuno-suppressive cells such as M2 TAMs and regulatory T cells (Tregs) 47,48 (Fig 2).

**Supplementary Files**

This is a list of supplementary files associated with this preprint. Click to download.

- Stable1.docx
- SPIRITChecklist.pdf