Phase I+Phase II Clinical Study of PRaG Therapy in Combination With Chemotherapy (AG Regimen) for Neoadjuvant Treatment of Locally Advanced Pancreatic Ductal Adenocarcinoma (PDAC) (NeoPRAG Study)

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Catalogue

1.0 Research Title

Phase I+Phase II Clinical Study of PRaG Therapy in Combination With Chemotherapy (AG Regimen) for Neoadjuvant Treatment of Locally Advanced Pancreatic Ductal Adenocarcinoma (PDAC) (NeoPRAG Study)

2.0 Purpose of the study

2.1 Primary purpose

Exploring the safety and efficacy of the PRaG treatment modality combined with chemotherapy neoadjuvant therapy for locally advanced pancreatic cancer

2.2 Secondary purpose

Exploring the local control and survival benefit of the PRaG treatment modality combined with neoadjuvant chemotherapy for locally advanced pancreatic cancer

Evaluating markers for efficacy prediction

3.0 Research endpoint

3.1 Primary study endpoints

Phase I: safety and tolerability

Adverse events，AE

Serious adverse events，AE

Phase II：1-year overall survival，OS

3.2 Secondary research endpoints

（1）Objective Response Rate (ORR): The percentage of the total number of patients exhibiting an optimal therapeutic response of Complete Response (CR) and Partial Response (PR) after treatment, out of the total number of evaluable cases with lesions evaluated according to RECIST 1.1 criteria.

（2） Disease Control Rate (DCR): The percentage of the total number of patients showing an optimal treatment response of CR, PR, and Stable Disease (SD) after treatment, out of the total number of evaluable cases, assessing the lesions according to RECIST 1.1 criteria.

（3）Progression Free Survival (PFS): The time from the start of treatment to the observation of disease progression or the occurrence of death from any cause. Patients alive at the time of analysis will have the date of their last contact as the cut-off date.

（4）**Overall Survival (OS):** The duration from the first date of enrollment to the time of death from any cause. Patients alive at the time of analysis will have the date of their last contact as the cut-off date.

（5）**R0 Excision Rate:** The rate at which the tumor was completely removed during surgery, with negative margins on microscopic examination.

（6）**Other Exploratory Research Endpoints:** The exploratory translational study metrics for this study include T-lymphocyte subsets, tumor-associated cytotoxic T-cells, activated cytotoxic T-lymphocytes, activated memory T-cells, monocytes, dendritic cells, and T-cell antigen receptor (TCR) examination. It is required that peripheral blood and/or tissue specimens be retained at study-specified time points whenever possible, and that these specimens be processed as specified in the protocol for subsequent translational studies.

4.0 Rationale for the project

4.1 Current status of surgical treatment of pancreatic ductal adenocarcinoma (PDAC)

Pancreatic ductal adenocarcinoma (PDAC) is highly malignant and aggressive, posing a significant threat to human health. According to GLOBOCAN data, in 2020, there were over 400,000 new cases of pancreatic cancer and related deaths globally, with an incidence rate of approximately 6.4 per 100,000 people. The incidence and mortality rates of pancreatic cancer are rising in most countries, with the 5-year survival rate in the United States at only 12% and in China at about 7.2% [1-3]. The standard treatment for PDAC involves surgical resection combined with chemotherapy. However, real-world clinical data indicate that the 5-year overall survival (OS) rate for patients who have undergone resection is around 20% (increased from less than 5% in 2011), while it remains less than 1% for those who have not had surgery (consistent with rates from 10 years ago) [4]. Large cohort studies report that approximately 20% of patients undergoing surgical resection experience recurrence within 6 months, and 40% within the first year post-surgery, even in cases with no residual tumor at the margin (R0) [5]. Consequently, there is a growing consensus that the biological nature of PDAC

differs from its early stages. Even in cases of resectable pancreatic ductal adenocarcinoma at an early stage, the disease is considered systemic, where surgery alone does not ensure complete tumor clearance. This necessitates a multimodal approach and an integrated, multidisciplinary model of treatment [6].

In 2006, the US National Comprehensive Cancer Network (NCCN) classified non-metastatic pancreatic ductal adenocarcinoma (PDAC) into three categories: resectable (R-PDAC), borderline resectable (BR-PDAC), and unresectable (UR-PDAC) [7]. Current guidelines recommend surgery as the initial treatment for patients with R-PDAC. For those with BR-PDAC, neoadjuvant chemotherapy (NACT) using 5-fluorouracil, calcium folinate, irinotecan, and oxaliplatin (mFOLFIRINOX), or Gemcitabine + Capecitabine, is the preferred regimen. NACT is not typically recommended for patients with UR-PDAC and metastatic PDAC (M-PDAC). However, recent advancements in neoadjuvant regimens have shown promising results, enabling more patients with locally advanced (LA) and metastatic tumors to undergo surgery. Conversion rates for surgery have ranged from 0% to 40% for LA-PDAC and from 4% to 9% for M-PDAC [5].

4.2 **Current status of research on neoadjuvant treatment modalities for pancreatic ductal adenocarcinoma (PDAC)**

Over the past five years, neoadjuvant therapy has emerged as a pivotal topic in the treatment of pancreatic cancer. Increasing high-quality, evidence-based medical research indicates that neoadjuvant therapy can suppress early micro-metastasis of tumors, enhance the rate of surgical resection, and subsequently improve patient prognosis. The benefits of neoadjuvant therapy include:

1. Early inhibition of micrometastases, aiding in controlling tumor recurrence and metastasis.

2. Reduction of tumor burden, facilitating R0 resection.

3. Improved patient tolerance and compliance, addressing intolerance to postoperative adjuvant therapy.

4. Pre-screening of patients with highly aggressive tumors to avert unnecessary surgery.

This development in neoadjuvant therapy represents a synergistic progression of medical and surgical procedures, broadening the surgical candidate pool and integrating resources for individualized treatment strategies.

5. Employing neoadjuvant therapy in healthier patients may allow a multimodal treatment approach for all patients, reducing the need for withdrawal due to postoperative complications.

BR-PDAC (borderline resectable pancreatic ductal adenocarcinoma) is defined by the International Society of Pancreatic Diseases based on anatomical, biological, and clinical criteria [7]. Anatomically, BR-PDAC involves lesions at high risk for positive R1 and R2 resections due to proximity to major blood vessels. Criteria include a contact angle $\geqslant 180^\circ$ with the portal vein or superior mesenteric vein (SMV), any contact with the inferior vena cava, and/or contact angle $<180^\circ$ with a major artery. This definition, unlike the NCCN guidelines, excludes jejunal branches extending into the SMV, mainly due to high anatomical variability [8].

The ESPAC-5F28 phase II study randomly assigned BR-PDAC patients to either initial surgery or NACT (using FOLFIRINOX or AG regimens) or to radiotherapy followed by surgery and AG. No significant difference was observed in the R0/R1 resection rate (44% vs. 41% post-NACT, P=0.668), nor in the number of patients receiving adjuvant therapy. However, 1-year overall survival (OS) significantly favored NACT (77% vs. 42%, HR=0.28; P<0.001), with 1-year OS rates of 84% for FOLFIRINOX, 79% for GA, and 64% for radiotherapy. Retrospective studies suggest comparable efficacy between FOLFIRINOX and GA, with no significant differences in median survival (37.3 vs. 31.9 months) or R0 resection rates (82.8% vs. 81.8%) [9]. FOLFIRINOX and GA are thus preferred NACT regimens for BR-PDAC when patient conditions permit [9].

UR-LA (locally advanced) PDAC includes cases with non-reconstructable venous involvement or contact $\geqslant 180^\circ$ with the superior mesenteric artery (SMA) or celiac artery, or involvement of the first jejunal branch artery of the SMA. In such cases, prognosis remains poor due to high rates of local recurrence and systemic progression, even if atherectomy is technically feasible [10]. Palliative systemic therapy is

recommended for UR-LA patients. However, various studies report successful surgical conversion post-chemotherapy, with or without radiation therapy. Median OS was notably higher in successful conversion surgeries (15.3 vs. 8.5 months, P<0.0001), irrespective of chemotherapy regimen [11]. It is important to note that these studies included only UR-PDAC, not BR-PDAC.

A cohort analysis of 680 patients, comprising 29.3% with BR-PDAC and 60.7% with UR-LA-PDAC, revealed that after clinical, radiological, and biological assessments, 23.9% underwent surgical exploration, with an overall resection rate of 15.1%. This rate represented 24.1% of BR-PDAC cases and 9% of UR-LAPDAC cases. Factors influencing resection included age, BR-PDAC status, completion of chemotherapy, and responsive imaging. The median OS was 12.8 months for the entire cohort, extending to 41.8 months for UR-LA-PDAC patients who underwent transformative surgery. No pre- or post-treatment factors were linked to survival post-pancreatectomy [12].

4.3 Current status of radiotherapy and immunotherapy in the neoadjuvant treatment modality of pancreatic ductal adenocarcinoma (PDAC)

Neoadjuvant radiotherapy is recommended for borderline resectable pancreatic ductal adenocarcinoma (BR-PDAC) in the US NCCN guidelines [8]. However, its value in BR-PDAC remains a subject of debate. Radiotherapy regimens and intensities differ significantly in their effects. Conventional radiotherapy, typically administered in small doses over 3 to 6 weeks with a broader irradiation range, contrasts with Stereotactic Body Radiotherapy (SBRT). SBRT, delivered in 3-5 doses over 1-2 weeks, uses larger doses and targets the tumor exclusively. High-dose irradiation, while effective in killing tumor cells, often alters local tissue reactivity and impacts local tumor resection. SBRT is increasingly adopted as a neoadjuvant approach for patients with BR/LA-PDAC. Reyngold et al. [13] demonstrated that increasing the radiotherapy dose and utilizing ablative radiotherapy, with either SBRT or conventional methods, enhances local control rates.

The CONKO-007 study, a phase III clinical trial involving 495 patients with locally advanced pancreatic cancer (LAPC), showed that adding radiotherapy post-

induction chemotherapy significantly increased the rate of negative peripheral margins and pathological remission. This improvement markedly enhanced the prognosis for patients with R0 resection, showing a 5-year survival rate of up to 35.9% [14]. While radiotherapy aids in local tumor control, further research is needed to determine the optimal radiotherapy regimen, intensity, and dose, minimizing serious complications. The impact of radiotherapy on overall survival also warrants further investigation through prospective studies.

Advances in precision radiotherapy technologies have popularized Hyperfraction Radiotherapy (HFRT) and SBRT in clinical settings. HFRT, compared to conventional fractionation doses, induces immune activation through direct actions on tumor cell DNA, in situ tumor seeding effects, and alterations in the tumor microenvironment, leading to irreversible DNA damage and resulting in apoptosis, necrosis, senescence, or mitotic failure of tumor cells [15,16]. Additionally, radiotherapy can elevate PD-L1 expression in tumor cells, potentially increasing their sensitivity to PD-1/PD-L1 inhibitors. The combination of radiotherapy with PD-1/PD-L1 inhibitors has shown promise in improving patient outcomes and prolonging survival, as evidenced by several clinical studies in various metastatic malignancies [15-19].

However, PDAC typically presents an immune-desert microenvironment, characterized by a high number of M2-type macrophages that suppress the immune system, a scarcity of lymphocytes and regulatory T-cells (Tregs), and a low tumor mutational burden (TMB), rendering it poorly responsive to immune checkpoint inhibitors (ICIs) [20]. Preclinical and metastatic clinical trial data suggest that cytotoxic chemotherapy and radiotherapy can enhance the immunogenicity of PDAC, potentially transforming the tumor microenvironment (TME) from an immunologically 'cold tumor' to a 'hot tumor' [21,22].

Recent studies, such as the one by Du et al., have explored this further. They published a single-arm, phase II exploratory trial of perioperative tirilizumab combined with GA and SBRT. Their pre-specified exploratory analyses included factors such as eosinophil count (associated with immunotherapy response in triple-negative breast cancer), CA 19-9 levels, circulating tumor DNA (ctDNA), neutrophil-to-lymphocyte

ratio (NLR), and TMB. Among the 29 patients with locally advanced (LA) or borderline resectable (BR) PDAC in the study, 25 completed the treatment. The results showed an objective response rate (ORR) of 60% and a disease control rate (DCR) of 100%. The 12-month overall survival (OS) rate was 72%, and the progression-free survival (PFS) rate was 64%, with no reported serious immune-related adverse events (irAEs) [23]. Further trials are planned or ongoing, focusing on neoadjuvant immune checkpoint inhibitors (ICIs), often in combination with standard care chemotherapy and/or radiotherapy.

NCT Number	Phase	ICI	Other Modalities	Status
NCT03572400	II	Durvalumab	Chemotherapy	Recruiting
NCT05132504	II	Pembrolizumab	Chemotherapy	Recruiting
NCT05462496	II	Pembrolizumab	Antibiotics	Not yet recruiting
NCT05562297	II	Sintilimab	None	Not yet recruiting
NCT03563248	II	Nivolumab	Chemotherapy, Radiation, Losartan	Active, not recruiting
NCT03245541	I/II	Durvalumab	Radiation therapy	Not yet enrolling
NCT02305186	I/II	Pembrolizumab	Chemotherapy and radiation	Enrolling
NCT04247165	I/II	Nivolumab, ipilimumab	Chemotherapy and radiation	Enrolling
NCT02930902	I/II	Pembrolizumab	Chemotherapy	Not yet enrolling
NCT03970252	I/II	Nivolumab	Chemotherapy	Enrolling

Figure 1 Ongoing foreign studies of neoadjuvant immunotherapy for pancreatic cancer

4.4 Pre-exploration of the PRaG Treatment Model

Dendritic cells' presentation of tumor antigens to T cells to activate adaptive immunity is a crucial step in the tumor immune cycle. Granulocyte-macrophage colony-stimulating factor (GM-CSF), a commonly used immunosensitizing cytokine, promotes the differentiation and activity of monocyte/M1 macrophages and dendritic cells (DCs), enhancing their activity, antigen presentation, and the overall immune effect. Combining GM-CSF with PD-1 inhibitors can also improve efficacy. Preliminary results for patients with advanced cholangiocarcinoma who received PD-1 inhibitors combined with GM-CSF showed a 35% progression-free survival (PFS) at 6 months of treatment and 7% grade 3 or higher adverse reactions. This suggests that the combination of PD-1 inhibitors with GM-CSF is safe and has achieved good near-term efficacy [24]. Additionally, GM-CSF can extend the immune effect of radiotherapy. Prospective clinical studies have shown that local radiotherapy combined with GM-

CSF for patients with advanced solid tumors induced a distant effect and improved patient prognosis [25].

Based on this, our center was the first to propose the "PRaG protocol" (PRaG 1.0) [26], which is the combination of a PD-1 inhibitor followed by GM-CSF with large fractionated radiotherapy in patients with advanced solid tumors that have progressed after first- and second-line chemotherapy. Fifty-four patients were enrolled in the study with a median follow-up of 16.4 months. The objective remission rate reached 16.7%, and the disease remission rate was 46.3%; the median PFS was 4.0 months, and the median overall survival (OS) was 10.5 months. The overall tolerability of patients receiving the Bragg regimen was good, with 5 patients experiencing grade 3 treatment-related adverse events (TRAEs) and only 1 patient experiencing a grade 4 TRAE, suggesting that the PRaG regimen is an effective therapeutic option for salvaging patients with advanced recurrent solid tumors.

4.5 Advances in Dual Immunity and Dual Antibody Research

Checkpoint inhibitors have proliferated in clinical use as immunotherapies targeting adaptive immune responses, unleashing the immune system and making it more potent in generating a response against tumor cells. The CTLA-4 and PD-1 pathways, linked to tumors' ability to evade the host immune system, are most widely used in clinical practice. However, the number of clinical cases and efficacy of anti-PD-1/PD-L1 or anti-CTLA-4 monotherapy in pancreatic cancer is limited. The objective response rates (ORRs) for monotherapy with immune checkpoint inhibitors and anti-PD-1/CTLA-4 combination therapy in pancreatic cancer were 0% and 3%, respectively. These disappointing results contrast with the remarkable effectiveness of immune checkpoint inhibitors in other solid tumors. In other tumors, the double-immunity treatment modality of nabulizumab combined with ibritumomab improved the ORR of malignant melanoma to 57%, significantly better than the 19% and 44% for monotherapy [27]. Dual-immunotherapy with nabulizumab combined with ipilizumab resulted in a higher ORR (23% vs. 10%) [28]. CheckMate 227 reported that the dual-immunotherapy modality of nabulizumab combined with ipilizumab resulted in 36.2 months of prolonged survival in advanced non-small cell lung cancer [29]. The

double-immunotherapy modality has also achieved good results in malignancies such as colorectal, renal, and gastric/esophageal cancers. Pancreatic cancer, a typical immunologically "cold tumor," requires a transformation from a "cold tumor" to a "hot tumor" to unlock the potential of immunotherapy treatment. The current methods to achieve this transformation include chemotherapy, radiotherapy, and other therapies [30-32]. Preclinical and small-sample clinical studies have shown that anti-PD-1 and anti-CTLA-4 antibodies enhance the immune response induced by ablative monotherapy by blocking regulatory checkpoints, offering a new strategy for immunotherapy in pancreatic cancer [33]. The 2021 ASCO reported data from a phase II clinical study of Cunningham & Gerard KN046 combined with chemotherapy in advanced pancreatic cancer, with an ORR of 55.6% and a disease control rate (DCR) of 88.9% [34]. The ORR of KN046 combination chemotherapy was significantly higher than that of the AG regimen alone. Subsequently, the NMPA approved a multicenter, randomized, double-blind phase III clinical study of the efficacy and safety of KN046 (anti-PD-L1/CTLA-4 dual-antibody) combined with albumin-paclitaxel and gemcitabine versus placebo combined with albumin-paclitaxel and gemcitabine in patients with advanced pancreatic cancer (ENREACH-PDAC-01, NCT05149326).

4.5.1 Mechanism of action of the investigational drug cardunolizumab

Cardunilizumab is a tetravalent IgG-ScFv bispecific antibody [35]. It contains a point mutation in its constant region that prevents the binding of complement protein C1q and the Fcγ receptor involved in cytotoxic effects. Expressed in a Chinese hamster ovary cell line, cardunilizumab has a total molecular weight of approximately 200 kDa, including oligosaccharides. It is a humanized immunoglobulin G1 (IgG1) bispecific antibody (BsAb) with a crystallizable fragment (Fc) mutation that eliminates Fc receptor and complement-mediated cytotoxic effects. Cardunilizumab binds both programmed cell death receptor-1 (PD-1) and cytotoxic T-lymphocyte-associated antigen-4 (CTLA-4), blocking the interactions of PD-1/programmed cell death ligand-1 (PD-L1), PD-1/PD-L2, CTLA-4/B7.1, and CTLA-4/B7.2. For more information on safety findings from clinical studies of combination therapy with anti-PD-1/L1 and anti-CTLA-4 antibodies, refer to the Investigator's Manual.

On 29 June 2022, China's State Drug Administration approved the New Drug Marketing Application for Kaitanib® (cardunculizumab injection), developed by Kangfang Bio. It is the world's first PD-1/CTLA-4 bispecific antibody tumor immunotherapy new drug for treating patients with recurrent or metastatic cervical cancer (R/MCC) who have failed prior platinum-containing chemotherapy treatment. Cetanib® is the first dual immune checkpoint inhibitor dual antibody for tumors approved for marketing globally, filling a gap in immune drug treatment for advanced cervical cancer in China and in the development of bispecific antibodies.

4.5.2 Pharmacokinetic and pharmacodynamic study of cardunolizumab

The pharmacokinetic properties of cardunilizumab were investigated in Crab-Eating Monkeys. After single intravenous administration of 1, 4, and 16 mg/kg of cardunilizum

ab to the cynomolgus monkeys, the observed clearance (Cl) was 2.2, 1.6, and 1.9 ml/h/kg, respectively, and the apparent volume of distribution (Vss_obs) was 91, 92, and 113 ml/kg, respectively. This suggests significant distribution of cardunilizumab in tissues. The half-life (t1/2) after administration of 4 mg/kg was 47.9 h, approximately 1/3 to 1/2 that of typical antibodies. The volume of distribution of cardunilizumab in the monkeys was significantly greater than that of the blood, indicating significant tissue distribution. Rapid clearance from the bloodstream reduces non-specific (off-target) cytotoxicity. The preferential distribution of cardunilizumab in tumor tissue may result in a better safety profile compared to conventional anti-PD-1 and anti-CTLA-4 antibodies.

An approximately proportional dose-to-dose increase in exposure (Cmax and AUC) was observed in the dose range of 1 to 16 mg/kg. The increase in exposure (AUClast) of cardunilizumab was dose-proportional with a power function model β of 1.05, suggesting a linear pharmacokinetic profile of cardunilizumab.

A multiple administration PK study of cardunilizumab was performed in crab-eating monkeys. After 4 weeks of weekly intravenous administration of 4 mg/kg, the ratios of AUC 0-168h and Cmax after the last and first administration were 0.554 and 0.230, respectively. This suggests that the antidrug antibody may interfere with the

detection of analytes or accelerate the clearance of cardunilizumab, resulting in lower exposure to the drug after the last administration compared with that after the first administration.

The dose determination of cardunilizumab at 6 mg/kg Q2W in this study was based on safety data from a Phase Ia dose-escalation trial conducted in Australia and a Phase I/II clinical study conducted in China. In the Australian study, cardunilizumab monotherapy was initiated at a ramp-up dose of 0.2 mg/kg, followed by dose escalations of 0.5 mg/kg, 1 mg/kg, 2 mg/kg, 4 mg/kg, 6 mg/kg, and 10 mg/kg Q2W, with extensions to both the 6 mg/kg and 450 mg dose groups. As of 7 January 2020, 97 subjects were enrolled in the 6 mg/kg Q2W dose group, no drug-related dose-limiting toxicities (DLTs) were observed, the majority of drug-related adverse reactions were Grade 1, Grade 3 or higher treatment-related adverse events (TRAEs) were observed in 11 patients (11.3%), and no Grade 5 TRAEs occurred. The safety profile of cardunilizumab monotherapy was well tolerated. Currently, the ORR of cardunculizumab monotherapy is about 20% in each tumor, and among 8 patients who failed previous PD-1/PD-L1 treatment, 3 cases showed lesion shrinkage. According to pharmacokinetic (PK) data, a 6mg/kg Q2W administration at that time could maintain the trough concentration at ~5ug/ml and ensure the saturation of receptor occupancy (RO) in the in vitro assay. Ki67, as a pharmacodynamic (PD) marker for CTLA-4 and PD-1 blockade, was dose-dependently increased in the expression of Ki67 in the peripheral blood CD4+ T-cells after administration of Cardunilizumab on the eighth day. Moreover, the approved dose of cardunilizumab for the indication of advanced recurrent cervical cancer is 6 mg/kg Q2W.

Therefore, this study will first consider the option of treatment with cardunilizumab 6 mg/kg Q2W, and if DLT occurs, the investigators will discuss and make a decision about continuing, revising, or discontinuing the study.

4.5.3 Safety summary of cardunolizumab

Combining preclinical studies conducted in China and abroad, as of 10 July 2020, a total of 277 patients were treated with cardunilizumab with dose escalation from 0.2 mg/kg Q2W to 25 mg/kg Q3W. Only one subject in the 1.0 mg/kg dose group

experienced a dose-limiting toxicity (DLT) event: a grade 3 aspartate aminotransferase (AST) elevation. For the 15 mg/kg Q3W dose climb, no DLT was observed. For the 25 mg/kg Q3W dose climb, no DLT was observed. The incidence of drug treatment-related adverse events (TRAEs) was 73.6%, and all TRAEs recovered and resolved with suspension of the drug as well as with symptomatic supportive care. As of 29 September 2020, 12 subjects with advanced solid tumors who had failed standard therapy were treated with cardunilizumab (15 mg/kg Q3W), with a 75% (9/12) incidence of treatment-related adverse events (TRAEs), all of which were Common Terminology Criteria for Adverse Events (CTCAE) grades 1-2, and only one grade 3 TRAE, colitis, which resolved rapidly with treatment. There were no TRAEs leading to drug discontinuation and no TRAEs leading to death. The incidence of Grade 3 and higher TRAEs was 12.6%. Common TRAEs (≥2 occurrences) during the study period in the 15 mg/kg Q3W dose group across all dose groups included rash, infusion reactions, fatigue, nausea, itching, elevated alanine transaminase (ALT)/AST, fever, and hyperthyroidism, the majority of which were Grade 1-2. Regarding the preliminary antitumor activity of the cardunilizumab 15mg/kg Q3W dose group, 5 out of 10 assessable subjects with advanced solid tumors who had failed systemic therapy achieved partial remission (PR) with an ORR of 50%. These results indicate that the cardunilizumab 15mg/kg Q3W dosing regimen has a good safety and tolerability profile, as well as significant antitumor activity.

4.5.4 Risk/benefit assessment

The potential risks associated with cardunilizumab and related molecules primarily involve immune-mediated reactions. These potential immune-related adverse events (irAEs) might resemble those arising from the use of anti-PD-1/L1 and/or anti-CTLA-4 drugs. Such reactions could include, but are not limited to, infusion-related reactions (fever, rash, pruritus, hypotension, dyspnea, chest discomfort, wheezing, tachycardia, rigors), dermatotoxicity (rash, pruritus, vitiligo), endocrine toxicity (hyperglycemia, hypothyroidism, hyperthyroidism, primary hypoadrenalism, pituitary gland inflammation), hepatotoxicity (hepatitis, elevated aspartate aminotransferase, elevated alanine aminotransferase, elevated bilirubin), gastrointestinal toxicity

(diarrhea, colitis), pulmonary toxicity (pneumonitis, pulmonary nodulosis), rheumatoid/skeletal muscle toxicity (rheumatoid arthritis, myositis, myalgia), neurotoxicity (myasthenia gravis, aseptic meningitis, encephalitis, transverse myelitis, Guillain-Barre syndrome), hematotoxicity (autoimmune hemolytic anemia, aplastic anemia, immune thrombocytopenia, acquired hemophilia), nephrotoxicity (nephritis, renal insufficiency), cardiotoxicity (myocarditis, pericarditis, cardiovascular anomalies), and ophthalmic toxicity (uveitis, scleritis). To date, the safety events identified in clinical studies with cardunilizumab are consistent with tumor immunotherapy targeting PD-1 and/or CTLA-4, with no new adverse events reported.

Preliminary data from the Phase Ia clinical trial of cardunilizumab indicate that it is safe and well-tolerated in patients with advanced tumors, showing preliminary antitumor activity and clear pharmacological activity. Given recent data suggesting significant benefits of anti-PD-1 and anti-CTLA-4 antibody double-immunity therapy in multiple tumor types, it is proposed that the anti-PD-1 and CTLA-4 bispecific antibody cardunilizumab may also be effective in similar patients.

Furthermore, tumor-infiltrating lymphocytes co-expressing PD-1 and CTLA-4 receptors exhibit higher levels of these receptors compared to lymphocytes in normal tissue and peripheral blood. Cardunilizumab, targeting both PD-1 and CTLA-4, has a tetravalent structure and a short half-life. Its low toxicity, as observed in the Crab-Eating Monkey study, suggests it might be more effective and/or safer than combination therapy with anti-PD-1/L1 and anti-CTLA-4 antibodies alone.

The current study is informed by the preliminary efficacy demonstrated by Corning Jericho KN046 (anti-PD-L1/CTLA-4 dual antibody) in combination with albumin paclitaxel and gemcitabine in patients with advanced pancreatic cancer. As of 26th May 2021, 22 patients had undergone at least one tumor assessment, showing an objective remission rate (ORR) of 50.0%, a disease control rate (DCR) of 95.5%, and a 6-month progression-free survival rate (PFS-6M Rate) of 62.3%. Four patients, who met the criteria for surgical resection by multidisciplinary team (MDT) assessment after 4-6 cycles of treatment, underwent surgery. The study explores the use of the approved and marketed cadaverine monoclonal antibody (PD-1/CTLA-4 dual-antibody) in

combination with mFOLFIRINOX chemotherapy for the translational treatment of patients with locally progressive pancreatic ductal adenocarcinoma.

In summary, the study aims to enroll patients with critically resectable pancreatic ductal adenocarcinoma to explore potential markers for patients suitable for neoadjuvant therapy. This approach will be through the Bragg treatment modality in combination with the neoadjuvant treatment modality of chemotherapy.

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5.0 Selection of study population

5.1 Inclusion Criteria

5.1.1 Age ⩾ 18 ⩽ 75 years；no gender limitations

5.1.2 Histopathologically and/or cytologically confirmed ductal adenocarcinoma of the pancreas, the patient has fresh pathological tissue and the tumour is located in the head and neck or body of the pancreas.

5.1.3 Locally advanced pancreatic cancer, borderline resectable or unresectable, without metastases.

5.1.4 Life expectancy >= 3 months.

5.1.5 ECOG score 0-1.

5.1.6 Have at least 1 measurable lesion according to RECIST 1.1 criteria.

5.1.7 No prior treatment with abdominal radiotherapy, chemotherapy and PD-1/PD-L1 antibody.

5.1.8 Adequate organs functions as defined by the following laboratory values (completed within 14 days prior to registration):

(1) haemoglobin >= 90 g/L (no blood transfusion within 14 days)；

(2) neutrophil count > 1.5x10^9/L；

(3) platelet count >= 100x10^9/L；

(4) total bilirubin <= 1.5xULN (upper limit of normal)；

(5) blood glutamic transferase (ALT) or blood glutamic transferase (AST) <= 2.5xULN

(6) endogenous creatinine clearance >= 60 ml/min (Cockcroft's AST). (ALT) or blood albumin transaminase (AST) <= 2.5xULN；

(6) endogenous creatinine clearance >= 60 ml/min (Cockcroft-Gault formula)；

(7) cardiac Doppler ultrasound assessment: left ventricular ejection fraction (LVEF) >= 50%.

(8) International normalised ratio (INR) of prothrombin time ≤ 1.5 and partial thromboplastin time (APTT) ≤ 1.5 times the upper limit of normal in patients who have not received anticoagulation. Patients receiving full or parenteral anticoagulant therapy may enter a clinical trial as long as the dose of anticoagulant has been stable for at least 2 weeks prior to entry into the clinical study and the results of coagulation assays are within the limits of local therapy.

5.1.9 No congestive heart failure, unstable angina, unstable arrhythmia in the last 6 months.

5.1.10 No previous severe haematopoietic, cardiac, pulmonary, hepatic or renal abnormalities or immunodeficiencies.

5.1.11 Patient must be able to understand the potential risks and benefits associated with this study. Patient able to give informed consent and would likely to comply with the study parameters.

Exclusion Criteria:

5.2.1 Pregnant or breastfeeding women.

5.2.2 Patients with a history of other malignant diseases in the last 5 years, except cured skin cancer and cervical cancer in situ.

5.2.3 Patients with a history of uncontrolled epilepsy, central nervous system disease or psychiatric disorders whose clinical severity, in the judgement of the investigator, may

prevent the signing of informed consent or affect the patient's adherence to drug therapy.

5.2.4 Severe heart disease, such as symptomatic coronary heart disease, New York Heart Association (NYHA) class II or worse congestive heart failure or severe arrhythmia requiring pharmacological intervention, or a history of myocardial infarction within the last 12 months.

5.2.5 Organ transplants requiring immunosuppressive therapy

5.2.6 Active infection or, in the investigator's judgement, significant haematological, renal, metabolic, gastrointestinal, endocrine function or metabolic disorders, or other serious uncontrolled concomitant disease

5.2.7 Allergy to any of the study drug ingredients.

5.2.8 History of immunodeficiency, including HIV-positive or other acquired or congenital immunodeficiency diseases, or history of organ transplantation, or other immune-related diseases requiring long-term oral hormone therapy.

5.2.9 During acute or chronic tuberculosis infection (patients with a positive T-spot test and suspicious tuberculosis foci on chest radiographs).

5.2.10 Other conditions considered by the investigator to be unsuitable for enrolment.

5.3 Exit criteria

5.3.1. Patients who, in the judgement of the investigator, will not benefit from continued medication after medical imaging has shown progression of the disease

5.3.2. Those whose toxicity remains intolerable to the patient after suspension of drug therapy;

5.3.3. The patient withdrew informed consent and asked to be withdrawn;

5.3.4. Other situations where the researcher felt it was necessary to withdraw from the study.

6.0 Research treatment

6.1 Research design

Phase I clinical study

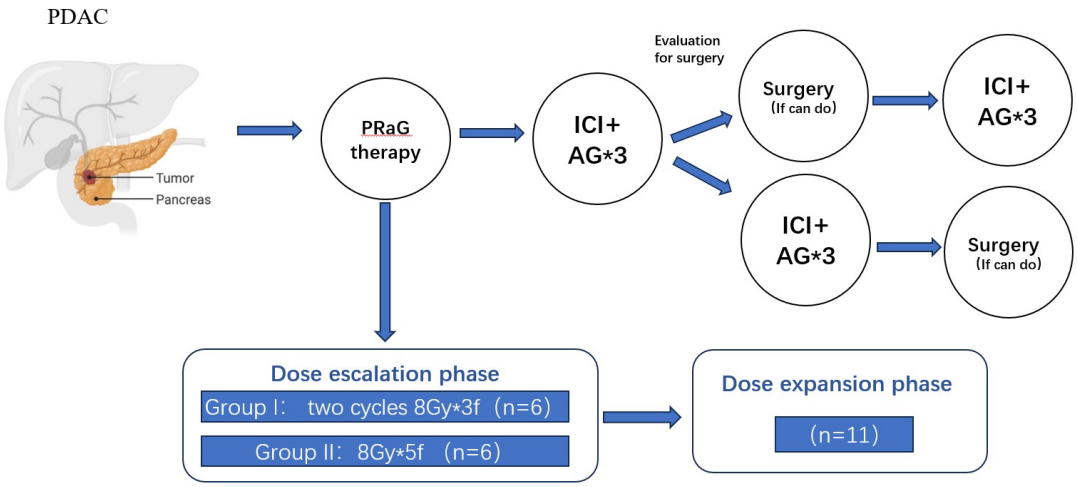

Fig. 2 Flowchart of the study design

Phase I clinical studies are divided into phases Ia+Ib. Phase Ia is a dose-climbing trial, which is divided into two cohorts of 3+3 patients each according to the radiotherapy dose. Phase Ib is a sample size expansion phase, for which priority is given to choosing the best-tolerated regimen in terms of safety among the phase Ia regimens, followed by choosing regimens with high surgical conversion rates.

6.2 Treatment programme

Dose-climbing experimental stage

6.2.1 Phase I: PRaG treatment

Group I n=3+3

First cycle of PRaG treatment

Radiotherapy：24Gy：8Gy*3f d4-d6

GM-CSF treatment: GM-CSF 200 μg subcutaneously daily for 7 days starting on the day of radiotherapy; d1-d7

Immunotherapy: cardunculizumab 375mg within one week after radiotherapy

Second cycle of PRaG treatment

Radiotherapy：24Gy：8Gy*3f d4-d6

GM-CSF treatment: GM-CSF 200 μg subcutaneously daily for 7 days starting on the day of radiotherapy; d1-d7

Immunotherapy: cardunculizumab 375mg within one week after radiotherapy

Group II n=3+3

Radiotherapy：40Gy：8Gy*5f d3-d7

GM-CSF treatment: GM-CSF 200 μg subcutaneously daily for 7 days starting on the day of radiotherapy; d1-d7

Immunotherapy: cardunculizumab 375mg within one week after radiotherapy

6.2.2 Phase II: 3 cycles of neoadjuvant immunotherapy combined with chemotherapy

After 3 weeks of phase I immunotherapy

Cardunolizumab 375mg d1

Albumin-bound paclitaxel 125mg/m2 d1,d8

Gemcitabine 1000mg/m2 d1,d8

6.2.3 Phase III: Surgical treatment

First Surgical Evaluation: After three cycles of neoadjuvant immuno-combination AG regimen, tumor indices and imaging examinations are improved, followed by a multidisciplinary discussion to decide on the feasibility of surgery.

Second Surgical Evaluation: For patients deemed inoperable after the first evaluation, an additional three cycles of the immuno-combination AG regimen are administered. Surgery is re-evaluated upon completion of these chemotherapy cycles.

For patients with preoperative total bilirubin ≤102.6 mmol/L, yellowing can be reduced with the help of PTCD or a biliary stent.

Patients who are resectable after neoadjuvant therapy undergo radical surgical treatment, such as radical pancreaticoduodenectomy, radical paracentesis modular pancreas splenectomy, and radical total pancreatectomy with regional lymph node dissection.

6.2.4 Phase IV: 3 cycles of immuno-combination chemotherapy

Patients who have undergone surgery, as well as those assessed as inoperable after three cycles of chemotherapy combined with immunotherapy, continue with three cycles of immune-combination chemotherapy.

Cardunolizumab 375mg d1

Albumin-bound paclitaxel 125mg/m2 d1,d8

Gemcitabine 1000mg/m2 d1,d8

6.2.5 Imaging Evaluation

Pre-treatment imaging assessment is performed, followed by the first imaging assessment after two cycles of treatment and the second imaging assessment after three cycles of AG combination immunotherapy. The third imaging assessment occurs after completion of six cycles of adjuvant therapy. Multi-stage enhanced CT is preferred, with CT thin-layer reconstruction performed whenever possible. However, imaging often fails to reflect biological attributes such as tumor heterogeneity, activity, blood supply, and immune cell infiltration. As pancreatic cancer is rich in mesenchyme, the tissue around the tumor also produces an inflammatory reaction and fibrosis after neoadjuvant therapy. Even if the therapy is effective, the size of the tumor and the extent of involvement of important blood vessels often do not significantly change. It is often difficult to accurately assess the effect of neoadjuvant therapy for pancreatic cancer and the resectability of the tumor with the RECIST 1.1 criteria. Therefore, dynamic enhanced MRI, PET-CT, and CA199 assessments are combined for comprehensive evaluation.

6.2.6 Pathological evaluation

Pathologists perform post-surgical imaging to assess margins, lymph node status, etc. For patients who do not undergo surgery, re-puncture for pathology retention is conducted at the end of six chemotherapy cycles.

6.2.7 Collection of specimens

Collection includes 10 ml of fresh pathological tissue and peripheral blood samples before treatment, 10 ml of peripheral blood samples after Bragg treatment, 10 ml of peripheral blood samples after the first immuno-combination chemotherapy, 10 ml of peripheral blood specimens after the fourth cycle of AG-combination immunotherapy, 10 ml of fresh surgical tissue specimens and peripheral blood after the surgery, and 10 ml of peripheral blood specimens after completion of six cycles of adjuvant therapy.

6.2.8 Decision-making in the dose-climbing phase

The Data Review Panel, consisting of investigators, medical supervisors, physicians, clinical representatives, and statisticians, determines whether to increase

the Phase I dose based on the presence or absence of DLTs. Before deciding to increase the dose, the panel reviews all relevant adverse event data, including non-DLT toxicities, laboratory evaluations, and other safety assessments, as well as any data described in the Dose Reduction Plan. Quality control of critical safety data is also outlined in the dose reduction plan, including ongoing study monitoring visits, review of clinical databases, and confirmation of data accuracy and completeness by site investigators. Dose reduction decisions and rationale are documented in writing and maintained at each study site.

Group I receives a radiotherapy dose of 8Gy*3f before surgical treatment. If no patient among the first three subjects develops DLT, the original dose continues for three more cases. If there is still no DLT or only one case develops DLT, enrollment in Group II proceeds. If one of the first three patients in Group I develops a DLT, then three additional patients receive the same dose. If two or three patients develop DLT, the dose is deemed to exceed the maximum tolerated dose (MTD) of the drug, and no further enrollment in Group II occurs. The mode and dose of Bragg treatment in the next extended sample size phase are determined based on the safety and tolerability of the two groups. If the safety profiles of the two groups are similar, the mode and dose for the next phase are decided based on the surgical conversion rate and R0 resection rate.

6.3 Radiotherapy delivery

The pancreas is selected for radiotherapy, administered once daily.

6.3.1 Posture fixation

The patient is placed in a comfortable position, fixed with a thermoplastic body wrap, and positioned under CT simulation. The scanning scope covers the target area and all critical organs, extending at least 5-10 cm to the cephalad and foot side of the target area boundary.

6.3.2 Definition of target area

Gross Tumor Volume (GTV): Tumors visible by imaging and clinical examination, including physical examination, fiberoptic colonoscopy, ultrasound, CT, MRI, and PET.

Internal Target Volume (ITV): Considers respiratory motion or organ-moving

tumor foci.

Planning Target Volume (PTV): GTV/ITV + postural error.

6.3.3 Prescribed dose for target area

Prescribed dose: 48Gy/8Gy/6f, irradiated once daily. Image-guided radiotherapy (IGRT) is required for each session.

6.3.4 Safe dose for normal tissues

Safe dose for normal tissues（Refer to AAPM Task Group 101 document）。

Tandem organ	Volumes	Maximum dose by volume（Gy）	Maximum point dose（Gy）	Adverse events (≥ grade 3)
cauda equina	<5cc	21.9Gy(7.3Gy/Fx)	24Gy(8Gy/Fx)	neuritis
sacral plexus	<5cc	22.5Gy(7.5Gy/Fx)	24Gy(8Gy/Fx)	neuropathy
esophagus	<5cc	17.7Gy(5.9Gy/Fx)	25.2Gy(8.4Gy/Fx)	Stenosis/fistula
brachial plexus	<3cc	20.4Gy(7.5Gy/Fx)	24Gy(8Gy/Fx)	neuropathy
Heart/Pericardium	<15cc	24Gy(8Gy/Fx)	30Gy(10Gy/Fx)	pericarditis
capillary blood vessels	<10cc	39Gy(13Gy/Fx)	45Gy(15Gy/Fx)	aneurysm
Trachea and main bronchi	<4cc	15Gy(5Gy/Fx)	30Gy(10Gy/Fx)	Stenosis/fistula
Bronchial branches	<0.5cc	18.9Gy(6.3Gy/Fx)	23.1Gy(7.7Gy/Fx)	stenosis with atelectasis
Ribs	<1cc <1cc	28.8Gy(9.6Gy/Fx) 30.0Gy(10.0Gy/Fx)	36.9Gy(12.3Gy/Fx)	Pain or fracture
Skin	<10cc	30Gy(10Gy/Fx)	33Gy(11Gy/Fx)	ulcers
Stomach	<10cc	16.5Gy(5.5Gy/Fx)	22.2Gy(7.4Gy/Fx)	Ulcers/fistulas
Bile ducts			35.7Gy(11.9Gy/Fx)	narrower
Duodenum	<5cc <10cc	16.5Gy(5.5Gy/Fx) 11.4Gy(3.8Gy/Fx)	22.2Gy(7.4Gy/Fx)	ulcers
Jejunum/Ileum	<5cc	17.7Gy(5.9Gy/Fx)	25.2Gy(8.4Gy/Fx)	Inflammation/obstruction
colon	<20cc	24Gy(8Gy/Fx)	28.2Gy(9.4Gy/Fx)	Colitis/fistula
rectum	<20cc	24Gy(8Gy/Fx)	28.2Gy(9.4Gy/Fx)	Proctitis/fistula
ureter			48.9Gy(16.3Gy/Fx)	narrower
Femoral head (left/right)	<10cc	21.9Gy(7.3Gy/Fx)		Necrosis
Renal portal/vascular	<2/3 volume	18.6Gy(6.2Gy/Fx)		malignant hypertension

trunk				
parallel organ	critical volume	Critical Volume Maximum Dose （Gy）		Adverse events (≥ grade 3)
Lungs (left/right)	1500cc	11.6Gy(2.9Gy/Fx)		baseline lung function
Lungs (left/right)	1000cc	12.4Gy(3.1Gy/Fx)		inflammation of the lungs
liver	700cc	19.2Gy(4.8Gy/Fx)		Basic liver function
Renal cortex (left/right)	200cc	16.0Gy(4.0y/Fx)		Basic renal function

6.4 Combination of drugs

1) Medications deemed consistent with the protocol by the Investigator, such as those used for treating disease-related symptoms or managing various adverse events (AEs) associated with the treatment, are permitted.

2) Subjects requiring long-term medication for pre-existing medical conditions, such as hypertension or diabetes, may continue their medication regimen.

3) The use of topical glucocorticoids, including dermal applications, eye drops, nasal sprays, and inhalations, is allowed.

4) Routine administration of non-steroidal anti-inflammatory drugs (NSAIDs) is permitted.

7.0 Research and Evaluation

7.1 Evaluation of toxic reactions during radiotherapy and immunotherapy

Toxicity reactions are evaluated using the Common Terminology Criteria for Adverse Events (CTCAE) version 5.0, the European Society for Medical Oncology (ESMO) Clinical Practice Guidelines for the Management of Toxicity in Immunotherapy, ESMO Diagnosis, Treatment and Follow-up, and the Chinese Society of Clinical Oncology (CSCO) Guidelines for the Management of Toxicity in Immunotherapy, as detailed in the Case Report Form (CRF) table.

7.2 Evaluation of the efficacy of treatment

Patients were examined periodically during and after treatment to assess their outcome and prognosis, and the items and time points are shown in the table below:

	Baseline before treatment	Before each treatment cycle during treatment	After 3 cycles of treatment	After 6 cycles of treatment	One month after surgery
clinical examination	X	X	X	X	X
routine blood test	X	X	X	X	X
liver and kidney function	X	X	X	X	X
Tumour indicators	X	X	X	X	X
Thyroid function	X	X	X	X	X
Myocardial Enzyme Profile	X	X	X	X	X
glycated haemoglobin	X	X	X	X	X
electrocardiography	X	/	X	X	/
cardiac ultrasound	X	/	X	X	/
spirometry	X	/	X	X	/
Enhanced CT Chest	X	/	X	X	X
Enhanced CT Abdomen	X	/	X	X	X
Enhanced CT of the pelvis	X	/	X	X	X
Abdominal Enhanced MR	unconditional	/	unconditional	unconditional	/
PET-CT	unconditional	/	unconditional	unconditional	/
Quality of life assessment	X	X	X	X	X
Toxicity response evaluation	X	X	X	X	X

Efficacy assessment: assessed using RECIST 1.1 criteria, respectively.

8.0 Annual Research Programme

2024.01-2024.10	Perform patient enrolment and follow-up
2024.11-2025.10	Perform patient enrolment and follow-up
2025.11-2026.10	Completion of patient enrolment and follow-up
2026.11-	Data collation and analysis, research summaries, publications

9.0 Research Design and Statistical Analysis

9.1 Study design and sample size calculation

Phase I: This study is a Phase I clinical trial, divided into Phase Ia and Ib. Phase Ia, a dose-escalation experiment, is split into two cohorts based on radiotherapy dosage, each with 3+3 patients, totaling 12 patients across three groups. Phase Ib, an expansion phase, plans to enroll 11 patients. From the three protocols in Phase Ia, the one with the highest safety tolerability is preferred, followed by the protocol with a high surgical conversion rate. It is proposed to enroll a total of 23 patients. Considering a 10% loss-to-follow-up rate, the total sample size is projected to be 26 patients.

Phase II Study: The primary endpoint is the 1-year overall survival (OS) rate. Based on the ESPAC-5F28 Phase II study, the 1-year OS rate for patients undergoing direct surgery is approximately 40%. This study aims to increase the 1-year OS rate to 60%. Using PASS software, Tests for One Proportion, P0=0.40, P1=0.60, α=0.05, 1-β=0.8, the sample size was calculated to be 36 cases. Factoring in a 10% loss-to-follow-up rate, a total of 40 patients are proposed to be enrolled.

9.2 Statistical analysis

Data analysis will be conducted using SPSS25.0 statistical software. The residuals will be tested for normality using the Shapiro-Wilk line test, with a test level α>0.05. Indicators conforming to normal distribution will be analyzed using random area group ANOVA, while those not conforming will be tested with the random area group design rank-sum test, with a test level α<0.05. This analysis will compare changes in leukocyte counts before and after radiotherapy, including granulocyte and lymphocyte count changes, and alterations in cytokines. In conjunction with survival time, Cox regression

will analyze the effects of changes in leukocyte counts, granulocyte counts, lymphocytes, tumor markers, their classified cell number alterations, and cytokine changes before and after radiotherapy on patient survival. The Kaplan-Meier method will be used to analyze the difference in survival rates between patients with and without various markers, and to study the relationship between patient survival rates and factors such as relevant cytokines and changes in tumor cells.

Signature of the principal investigator：

Date：