BMJ Open

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Journal:	*BMJ Open*
Manuscript ID	bmjopen-2022-067767
Article Type:	Protocol
Date Submitted by the Author:	06-Sep-2022
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Keywords:	Clinical trials < THERAPEUTICS, Gynaecological oncology < GYNAECOLOGY, IMMUNOLOGY, CHEMOTHERAPY

Neoadjuvant Camrelizumab plus Chemotherapy for Locally Advanced Cervical Cancer (NACI study): A Study Protocol of Prospective, Single-arm, Phase II trial

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Keywords: Neoadjuvant chemotherapy, Immunotherapy, Locally Advanced Cervical Cancer (LACC), Camrelizumab, PD-1 inhibitor

Running tittle: Neoadjuvant chemo-immunotherapy for LACC.

Abstract

Introduction: Neoadjuvant chemotherapy (NACT) is an emerging approach for locally advanced cervical cancer (LACC), however, the clinical response and postoperative adjuvant radiation or chemoradiation trimodality treatment resulting in controversy. PD-1 inhibitors have shown promising role in recurrent or metastatic cervical cancer, and preclinical evidence of the activation and synergistic effects of NACT on PD-1 inhibitors. This study aims to evaluate the efficacy and safety of the preoperative PD-1 inhibitor camrelizumab combined neoadjuvant therapy for LACC.

Methods and analysis: The study is designed as a multicenter, open-label, single-arm, prospective phase II study. A total of 82 patients will receive neoadjuvant chemo-immunotherapy, defined as one cycle of cisplatin (75-80 mg/m2, iv) plus nab-paclitaxel (260 mg/m2, iv) NACT and subsequent two cycles of camrelizumab

(200mg, iv) combined NACT. After neoadjuvant chemo-immunotherapy, patients exhibiting complete response (CR) and partial response (PR) will undergo radical surgery and subsequent adjuvant therapy, whereas patients with stable disease (SD) and tumor progression (PD) will transfer to concurrent chemoradiotherapy (CCRT). Following surgery, patients will receive adjuvant CCRT or radiotherapy. The primary endpoint is the objective response rate (ORR), and the secondary endpoints are the pathological complete response (pCR), patients requiring postoperative adjuvant therapy, safety of neoadjuvant chemo-immunotherapy, surgical complication, event-free survival and overall survival. An additional aim is to dynamically evaluate of peripheral immune responses and local immunological microenvironments and their association with neoadjuvant immunotherapy.

Ethics and dissemination: This trial was approved by The Medical Ethics committee of Tongji Medical College, HUST (S2020-112). This is one of the first study to evaluate the efficacy and safety of neoadjuvant chemo-immunotherapy in LACC, and the findings of this research will promote neoadjuvant anti-PD-1 immunotherapy with radical surgery as new therapeutic strategy.

Trial registration number: NCT04516616 (ClinicalTrials.gov).

Strengths and limitations of this study

- This is one of the first multi-center, prospective cohort study to evaluate the efficacy and safety of neoadjuvant chemo-immunotherapy in locally advanced cervical cancer (LACC).
- Induction chemotherapy will be used to recondition the tumor immune microenvironment in prior to anti-PD-1 combined chemotherapy, giving the time window for PD-L1 detection and clinical feasibility.
- Paclitaxel was replaced with nab-paclitaxel as a chemotherapy regimen, avoiding the combination use of glucocorticoids that may affect the efficacy of PD-1 inhibitors.
- Postoperative adjuvant therapy is in accordance with the international guidelines.
- Blood samples and biopsies of the tumor tissue will be collected into a translational research program.

INTRODUCTION

Cervical cancer is the fourth most frequently diagnosed cancer and the fourth most common cause of cancer-related deaths among women worldwide, accounting for approximately 604,000 new cases and 342,000 deaths in 2020.[1] Locally advanced

cervical cancer (LACC) is an early-stage cervical cancer characterized by a local tumor diameter ≥4 cm with a relatively poor prognosis. The large extent of malignant tissue makes complete surgical resection very difficult. Concurrent chemoradiotherapy has been the standard treatment for LACC for several years. Nevertheless, the prognosis has not improved significantly over the past decades, with an average 5-year survival rate of 60-82.3%. Furthermore, treatment options that improve survival in this subgroup of patients continue to be a major public health concern.[2-4] Hence, effective therapies for further improvement in survival must be developed.

Platinum-based neoadjuvant chemotherapy (NACT) followed by radical hysterectomy has been proposed as an alternative approach in areas where radiotherapy apparatus is not available, which increase the opportunities for radical surgery and indicates a similar prognosis compared with concurrent chemoradiotherapy (CCRT). The NACT, however, is only effective in two-thirds of patients, and those who do not respond receive little benefit from it.[5 6] Moreover, 32.2% of patients required postoperative adjuvant radiation or chemoradiation, resulting in controversy in terms of health economics and trimodality treatment.[3] Therefore, although NACT has been widely used in many countries, it is not recommended as a first-line treatment for LACC.

Recently, immune checkpoint inhibitors (ICIs) have made remarkable advances in the field of immunotherapy for cervical cancer and pembrolizumab combined chemotherapy has been approved as a first-line treatment for advanced PD-L1-positive cervical cancer.[7 8] Previous clinical trials have shown that immunotherapy, with a favorable toxicity profile and durable responses, has made a tremendous breakthrough in the treatment of cervical cancer, whereas immuno-monotherapy shows a relatively low response rate. In advanced or recurrent cervical cancer, ICIs monotherapy has a response rate of approximately 15%, and combination therapy with chemotherapy, radiotherapy, or other immune-targeted therapies have improved response rates to 50%-60%.[9 10]

Neoadjuvant chemo-immunotherapy has attained milestones in the treatment of many solid tumors, whereas in terms of cervical cancer, the majority of research has focused on immunotherapy in patients with metastatic or recurrent disease, and very few studies have explored neoadjuvant immunotherapy in LACC. Recent theoretical developments have revealed that chemotherapeutic agents possess immunomodulatory properties, modulating immune cells and shaping their functions. Conventional chemotherapeutics have been known to promote immunogenicity of the tumor due to the modulation of the anti-tumor T cell response through triggering the release of tumor-derived antigens, inducing immunogenic cell death, disrupting the immunosuppressive microenvironment and enhancing the effector T-cell response.[11] In addition, clinical research supports that NACT can increase PD-L1 expression in cervical cancer, and over-expression of PD-L1 has been shown to be

significantly associated with a better response to ICIs, such as the PD-1/PD-L1 blockade.[12] These findings suggest that reprogramming the tumor immunologic microenvironment using NACT may potentially sensitize cervical tumors to immunotherapy and act synergistically with ICIs, similar to early triple-negative breast cancer (TNBC) and non-small cell lung cancer (NSCLC).[13 14]

Camrelizumab is a human immunoglobulin G4 monoclonal antibody that targets the PD-1 immune checkpoint receptor. Favorable efficacy and tolerable safety profiles have been demonstrated in several advanced tumors. We perform this pilot study to evaluate the clinical activity and safety of NACT combined with PD-1 inhibitors in patients with LACC. Furthermore, we will also explore the relationship between the tumor microenvironment landscape and response to therapy, as well as dynamic changes in intratumoral and peripheral immune microenvironments.

METHODS AND ANALYSIS

Trial design

The study is designed as a multicenter, open-label, single-arm, prospective phase II study. A total of 82 patients will receive neoadjuvant immunotherapy, defined as one cycle of cisplatin plus nab-paclitaxel NACT and subsequent 2 cycles of PD-1 antibody combined with NACT. Figure 1 shows the flowchart of the trial. Patients will be recruited from large 3A hospitals and a list of all participating centers is added as Supplementary Table 1.

The objective of the study

This study aims to evaluate the effects of neoadjuvant chemo-immunotherapy on tumor response (including clinical response and pathological response), the proportion of patients requiring postoperative adjuvant therapy, treatment-related toxicity, subsequent surgical complications, and survival (including event-free survival (EFS) and overall survival (OS)) of patients with LACC. An additional aim of this study is to dynamically evaluate peripheral immune responses and local immunological microenvironments, and their association with neoadjuvant immunotherapy efficacy.

Endpoints of the study

The primary endpoint is the objective response rate (ORR), defined as the percentage of the participants who achieve a complete response (CR) or partial response (PR). Participants who receive camrelizumab therapy and have at least one post-baseline tumor assessment will be included in the primary endpoint analysis. The ORR will be assessed by a blind independent central reviewer per Response Evaluation Criteria in Solid Tumors V.1.1 (RECIST 1.1). The secondary endpoints are the rate of pathological complete response (pCR), the number of patients with treatment-related

adverse events (AEs), serious AEs or immune-related AEs (irAEs), the number of patients with surgical complications and postoperative adjuvant therapy, EFS, and OS. Histological examination of the entire resected specimen will be performed to identify patients with pCR, defined as the absence of viable tumor cells on all slides. Based on the Buda criteria, the optimal response defines as a complete disappearance of the tumor in the cervix with negative nodes or a residual disease with less than 3 mm stromal invasion, including in situ carcinoma.[15] AEs after the first dose of chemotherapeutic drugs to 30 days after the last dose is evaluated using the Common Terminology Criteria for Adverse Events (CTCAE) (V.5.0). The safety set used for the safety evaluation will include patients using camrelizumab at least once and with relatively complete medical records. Postoperative complication defines as any clinically significant deviation from a normal postoperative course. EFS is defined as the time interval from the date of randomization to disease progression, local or distant recurrence (in patients undergoing surgery), or death due to any cause. OS is defined as the date of randomization until the date of death due to any cause or the last follow-up visit. Additionally, in participants who undergo radical surgery, disease-free survival (DFS) is calculated separately from the date of completing prescribed treatment to the date of disease relapse or death, irrespective of the cause.

Study Procedures

Patient selection/screening

Eligible patients are18-70 years of age and have newly diagnosed cervical cancer of stage IB3, IIA2, or IIB/IIIC1r with tumor diameter ≥4cm (FIGO, 2018). All patients will be PD-L1 positive, with pathologically confirmed squamous cell carcinoma, adenocarcinoma or adenosquamous carcinoma, and have normal organ function. The detailed inclusion and exclusion criteria are listed in Table 1. Informed consent will be obtained before PD-L1 immunohistochemistry assays and the participant will receive one cycle of cisplatin and nab-paclitaxel treatment. PD-L1 expression is evaluated using the 22C3 antibody clone (Agilent, Dako). Participants who do not satisfy the inclusion criteria or meet the exclusion criteria after randomization will be excluded.

Table 1 Inclusion and exclusion criteria

Inclusion criteria	Exclusion criteria
FIGO stage IB3, IIA2, or IIB/IIIC1r (tumor size≥4cm) cervical cancer and without any treatment	The active autoimmune disease requiring a systemic treatment
Histologically confirmed squamous cell carcinoma, adenocarcinoma or adenosquamous carcinoma of the cervix	Previous treatment with checkpoint inhibitors or hypersensitivity to study drug

PD-L1 positive (Combined positive score≥1)	Patients requiring treatment with systemic corticosteroids (over 10 mg/day dose) or immunosuppressants within 14 days before enrolment
Females 18-70 years of age	Inoculating live vaccine or attenuated live vaccine within 4 weeks
ECOG score 0-1	History of allogeneic tissue/solid organ transplant
WBC≥3.5*10^9/L, NEU≥1.5*10^9/L, Platelet≥80×10^9 /L; AST and ALT ≤1.5 times normal upper limit; Total bilirubin ≤1.5 times the upper limit of normal value; serum creatinine and blood urea nitrogen ≤the upper limit of normal value	Known active HBV, HCV or HIV infection
	Patients who have serious or uncontrollable disease
	Patients who have a serious or uncontrollable disease
	Pregnant or lactating female patients
Willing to participate in this study and signed the informed consent Patients who observed the rules about scheduled visit, study schedule and medical examination	Drug or alcohol abuse
	Participate in clinical trials at the same time
Patients who observed the rules about scheduled visit, study schedule and medical examination	Unable or unwilling to sign informed consents
	Not eligible for the study judged by researchers

ECOG, Eastern Cooperative Oncology Group; WBC, White Blood Cell; NEU, neutrophils; AST, Aspartate Aminotransferase; ALT, Alanine Aminotransferase; HBV, Hepatitis B virus; HCV, Hepatitis C Virus; HIV, Human Immunodeficiency Virus.

Treatment

Patients enrolled in this cohort will first receive one cycle of platinum-based doublet NACT. The regimens are cisplatin 75-80 mg/m2 and nab-paclitaxel 260 mg/m2, administering intravenously (iv). After three weeks, participants will receive two cycles of PD-1 inhibitor combined NACT once every three weeks. The regimens are cisplatin 75-80 mg/m2 (iv) plus nab-paclitaxel 260 mg/m2 (iv) on day 1 and camrelizumab 200 mg (iv) on day 2. Tumor response will be assessed by magnetic resonance imaging (MRI) using RECIST criteria 1.1 three weeks after the last dose of neoadjuvant therapy. If patients achieve CR or PR, open laparotomy radical hysterectomy plus pelvic lymphadenectomy will perform, with or without para-aortic lymphadenectomy. Following surgery, patients will receive therapy as recommended by the national comprehensive cancer network (NCCN) guidelines; that is, patients with high-risk factors (pelvic lymph node-positive, margin positive, or parametrial infiltration) are recommended to be treated with irradiation plus cisplatin concurrent

chemotherapy (CCRT), while those with intermediate risk factors as in the Sedlis criteria will receive adjuvant radiotherapy. However, patients with stable disease (SD) or tumor progression (PD) will receive concurrent chemoradiotherapy (CCRT). In the case of grade 3-4 AEs, the treatment should be suspended, and the AEs should be actively treated until they return to grade 1-2. The dose of cisplatin and nab-paclitaxel may be reduced in the treatment in case of serious AE (SAE), but discontinuation of camrelizumab is recommended if irAEs do not recovery to grade 2 or lower before starting the second cycle of combined therapy.

Data collection procedure

Informed consent forms will be obtained from each participant before the screening evaluations. Baseline data will be recorded at the screening visit and completed within 28 days before enrolment. In addition, data will be collected at baseline, during the course of neoadjuvant immunotherapy, surgery, and postoperative adjuvant therapy, or CCRT. Table 2 provides an overview for collection of the outcomes.

Follow-up

After completion of the initially recommended therapy, routine follow-up will be scheduled every 3 months for one year and every 6 months thereafter. The patients will be followed up for at least 5 years. Follow-up includes pelvic examinations, imaging examinations, serum tumor markers, and cytology. Imaging examinations include abdominal and pelvic computed tomography (CT) or MRI, and abdominal imaging are allowed to perform every 6 months in the first year. Vaginal stump brushing cytology and HPV-testing are performing simultaneously. The serum tumor markers are squamous cell carcinoma antigen (SCCA) for squamous cell carcinoma patients, CA125 for adenocarcinoma, and both for adenosquamous carcinoma. Systematic examination revealed tumor recurrence will result in whole body restaging, further individual examination, post-recurrence anti-tumor therapy, and survival follow-up are need to be obtained.

Translational research

Blood samples are obtained from patients before initiation of and after every cycle of chemotherapy and cervical cancer biopsy samples are additionally obtained immediately before every cycle of therapy. The blood samples of patients undergoing radical surgery are collected before and 7 days after the surgery, if any, after the administration of postoperative adjuvant treatment. Additionally, surgically resected tumor tissues and adjacent normal tissues will be collected. On the other hand, blood samples and cervical cancer biopsy samples will be taken before CCRT, and blood samples will be taken again at the end of the therapy in patients with SD/PD who transfer to CCRT. The detailed sample collection process is presented in Figure 1.

Table 2 A plan of collection of the different outcomes.

Item required	Sceening		Treatment					Follow-up	
	Within 1 month	Within 7D	NT	P-NACT1	P-NACT2	□Surgery [1] □CCRT	End of therapy [2]	S1/S3	S2 ~ S12
			D1	D1±7D	D1±7D		±7D	±30D	±30D
Baseline characteristics									
Informed Consent	√								
Medical history [3]	√								
Medication history [4]	√		√	√	√	√		√	√
Lab examination									
Blood routine		√		√	√	√	√	√	
Urine Routine		√							
Blood biochemistry		√		√	√	√	√	√	
Coagulation function		√							
Myocardial enzyme		√							
HBV/HCV/HIV	√								
Tumor marker [5]	√		√	√	√	√	√	√	√
HPV and TCT	√							√	√
Imaging examinations									
Chest X-ray/CT	√		√	√	√	√	√		
Pelvic [6]	MRI		MRI			MRI	MRI	MRI/CT	MRI/CT
Whole Abdomen [7]	MRI		MRI	B/MRI	B/MRI	MRI	B/MRI		MRI/CT

Other examination									
Electrocardiograph		√		√	√	√	√		
Cervical pathology [8]	√					√			
The study drug [9]			√	√	√				
Clinical evaluation									
Physical examination [10]		√		√	√	√	√	√	√
ECOG score		√							
Adverse event [11]			√	√	√	√	√	√	√
Quality of Life		√		√		√	√	√	√
Survival analysis									
Recurrence [12]								√	√
Death [13]								√	√

All examinations and tests were performed in accordance with the study schedule. Unless otherwise specified, all items were recorded as pretreatment results for each cycle.

[1] Choose the Treatment according to neoadjuvant immunotherapy response. Surgical patients with postoperative adjuvant treatment should have their time and dose of radiotherapy, time and dose of chemotherapy administration, adverse events, radiotherapy, or concurrent chemoradiotherapy recorded. The time and dose of CCRT and adverse events should be collected for patients undergoing CCRT.

[2] Definition of end of therapy. In patients undergoing surgery, if they have high-risk factors or meet the Sedlis criteria, it means completion of all postoperative adjuvant therapy, and if postoperative risk factors do not meet the NCCN guidelines for treatment, it means that the operation is completed. In patients undergoing CCRT, it means concurrent radiochemotherapy completed.

[3] Patients during pregnancy and lactation patients are excluded.

[4] Record use of drugs, in addition to the study drugs, are recorded.

[5] Squamous cell carcinoma antigen (SCCA) should be detected for the type of squamous cell carcinoma, CA125 for adenocarcinoma, and both for adenosquamous carcinoma.

[6] If there is contraindicated, contrast-enhanced CT is recommended. If pelvic MRI shows an enlargement of the retroperitoneal pelvic lymph node (short diameter > 15 mm), an entire abdominal enhanced CT should be performed. MRI/CT means both are allowed. MRI/CT is required at the end of treatment only in the CCRT group (not in the surgery group), and the CCRT group should be followed up by MRI.

[7] B/MRI and MRI/CT were allowed.
[8] Including vaginal biopsy, histopathological examination, or pathological examination of tumor tissue after surgery.
[9] Including the time and dose of cisplatin, nab-paclitaxel, and camrelizumab.
[10] Including gynecological examinations.
[11] Collecting of intraoperative and postoperative complications in patients undergoing surgery.
[12] The Recurrence, recurrence time, recurrence location, and anti-tumor therapy after recurrence are recorded.
[13] Including the death time and cause of death.
NT, induction chemotherapy cycle; P-NACT; PD-1 inhibitor combined neoadjuvant chemotherapy; D, days; CCRT, concurrent chemoradiotherapy; CT, computed tomography; CT, computed tomography; MRI, magnetic resonance imaging; B, B-ultrasonography; ECOG, Eastern Cooperative Oncology Group.

All the patients participating in the trial may be included in a translational research program which are stated in informed consent. Among major interests is the analysis of the predictive value of available immune microenvironmental factors in responders, possible therapy-associated changes in different tumor regions, tumor microenvironment, and peripheral blood. Detailed research will be conducted to further investigate the immunotherapy reaction with tumor gene mutations and defects, the tumor immune microenvironment and mesenchymal cells. Dynamic panoramas of intratumoral infiltrating immune cells and peripheral blood will be performed with multiple omics research.

Statistical Analysis

Sample size calculation

The clinical response rate in patient with LACC after NACT is approximately 70%.[16] [17] With a one-sided binomial test at an overall type I error rate of a = 0.05, a sample size of 69 patients is needed to maintain 91.4% power under the hypothesis that the overall response rate of neoadjuvant immunotherapy is expected to increase to 85%. Eighty-two patients were included in the study, with an estimated dropout rate of 15%.

Data Analyses

Participants who receive camrelizumab and have at least one post-baseline tumor assessment will be included in the efficacy analysis. The participants undergo surgery will be evaluated for surgical pathological findings and any operative complications. For ORR and pathological response rate, confidence intervals (CIs) will be estimated using the Clopper-Pearson method. EFS, DFS and OS analysis will be performed by the Kaplan Meier curve, providing the median time to event and 95% CIs are calculated using the Greenwood formula. The enrolled patients who receive at least one dose of camrelizumab will be included in the safety analysis. In general, descriptive measures are used to summarize continuous variables (average, standard deviation, median, maximum, and minimum values). Categorical variables are expressed as frequencies and percentages. For treatment sensitivity analysis of the biomarkers, Cox univariate and multivariate analyses are used to analyze the correlation between biomarker expression, clinical efficacy, and prognosis. All data collected on the case report form (CRF) will be listed on a per-patient basis. Any deviations from the statistical methods given in the protocol will be reported in the final report as appropriate. Outcomes with $p<0.05$ is considered statistically significant. Furthermore, no interim analysis of the data will be conducted.

Data management and monitoring

Detailed data obtained from the patients will be recorded in the CRF and a central database are responsible for data collection and management. All study records and original documents will be maintained and stored according to relevant regulations and guidelines, or by the research institution's rules. The investigator accessing the relevant raw data of the clinical study are responsible for reviewing the CRF to determine the completeness, accuracy, and consistency of the information with the source data. Throughout the trial, a trained and qualified central monitoring group will

periodically visit each participating center to ensure data submission, patient eligibility and protocol compliance.

Patient and Public Involvement statement

Patients or the public were not involved in the design, or conduct, or reporting of this clinical trial.

ETHICS AND DISSEMINATION

The Medical Ethics committee of Tongji Medical College, Huazhong University of Science and Technology, China approved the study on June 10, 2020 (approval number: S2020-112). The protocol of this study has been registered at ClinicalTrials.gov (identification number: NCT04516616). Participants who suffer harm from trial participation will receive compensation from insurance company. As the study still in progress, results will be published when the last enrolled patient finished primary endpoint evaluation.

DISCUSSION

To the best of our knowledge, this is one of the first multi-center, prospective cohort study to evaluate the efficacy and safety of neoadjuvant chemo-immunotherapy in LACC. The results of this research will provide the foundation for developing alternative therapeutic strategies for LACC, and provide molecular biology of NACT and immunotherapy on the intratumoral and peripheral immune microenvironments in patients with cervical cancer. Although several similar clinical trials have been carried out since we registered the protocol, the unique characteristic of this clinical trial is that participants will receive upfront induction chemotherapy followed by PD-1 inhibitor combined chemotherapy.

Although CCRT is the standard primary treatment for LACC, several challenges remain to be overcome. Due to the high cost of radiotherapy facilities, difficulties exist in the implementation of standard radiation schedules in low- and middle-income countries. Additionally, patients would experience serious side effects, including gastrointestinal toxicity, sexual dysfunction, and fertility concerns in young patients, which severely affect their quality of life.[18 19] Next, radiation-induced pelvic fibrosis increases surgical difficulty and risk, and in case of progression or recurrence after radiation, treatment options are extremely limited. Finally, radioresistant cervical cancer has a high potential for recurrence, including regional and distant metastasis; thus, early initiation of systemic therapy is imperative to further improve survival. Over the past two decades, NACT combined with surgery has been the preferred treatment for LACC in low- and middle-income countries that lacking radiotherapy equipment. NACT can effectively reduce the size of bulky tumors and improve the chances of performing radical surgery; however, survival benefits have only been demonstrated in patients with clinical responses.[20] In addition, more than one-third of patients treated with NACT and surgery still require postoperative radiotherapy, thereby increasing the risk of treatment-related complications.[21] Therefore, therapeutic options that improve clinical response and avoid postoperative adjuvant radiotherapy based on reduced surgical pathological findings are warranted.

Since interleukin-2 (IL-2), the first immunotherapy approved for the treatment of

metastatic melanoma in 1998,[22] the promising role of immunotherapy in solid tumors has gained increasing attention, especially in recurrent or metastatic disease. The FDA has approved pembrolizumab, a PD-1 inhibitory antibody for cervical cancer following the outcome of the KEYNOTE-158 clinical trial.[8] A series of clinical trials have demonstrated the clinical benefits of ICIs in patients with recurrent or metastatic cervical cancer, showing promising overall response rates and well-tolerated toxicity profiles.[23 24] With the new progress in the molecular mechanism of cancer treatment, immunotherapy is recommended as the initial therapy for several solid tumors, apart from second-line treatment in recurrent/metastatic cases. Recent publications indicated that ICIs neoadjuvant therapy was superior to chemotherapy alone in NSCLC, with reported major pathological response rates of 20-85%.[25-27] Moreover, studies have shown that neoadjuvant immunotherapy in combination with chemotherapy improves the pCR and EFS rates in early TNBC. The KEYNOTE-522 study showed that the pembrolizumab-chemotherapy group had significantly higher EFS (84.5%) at 36 months than the chemotherapy group (76.8 %).[28] Based on the results of this study, pembrolizumab has been recommended as a preoperative neoadjuvant and postoperative adjuvant combination treatment for TNBC in several countries.

However, ICIs monotherapy does not maximize the benefits; researchers have also explored the optimized neoadjuvant therapy scheme of ICIs combined with chemotherapy. PD-1 inhibitors monotherapy has achieved a 40-45% major pathological response and 15-16% pCR in resectable NSCLC,[25 29] whereas in the NADIM study, patients receiving nivolumab combined neoadjuvant chemotherapy showed a major pathological response of 83%, of whom 63% had a complete pathological response.[27] Similarly, in breast cancer, a remarkably higher pCR rate for TNBC was observed in a series of studies on PD-1/PD-L1 inhibitors plus chemotherapy as NACT regimens, which increased from 22%-51.2% to 58%-64%.[30-33] Collectively, these data highlight the therapeutic potential of PD-1/PD-L1 inhibitors in the field of neoadjuvant therapy and provides the basis for the rational design of neoadjuvant chemo-immunotherapies for cervical cancer, even though no previous evidence is available.

The therapeutic activity of conventional chemotherapy is known to induce anti-cancer immunity by immunogenic cell death (ICD), which promotes the antigenicity and immunogenicity of tumors or by reprogramming tumor-reactive T cells and enhanicing anti-tumor T cell responses. The standard therapies for cervical cancer include platinum-based drugs and taxanes, both of which are known to have immunomodulatory effects. Cisplatin has been shown to up-regulate MHC-I expression in cancer cells, thereby mediating antigen presenting to T-cells; it can also remodel the immunosuppressive tumor microenvironment by depleting regulatory T (Treg) cells and myeloid-derived suppressor cells (MDSCs).[34 35] Meanwhile, cisplatin treatment upregulated PD-L1 expression in both tumor and immune cells, thereby escaping from immune recognition and attack, but making an opportunity for the use of PD-1/PD-L1 inhibitors.[36] In addition, docetaxel and paclitaxel have been shown to selectively reduce the number of MDSCs and Tregs without affecting the CD4+ and CD8+ T-cell activity.[37] By upregulating of mannose-6-phosphate receptors on tumor cells, cisplatin plus paclitaxel therapy makes tumor cells more susceptible to the cytotoxic effect of cytotoxic T lymphocytes (CTLs) by increasing their permeability to Granzyme B.[38] A similar conclusion was reached from clinical studies that cisplatin

and paclitaxel treatment significantly decreased FoxP3+ Tregs and increased cytotoxic CD8+ T cells in the cervical tumor stroma,[39] and neoadjuvant chemotherapy was associated with increased CD4, CD8, CD20, and CD56 signals, most prominently in good responders.[40] Recently, we conducted a study to systematically assess the immune microenvironment before and after neoadjuvant chemotherapy, which showed that following chemotherapeutic intervention, tumor-infiltrating immunostimulating cell concentrations were increased, including CD3+, CD4+, and CD8+ T-cell, dendritic cells and macrophages, whereas the expression of the immunosuppressive factosr FOXP3, IDO, and PD-L1 was down-regulated expression (Figure 2). In general, chemotherapy reprograms the cervical tumor's immunological microenvironment and makes the tumor more sensitive to immunotherapy.

Based on the promising clinical use and compact mechanisms of ICIs used in combination with chemotherapy, we conducted the first registered cohort study to evaluate the efficacy of neoadjuvant chemo-immunotherapy in LACC. First, induction chemotherapy will be used to recondition the tumor immune microenvironment in prior to anti-PD-1 combined chemotherapy. Next, PD-L1 detection is performed after enrollment and at the same time, patients receive platinum-based doublets of induction chemotherapy. Approximately 10 days later, the result of PD-L1 combined positive score (CPS) is used to determine whether they are screened in the clinical trial, without delaying the initial treatment of patients and increasing clinical feasibility. Third, paclitaxel was replaced with nab-paclitaxel as a chemotherapy regimen, avoiding the combination use of glucocorticoids that may affect the efficacy of PD-1 inhibitors, and can bring therapeutic benefits into fuller play. Besides, previous literature suggests that 2-3 cycles of neoadjuvant chemotherapy could result in tumor shrinkage in 69.4-79.3% of patients with LACC;[5 16] therefore, the one cycle of induction chemotherapy plus two courses of chemo-immunotherapy in our study are theoretically adequate to assess the short-term efficacy without delaying the treatment for PD/SD patients. Finally, the postoperative treatments in this study are based on surgical pathological findings specified in the NCCN guidelines, rather than treating all patients with adjuvant therapy, as in other researches,[41-44] which is not only in accordance with the international guidelines, but also avoids overtreatment suspicions and impact of different subsequent treatments on patient survival.

The present study has some limitations. First, this is a single-arm study without a control group. Therefore, randomized controlled clinical trials involving large sample sizes and high quality should be conducted in the near future. According to ethical requirements, this study focuses on the PD-L1-positive subgroup, and the results should be generalized with great caution. However, a large number of literatures have suggested that PD-1 inhibitors are not limited to populations with positive PD-L1 expression,[45] and subsequent studies will further expand the population.

In summary, the findings of this study will promote neoadjuvant anti-PD-1 combined chemo-immunotherapy with radical surgery as a new therapeutic strategy for patients with LACC. With the continuous improvement of medical care, treatment for cervical cancer is no longer monotherapy of radical surgery, chemotherapy, or radiation, and the integration of multiple treatments may achieve better cancer treatment effects. Further clinical trials and translational research are warranted to elucidate potential predictive biomarkers and investigate the mode of tumor microenvironment remodeling of anti PD-1 therapies in cervical cancer. In this regard, patient

stratification and individualized precision therapy can be achieved.

Contributors

JC and YH contributed equally to this trial and write the article. KL, GC, and CS developed the study concept and protocol. YH, XF, XM and SG assisted in further development of the protocol. KL are responsible for the supervision of the clinical trial and has access to the final trial dataset. All authors contributed to the article and approved the submitted version.

Funding

This trial is funded by Jiangsu Hengrui Medicine, Lianyungang, China (MA-CervC-II-002). The funder was not involved in the study design and the writing of this article or the decision to submit it for publication.

Competing interests statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Figure legend

Figure 1 The overall trial flow of the study. CPS, combined positive score; MRI, magnetic resonance imaging; Q3W, every 3 weeks; CR, complete response; PR, partial response; SD, stable disease; PD, progression disease; CT, computed tomography; CCRT, concurrent chemoradiotherapy; NACIT, neoadjuvant chemo-immunotherapy; Q3M, every 3 months; Q6M, every 6 months; [1], if there are contraindications for MRI, CT is recommended; [2], in priming phase and NACIT phases, blood samples are collected before (～24h) and after (～24h) each cycle; in patients with surgery, blood samples are collected before and then 7 days after radical surgery, if any, after administration of postoperative adjuvant treatment; in patients with CCRT, blood samples are collected before and immediately after CCRT; [3], in priming phase and NACIT phases, biopsy sample of tumor tissue are collected before each cycle; in patients undergoing surgery, the resected tumor tissue and a paracancer normal tissue will be collected; in patients with CCRT, biopsy sample is collected before CCRT.

Figure 2 Representative image of a multiplex immunohistochemistry of the immune context of cervical cancer patient tumors pre- and post-neoadjuvant chemotherapy (NACT).

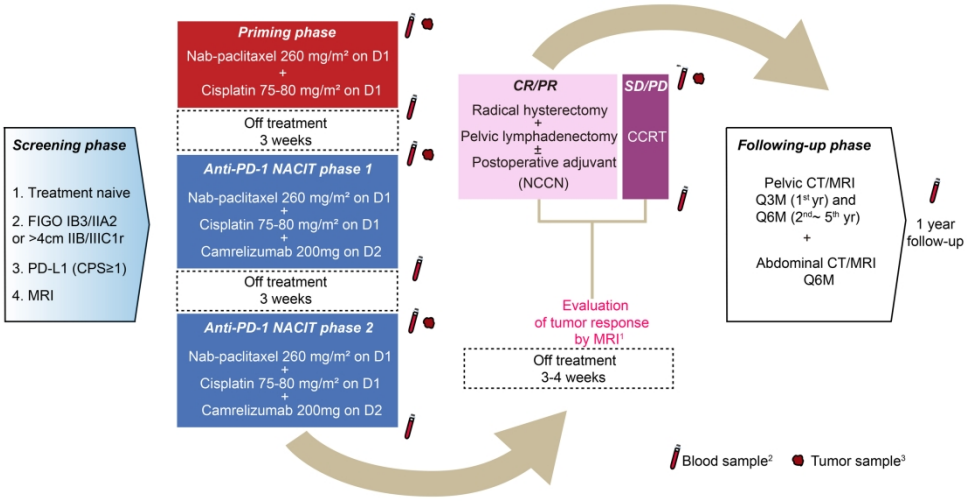

Figure 1 The overall trial flow of the study. CPS, combined positive score; MRI, magnetic resonance imaging; Q3W, every 3 weeks; CR, complete response; PR, partial response; SD, stable disease; PD, progression disease; CT, computed tomography; CCRT, concurrent chemoradiotherapy; NACIT, neoadjuvant chemo-immunotherapy; Q3M, every 3 months; Q6M, every 6 months; 1, if there are contraindications for MRI, CT is recommended; 2, in priming phase and NACIT phases, blood samples are collected before (~24h) and after (~24h) each cycle; in patients with surgery, blood samples are collected before and then 7 days after radical surgery, if any, after administration of postoperative adjuvant treatment; in patients with CCRT, blood samples are collected before and immediately after CCRT; 3, in priming phase and NACIT phases, biopsy sample of tumor tissue are collected before each cycle; in patients undergoing surgery, the resected tumor tissue and a paracancer normal tissue will be collected; in patients with CCRT, biopsy sample is collected before CCRT.

247x123mm (300 x 300 DPI)

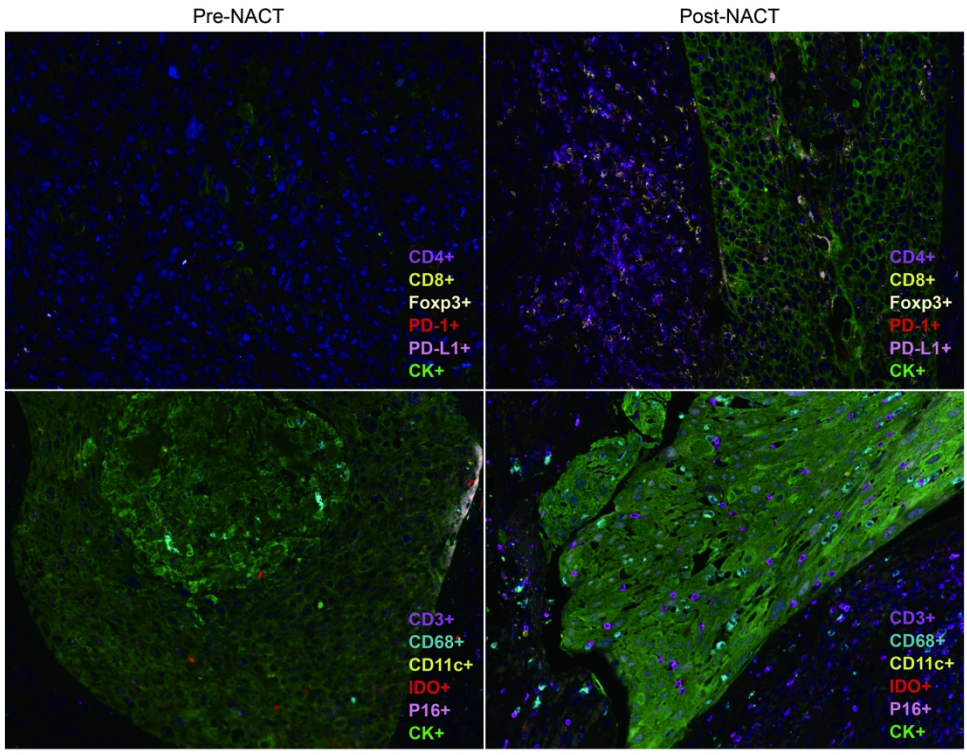

Figure 2 Representative image of a multiplex immunohistochemistry of the immune context of cervical cancer patient tumors pre- and post-neoadjuvant chemotherapy (NACT).

Supplementary Table 1 List of all participating centers

No.	Organization	Principal Investigator
1	Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology	Ding Ma, Kezhen Li, Gang Chen
2	Women's Hospital, School of Medicine, Zhejiang University	Weiguo Lu、Yuanming Shen
3	The Southwest Hospital of Army Medical University	Yanzhou Wang
4	Qilu Hospital of Shandong University	Kun Song
5	Obstetrics & Gynecology Hospital of Fudan University	Xiaojun Chen
6	Anhui Province Cancer Hospital	Bairong Xia
7	Chongqing University Cancer Hospital	Dongling Zou
8	Tianjin Medical University General Hospital	Yingmei Wang

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description
Administrative information		
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym Page 1 Line 1-3
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry Page 2 Line 50
	2b	All items from the World Health Organization Trial Registration Data Set Page 2 Line 50
Protocol version	3	Date and version identifier Page 12 Line 304-305
Funding	4	Sources and types of financial, material, and other support Page 15 Line 442-450
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors Page 15 Line 436-441
	5b	Name and contact information for the trial sponsor Page 1 Line 9-17
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities Page 15 Line 442-450
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee) Page 11 Line 289-298
Introduction		
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention Page 2-4
	6b	Explanation for choice of comparators Page 2-4
Objectives	7	Specific objectives or hypotheses Page 4 Line 128-135

Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory) Page 4 Line 121-127
Methods: Participants, interventions, and outcomes		
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained Supplementary Table 1
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists) Table 1
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered Page 6-7 Line 178-199
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease) Page 7 Line 194-199
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests) Page 7 Line 200-205
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial Page Page 7 Line 194-199
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended Page 4-5 Line 136-161
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure) Figure 1 and Table 2
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations Page 11 Line 263-270
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size Page 3 Line 125-127
Methods: Assignment of interventions (for controlled trials) This is a single arm trials		

Allocation:		
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions NA
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned NA
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions NA
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how NA
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial NA
Methods: Data collection, management, and analysis		
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol Page 7 Line 200-205, Table 2
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols Page 7 Line 206-218, Table 2
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol Page 11 Line 290-299
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol Page 11 Line 271-288
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses) Page 11 Line 271-288

	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation) Page 11 Line 271-288
Methods: Monitoring		
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed Page 11 Line 290-299
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial Page 11 Line 293-296
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct Page 11 Line 290-299
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor Page 11 Line 290-299
Ethics and dissemination		
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval Page 12 Line 303-305
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators) Page 11 Line 290-299
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32) Page 5 Line 168-170
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable Page 11 Line 254-255
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial Page 11 Line 290-299
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site Page 15 Line 446-448

Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators Page 11 Line 290-299
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation Page 12 Line 306-307
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions Page 12 Line 308-309
	31b	Authorship eligibility guidelines and any intended use of professional writers Page 15 Line 436-441
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code NA
Appendices		
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates Page 7 Line 201-202
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable Figure 1

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

BMJ Open

Neoadjuvant Camrelizumab plus Chemotherapy for Locally Advanced Cervical Cancer (NACI study): A Study Protocol of Prospective, Single-arm, Phase II trial

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2022-067767.R1
Article Type:	Protocol
Date Submitted by the Author:	13-Mar-2023
Complete List of Authors:	Chen, Jing; Tongji Hospital of Tongji Medical College of Huazhong University of Science and Technology, Han, Yingyan; Tongji Hospital of Tongji Medical College of Huazhong University of Science and Technology Hu, Yingjie; Tongji Hospital of Tongji Medical College of Huazhong University of Science and Technology Feng, Xue; Tongji Hospital of Tongji Medical College of Huazhong University of Science and Technology Meng, Xiaolin; Tongji Hospital of Tongji Medical College of Huazhong University of Science and Technology Guo, Shuaiqingying; Tongji Hospital of Tongji Medical College of Huazhong University of Science and Technology Sun, Chaoyang; Tongji Medical College of Huazhong University of Science and Technology Chen, Gang; Tongji Hospital of Tongji Medical College of Huazhong University of Science and Technology Li, Kezhen; Tongji Hospital of Tongji Medical College of Huazhong University of Science and Technology
Primary Subject Heading:	Oncology
Secondary Subject Heading:	Obstetrics and gynaecology
Keywords:	Clinical trials < THERAPEUTICS, Gynaecological oncology < GYNAECOLOGY, IMMUNOLOGY, CHEMOTHERAPY

Neoadjuvant Camrelizumab plus Chemotherapy for Locally Advanced Cervical Cancer (NACI study): A Study Protocol of Prospective, Single-arm, Phase II trial

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Keywords: Neoadjuvant chemotherapy (NACT), Immunotherapy, Locally Advanced Cervical Cancer (LACC), Camrelizumab, PD-1 inhibitor

Running tittle: Neoadjuvant chemo-immunotherapy for LACC.

Abstract

Introduction: Neoadjuvant chemotherapy (NACT) is an emerging approach for locally advanced cervical cancer (LACC). However, the clinical response and postoperative adjuvant radiation or chemoradiation trimodality treatment resulted in controversy. PD-1 inhibitors have shown promising role in recurrent or metastatic cervical cancer, and preclinical evidence of the activation and synergistic effects of NACT on PD-1 inhibitors. This study aims to evaluate the efficacy and safety of the preoperative PD-1 inhibitor camrelizumab combined neoadjuvant therapy for LACC.

Methods and analysis: The study is designed as a multicenter, open-label, single-arm, prospective phase II study. A total of 82 patients will receive neoadjuvant chemo-immunotherapy, defined as one cycle of cisplatin (75-80 mg/m2, iv) plus nab-paclitaxel (260 mg/m2, iv) NACT and subsequent two cycles of camrelizumab (200mg, iv) combined NACT. After neoadjuvant chemo-immunotherapy, patients exhibiting complete response (CR) and partial response (PR) will undergo radical surgery and subsequent adjuvant therapy. In contrast, patients with stable disease (SD) and progressive disease (PD) will transfer to concurrent chemoradiotherapy (CCRT). Following surgery, patients will receive adjuvant CCRT or radiotherapy. The primary endpoint is the objective response rate (ORR). The secondary endpoints are the

pathological complete response (pCR), patients requiring postoperative adjuvant therapy, safety of neoadjuvant chemo-immunotherapy, surgical complication, event-free survival and overall survival. An additional aim is to dynamically evaluate peripheral immune responses and local immunological microenvironments and their association with neoadjuvant immunotherapy.

Ethics and dissemination: This trial was approved by The Medical Ethics committee of Tongji Medical College, HUST (S2020-112). This study is among the first to evaluate the efficacy and safety of neoadjuvant chemo-immunotherapy in LACC. The findings of this research will promote neoadjuvant anti-PD-1 immunotherapy with radical surgery as a new therapeutic strategy.

Trial registration number: NCT04516616 (ClinicalTrials.gov).

Strengths and limitations of this study

- This is a multi-center, prospective cohort study to evaluate the efficacy and safety of neoadjuvant chemo-immunotherapy in locally advanced cervical cancer.
- Induction chemotherapy will be used before anti-PD-1 combined chemotherapy.
- Paclitaxel is replaced with nab-paclitaxel as a chemotherapy regimen, avoiding the combination use of glucocorticoids.
- Blood samples and tumor tissue biopsies will be collected into a translational research program.
- Since this study is a single-arm study, it may include some bias.

INTRODUCTION

Cervical cancer is the fourth most frequently diagnosed cancer and the fourth most common cause of cancer-related deaths among women worldwide, accounting for approximately 604,000 new cases and 342,000 deaths in 2020.[1] Locally advanced cervical cancer (LACC) includes patients with stage IB3 to IVA disease, of which concurrent chemoradiotherapy is the standard treatment, although definitive surgery can also be performed in patients with stage IB3 or IIA disease.[2,3] Characterized by the large extent of malignant tissue, it's very difficult to complete surgical resection. Furthermore, the prognosis of LACC has not improved significantly over the past decades, with an average 5-year survival rate of 60-82.3%.[4-6] Hence, treatment options that improve survival in this subgroup of patients continue to be a major public health concern and effective therapies for further improvement in survival must be developed.

In areas where radiotherapy apparatus is not available, platinum-based neoadjuvant chemotherapy (NACT) followed by radical hysterectomy has been proposed as an alternative approach. This alternative approach increases the opportunities for radical surgery and indicates a similar prognosis compared with concurrent chemoradiotherapy (CCRT). The NACT, however, is only effective in two-thirds of

patients, and those who do not respond receive little benefit from it.[7,8] Moreover, 32.2% of patients required postoperative adjuvant radiation or chemoradiation, resulting in controversy in terms of health economics and trimodality treatment.[5] Therefore, although NACT has been widely used in many countries, it is not recommended as a first-line treatment for LACC.

Recently, immune checkpoint inhibitors (ICIs) have made remarkable advances in immunotherapy for cervical cancer. Additionally, pembrolizumab combined chemotherapy has been approved as a first-line treatment for advanced PD-L1-positive cervical cancer.[9,10] Previous clinical trials have shown that immunotherapy, with a favorable toxicity profile and durable responses, has made a tremendous breakthrough in the treatment of cervical cancer. Contrarily, immuno-monotherapy shows a relatively low response rate. In advanced or recurrent cervical cancer, ICIs monotherapy has a response rate of approximately 15%, and combination therapy with chemotherapy, radiotherapy, or other immune-targeted therapies have improved response rates to 50%-60%.[11,12]

Neoadjuvant chemo-immunotherapy has attained milestones in the treatment of many solid tumors. In contrast, most cervical cancer research has focused on immunotherapy in patients with metastatic or recurrent disease, with very few studies exploring neoadjuvant immunotherapy in LACC. Recent theoretical developments have revealed that chemotherapeutic agents possess immunomodulatory properties, modulating immune cells and shaping their functions. Conventional chemotherapeutics have been known to promote immunogenicity of the tumor because of the modulation of the anti-tumor T cell response through triggering the release of tumor-derived antigens, inducing immunogenic cell death, disrupting the immunosuppressive microenvironment and enhancing the effector T-cell response.[13] In addition, clinical research supports that NACT can increase PD-L1 expression in cervical cancer, and over-expression of PD-L1 is significantly associated with a better response to ICIs, such as the PD-1/PD-L1 blockade.[14] These findings show that reprogramming the tumor immunologic microenvironment using NACT may potentially sensitize cervical tumors to immunotherapy and act synergistically with ICIs, similar to early triple-negative breast cancer (TNBC) and non-small cell lung cancer (NSCLC).[15,16]

Camrelizumab is a human immunoglobulin G4 monoclonal antibody that targets the PD-1 immune checkpoint receptor. Favorable efficacy and tolerable safety profiles have been demonstrated in several advanced tumors. We perform this pilot study to evaluate the clinical activity and safety of NACT combined with PD-1 inhibitors in patients with LACC. Furthermore, we will explore the relationship between the tumor microenvironment landscape and response to therapy, as well as dynamic changes in intratumoral and peripheral immune microenvironments.

METHODS AND ANALYSIS

Trial design

The study is designed as a multicenter, open-label, single-arm, prospective phase II study. Eighty-two patients will receive neoadjuvant immunotherapy, defined as one cycle of cisplatin plus nab-paclitaxel NACT and subsequent 2 cycles of PD-1 antibody combined with NACT. Figure 1 shows the flowchart of the trial. Patients

will be recruited from large 3A hospitals and a list of all participating centers is added as Supplementary Table 1. The patient registration process began from December 2020 to December 2022. The 5-year follow-up is designed for all patients, and the final study report will be prepared within 6 months. Therefore, this study is scheduled to end in July 2028.

The objective of the study

This study aims to evaluate the effects of neoadjuvant chemo-immunotherapy on tumor response (including clinical response and pathological response), the proportion of patients requiring postoperative adjuvant therapy, treatment-related toxicity, subsequent surgical complications, and survival (including event-free survival (EFS) and overall survival (OS)) of patients with LACC. An additional aim of this study is to dynamically evaluate peripheral immune responses and local immunological microenvironments, and their association with neoadjuvant immunotherapy efficacy.

Endpoints of the study

The primary endpoint is the objective response rate (ORR), defined as the percentage of the participants who achieve a complete response (CR) or partial response (PR). The primary endpoint analysis will include participants who receive camrelizumab therapy and have at least one post-baseline tumor assessment. The ORR will be assessed by a blind independent central reviewer per Response Evaluation Criteria in Solid Tumors V.1.1 (RECIST 1.1). The secondary endpoints are the rate of pathological complete response (pCR), the number of patients with treatment-related adverse events (AEs), serious AEs or immune-related AEs (irAEs), the number of patients with surgical complications and postoperative adjuvant therapy, EFS, and OS. Histological examination of the entire resected specimen will be performed to identify patients with pCR, defined as the absence of viable tumor cells on all slides. The optimal response, based on the Buda criteria, is a complete disappearance of the tumor in the cervix with negative nodes or a residual disease with less than 3 mm stromal invasion, including in situ carcinoma.[17] The Common Terminology Criteria for Adverse Events (CTCAE) (V.5.0) is used to evaluate AEs after the first dose of chemotherapeutic drugs to 30 days after the last dose. The safety set used for the safety evaluation will include patients using camrelizumab at least once and with relatively complete medical records. Postoperative complication defines as any clinically significant deviation from a normal postoperative course. EFS is defined as the time interval from the date of randomization to disease progression, local or distant recurrence (in patients undergoing surgery), or death due to any cause. OS is defined as the date of randomization until the date of death due to any cause or the last follow-up visit. Additionally, in participants who undergo radical surgery, disease-free survival (DFS) is calculated separately from the date of completing prescribed treatment to the date of disease relapse or death, irrespective of the cause.

Study Procedures

Patient selection/screening

Eligible patients are 18-70 years of age and have newly diagnosed cervical cancer of stage IB3, IIA2, or IIB/IIIC1r with a tumor diameter ≥4cm (FIGO, 2018). All patients will be PD-L1 positive, with pathologically confirmed squamous cell carcinoma,

adenocarcinoma or adenosquamous carcinoma, and have normal organ function. The detailed inclusion and exclusion criteria are listed in Table 1. Informed consent will be obtained before PD-L1 immunohistochemistry assays and the participant will receive one cycle of cisplatin and nab-paclitaxel treatment. PD-L1 expression is evaluated using the 22C3 antibody clone (Agilent, Dako). After randomization, participants who do not satisfy the inclusion or exclusion criteria will be excluded. From published data, 30%～88% cervical cancer patients are PD-L1 positive,[18,19] and it's feasible that patients turned out with PD-L1(-) will be excluded.

Table 1 Inclusion and exclusion criteria

Inclusion criteria	Exclusion criteria
FIGO stage IB3, IIA2, or IIB/IIIC1r (tumor size≥4cm) cervical cancer and without any treatment	The active autoimmune disease requires a systemic treatment[a]
Histologically confirmed squamous cell carcinoma, adenocarcinoma or adenosquamous carcinoma of the cervix	Previous treatment with checkpoint inhibitors or hypersensitivity to study drug
PD-L1 positive (Combined positive score≥1)	Patients requiring treatment with systemic corticosteroids (over 10 mg/day dose) or immunosuppressants within 14 days before enrolment
Females 18-70 years of age	Inoculating live vaccine or attenuated live vaccine within 4 weeks
ECOG score 0-1	History of allogeneic tissue/solid organ transplant
WBC≥3.5*10^9/L, NEU≥1.5*10^9/L, Platelet≥80×10^9 /L; AST and ALT ≤1.5 times normal upper limit; Total bilirubin ≤1.5 times the upper limit of normal value; serum creatinine and blood urea nitrogen ≤the upper limit of normal value	Known active HBV, HCV or HIV infection
	Patients who have a serious or uncontrollable disease[b]
	Any primary malignancy within 5 years
	Pregnant or lactating female patients
Willing to participate in this study and signed the informed consent	Drug or alcohol abuse
	Participate in clinical trials at the same time
Patients who observed the rules about the scheduled visit, study schedule and medical examination	Unable or unwilling to sign the informed consents
	Not eligible for the study judged by researchers

[a] as follows but not limited to autoimmune hepatitis, interstitial pneumonia, uveitis, enteritis, hepatitis, pituitary inflammation, vasculitis, nephritis, hyperthyroidism, thyroid dysfunction, asthma requiring intervention with bronchodilators. [b] including but not limited to: heart diseases (grade III-IV cardiac insufficiency (NYHA standard); uncontrolled high blood

pressure; cerebrovascular accident or brain disease within 6 months or incapacity; hematological diseases, including abnormal coagulation (INR> 2.0, PT>16s), bleeding tendency, receiving thrombolytic or anticoagulant therapy; organic abnormalities of the liver and kidney or surgical history; any active infection requiring systemic anti-infective therapy within 14 days.
ECOG, Eastern Cooperative Oncology Group; WBC, White Blood Cell; NEU, neutrophils; AST, Aspartate Aminotransferase; ALT, Alanine Aminotransferase; HBV, Hepatitis B virus; HCV, Hepatitis C Virus; HIV, Human Immunodeficiency Virus.

Treatment

Patients enrolled in this cohort will first receive one cycle of platinum-based doublet NACT. The regimens are cisplatin 75-80 mg/m2 and nab-paclitaxel 260 mg/m2, to be administered intravenously (iv). After three weeks, participants will receive two cycles of PD-1 inhibitor combined with NACT once every three weeks. The regimens are cisplatin 75-80 mg/m2 (iv) plus nab-paclitaxel 260 mg/m2 (iv) on day 1 and camrelizumab 200 mg (iv) on day 2. Tumor response will be assessed by magnetic resonance imaging (MRI) using RECIST criteria 1.1 three weeks after the last dose of neoadjuvant therapy. If patients achieve CR or PR, an open laparotomy radical hysterectomy plus pelvic lymphadenectomy will perform, with or without para-aortic lymphadenectomy. Following surgery, patients will receive therapy as the national comprehensive cancer network (NCCN) guidelines recommended. Patients with high-risk factors (pelvic lymph node-positive, margin positive, or parametrial infiltration) should be treated with irradiation plus cisplatin concurrent chemotherapy (CCRT)., Conversely, those with intermediate risk factors as in the Sedlis criteria will receive adjuvant radiotherapy. However, patients with stable disease (SD) or tumor progression (PD) will receive concurrent chemoradiotherapy (CCRT). In the case of grade 3-4 AEs, the treatment should be suspended, and the AEs should be actively treated until they return to grades 1-2. The dose of cisplatin and nab-paclitaxel may be reduced in the treatment in case of serious AE (SAE). However, discontinuation of camrelizumab is recommended if irAEs do not recover to grade 2 or lower before starting the second cycle of combined therapy.

Data collection procedure

Informed consent forms will be obtained from each participant before the screening evaluations. Baseline data will be recorded at the screening visit and completed within 28 days before enrolment. In addition, data will be collected at baseline, during neoadjuvant immunotherapy, surgery, and postoperative adjuvant therapy, or CCRT. Supplementary Table 2 provides an overview of the collection of the outcomes.

Follow-up

After the initially recommended therapy is completed, routine follow-up will be scheduled every 3 months for one year and every 6 months after that. The patients will be followed up for at least 5 years. Follow-up includes pelvic examinations, imaging examinations, serum tumor markers, and cytology. Imaging examinations include abdominal and pelvic computed tomography (CT) or MRI, and abdominal imaging is allowed to perform every 6 months in the first year. Vaginal stump brushing cytology and HPV-testing are performed simultaneously. The serum tumor markers are squamous cell carcinoma antigen (SCCA) for squamous cell carcinoma patients,

CA125 for adenocarcinoma, and both for adenosquamous carcinoma. Systematic examination revealed tumor recurrence would result in whole-body restaging. Further individual examination, post-recurrence anti-tumor therapy, and survival follow-up need to be obtained.

Translational research

Blood samples are obtained from patients before initiation of and after every cycle of chemotherapy and cervical cancer biopsy samples are additionally obtained immediately before every cycle of therapy. The blood samples of patients undergoing radical surgery are collected before and 7 days after the surgery, if any, after administering postoperative adjuvant treatment. Additionally, surgically resected tumor tissues and adjacent normal tissues will be collected. On the other hand, blood and cervical cancer biopsy samples will be taken before CCRT, and blood samples will be taken again at the end of the therapy in patients with SD/PD who transfer to CCRT. The detailed sample collection process is presented in Figure 1.

All the patients participating in the trial may be included in a translational research program as stated in the informed consent. the analysis of the predictive value of available immune microenvironmental factors in responders, possible therapy-associated changes in different tumor regions, tumor microenvironment, and peripheral blood are among the major interests. Detailed research will be conducted to further investigate the immunotherapy reaction with tumor gene mutations and defects, the tumor immune microenvironment and mesenchymal cells. Dynamic panoramas of intratumoral infiltrating immune cells and peripheral blood will be performed with multiple omics research.

Statistical Analysis

Sample size calculation

The clinical response rate in patients with LACC after NACT is approximately 70%.[20,21] With a one-sided binomial test at an overall type I error rate of a = 0.05, a sample size of 69 patients is needed to maintain 91.4% power under the hypothesis that the overall response rate of neoadjuvant immunotherapy is expected to increase to 85%. Eighty-two patients are included in the study, with an estimated dropout rate of 15%.

Data Analyses

The efficacy analysis will include participants who receive camrelizumab and have at least one post-baseline tumor assessment. The participants undergoing surgery will be evaluated for surgical pathological findings and any operative complications. Confidence intervals (CIs) will be estimated for ORR and pathological response rate using the Clopper-Pearson method. EFS, DFS and OS analysis will be performed by the Kaplan-Meier curve, providing the median time to the event and 95% CIs are calculated using the Greenwood formula. The enrolled patients who receive at least one dose of camrelizumab will be included in the safety analysis. In general, descriptive measures are used to summarize continuous variables (average, standard deviation, median, maximum, and minimum values). Categorical variables are expressed as frequencies and percentages. Cox univariate and multivariate analyses

are used to analyze the correlation between biomarker expression, clinical efficacy, and prognosis for treatment sensitivity analysis of the biomarkers All data collected on the case report form (CRF) will be listed per patient. Any deviations from the statistical methods given in the protocol will be reported in the final report as appropriate. Outcomes with $p<0.05$ are considered statistically significant. Furthermore, no interim analysis of the data will be conducted.

Data management and monitoring

Detailed data obtained from the patients will be recorded in the CRF and a central database is responsible for data collection and management. All study records and original documents will be maintained and stored according to relevant regulations and guidelines, or by the research institution's rules. The investigator accessing the relevant raw data of the clinical study is responsible for reviewing the CRF to determine the information's completeness, accuracy, and consistency with the source data. A trained and qualified central monitoring group will periodically visit each participating center throughout the trial to ensure data submission, patient eligibility, and protocol compliance.

Patient and Public Involvement statement

Patients or the public are not involved in this clinical trial's design, conduct, or reporting.

ETHICS AND DISSEMINATION

The Medical Ethics committee of Tongji Medical College, Huazhong University of Science and Technology, China approved the study on June 10, 2020 (approval number: S2020-112). An amendment was approved on Nov 30, 2022 (approval number: S2020-112-3). The protocol of this study has been registered at ClinicalTrials.gov (identification number: NCT04516616). Participants who suffer harm from trial participation will receive compensation from the insurance company. As the study is still in progress, results will be published when the last enrolled patient finishes the primary endpoint evaluation, finish completion of 3-year and 5-year follow-ups. The findings from the analysis of the trial will be disseminated through scientific and professional conferences, and published manuscripts in peer-reviewed journals.

DISCUSSION

This study is one of the first multi-center, prospective cohort studies to evaluate the efficacy and safety of neoadjuvant chemo-immunotherapy in LACC. The results of this research will provide the foundation for developing alternative therapeutic strategies for LACC, and provide molecular biology of NACT and immunotherapy on the intratumoral and peripheral immune microenvironments in patients with cervical cancer. Although several similar clinical trials have been carried out since we registered the protocol, the unique characteristic of this clinical trial is that participants will receive upfront induction chemotherapy followed by PD-1 inhibitor combined chemotherapy.

Although CCRT is the standard primary treatment for LACC, several challenges

remain to be overcome. Difficulties exist in implementing standard radiation schedules in low- and middle-income countries because of the high cost of radiotherapy facilities. Additionally, patients would experience serious side effects, including gastrointestinal toxicity, sexual dysfunction, and fertility concerns in young patients, severely affecting their quality of life.[22,23] Next, radiation-induced pelvic fibrosis increases the surgical difficulty and risk, and in case of progression or recurrence after radiation, treatment options are extremely limited. Finally, radioresistant cervical cancer has a high potential for recurrence, including regional and distant metastasis; thus, early initiation of systemic therapy is imperative to further improve survival. Over the past two decades, NACT combined with surgery has been the preferred treatment for LACC in low- and middle-income countries that lacking radiotherapy equipment. NACT can effectively reduce the size of bulky tumors and improve the chances of performing radical surgery. However, survival benefits have only been demonstrated in patients with clinical responses.[24] In addition, more than one-third of patients treated with NACT and surgery require postoperative radiotherapy, increasing the risk of treatment-related complications.[25] Therefore, therapeutic options that improve clinical response and avoid postoperative adjuvant radiotherapy based on reduced surgical pathological findings are warranted.

Since interleukin-2 (IL-2), the first immunotherapy approved for metastatic melanoma in 1998,[26] the promising role of immunotherapy in solid tumors has gained increasing attention, especially in recurrent or metastatic disease. The FDA has approved pembrolizumab, a PD-1 inhibitory antibody for cervical cancer following the outcome of the KEYNOTE-158 clinical trial.[10] A series of clinical trials have demonstrated the clinical benefits of ICIs in patients with recurrent or metastatic cervical cancer, showing promising overall response rates and well-tolerated toxicity profiles.[27,28] Immunotherapy is recommended as the initial therapy for several solid tumors, apart from second-line treatment in recurrent/metastatic cases. with the new progress in the molecular mechanism of cancer treatment. Recent publications indicated that ICIs neoadjuvant therapy was superior to chemotherapy alone in NSCLC, with reported major pathological response rates of 20-85%.[29-31] Moreover, studies have shown that neoadjuvant immunotherapy in combination with chemotherapy improves the pCR and EFS rates in early TNBC. The KEYNOTE-522 study showed that the pembrolizumab-chemotherapy group had significantly higher EFS (84.5%) at 36 months than the chemotherapy group (76.8 %).[32] Pembrolizumab has been recommended as a preoperative neoadjuvant and postoperative adjuvant combination treatment for TNBC in several countries based on the results of this study.

However, ICIs monotherapy does not maximize the benefits; researchers have also explored the optimized neoadjuvant therapy scheme of ICIs combined with chemotherapy. PD-1 inhibitors monotherapy has achieved a 40-45% major pathological response and 15-16% pCR in resectable NSCLC.[29,33] Contrarily, in the NADIM study, patients receiving nivolumab combined neoadjuvant chemotherapy showed a major pathological response of 83%, of whom 63% had a complete pathological response.[31] Similarly, in breast cancer, a remarkably higher pCR rate for TNBC was observed in a series of studies on PD-1/PD-L1 inhibitors plus chemotherapy as NACT regimens, which increased from 22%-51.2% to 58%-64%.[34-37] Collectively, these data highlight the therapeutic potential of PD-1/PD-L1 inhibitors in neoadjuvant therapy and provides the basis for the rational design of

neoadjuvant chemo-immunotherapies for cervical cancer, even though no previous evidence is available. Selected ongoing trials of chemo-immunotherapies for LACC are summarized in Supplementary Table 3.

The therapeutic activity of conventional chemotherapy is known to induce anti-cancer immunity by immunogenic cell death (ICD), which promotes the antigenicity and immunogenicity of tumors or by reprogramming tumor-reactive T cells and enhanicing anti-tumor T cell responses. The standard therapies for cervical cancer include platinum-based drugs and taxanes, both known to have immunomodulatory effects. Cisplatin has been shown to up-regulate MHC-I expression in cancer cells, thereby mediating antigen presenting to T-cells. It can also remodel the immunosuppressive tumor microenvironment by depleting regulatory T (Treg) cells and myeloid-derived suppressor cells (MDSCs).[38,39] Meanwhile, cisplatin treatment upregulated PD-L1 expression in both tumor and immune cells, thereby escaping from immune recognition and attack, but making an opportunity for the use of PD-1/PD-L1 inhibitors.[40] In addition, docetaxel and paclitaxel have been shown to selectively reduce the number of MDSCs and Tregs without affecting the CD4+ and CD8+ T-cell activity.[41] Cisplatin plus paclitaxel therapy makes tumor cells more susceptible to cytotoxic T lymphocytes (CTLs) cytotoxic effect by increasing their permeability to Granzyme B by upregulating of mannose-6-phosphate receptors on tumor cells.[42] A similar conclusion was reached from clinical studies that cisplatin and paclitaxel treatment significantly decreased FoxP3+ Tregs and increased cytotoxic CD8+ T cells in the cervical tumor stroma,[43] and neoadjuvant chemotherapy was associated with increased CD4, CD8, CD20, and CD56 signals, most prominently in good responders.[44] Recently, we conducted a study to systematically assess the immune microenvironment before and after neoadjuvant chemotherapy, which showed that following the chemotherapeutic intervention, tumor-infiltrating immunostimulating cell concentrations were increased, including CD3+, CD4+, and CD8+ T-cell, dendritic cells and macrophages. In contrast, the expression of the immunosuppressive factors FOXP3, IDO, and PD-L1 was down-regulated expression (Figure 2). In general, chemotherapy reprograms the cervical tumor's immunological microenvironment and makes the tumor more sensitive to immunotherapy.

We conducted the first registered cohort study to evaluate the efficacy of neoadjuvant chemo-immunotherapy in LACC based on the promising clinical use and compact mechanisms of ICIs combined with chemotherapy. First, induction chemotherapy will be used to recondition the tumor's immune microenvironment before anti-PD-1 combined chemotherapy. Next, PD-L1 detection is performed after enrollment and at the same time, patients receive platinum-based doublets of induction chemotherapy. Approximately 10 days later, the PD-L1 combined positive score (CPS) result is used to determine whether they are screened in the clinical trial, without delaying the initial treatment of patients and increasing clinical feasibility. Third, paclitaxel is replaced with nab-paclitaxel as a chemotherapy regimen, avoiding the combination use of glucocorticoids that may affect the efficacy of PD-1 inhibitors, and can bring therapeutic benefits into fuller play.[45,46] Besides, previous literature shows that 2-3 cycles of neoadjuvant chemotherapy could result in tumor shrinkage in 69.4-79.3% of patients with LACC.[7,20] Therefore, the one cycle of induction chemotherapy plus two courses of chemo-immunotherapy in our study are theoretically adequate to assess short-term efficacy without delaying the treatment for PD/SD patients. Finally, the

postoperative treatments in this study are based on surgical pathological findings specified in the NCCN guidelines, rather than treating all patients with adjuvant therapy, as in other research,[47-50] which is not only according to the international guidelines, but also avoids overtreatment suspicions and impact of different subsequent treatments on patient survival.

This study has some limitations. First, this is a single-arm study without a control group. Therefore, randomized controlled clinical trials involving large sample sizes and high quality should be conducted soon. According to ethical requirements, this study focuses on the PD-L1-positive subgroup, and the results should be generalized with great caution. However, broad literature has shown that PD-1 inhibitors are not limited to populations with positive PD-L1 expression,[51] and subsequent studies will further expand the population.

In summary, the findings of this study will promote neoadjuvant anti-PD-1 combined chemo-immunotherapy with radical surgery as a new therapeutic strategy for patients with LACC. With the continuous improvement of medical care, treatment for cervical cancer is no longer monotherapy of radical surgery, chemotherapy, or radiation. The integration of multiple treatments may achieve better cancer treatment effects. Further clinical trials and translational research are warranted to elucidate potential predictive biomarkers and investigate the mode of tumor microenvironment remodeling of anti-PD-1 therapies in cervical cancer. In this regard, patient stratification and individualized precision therapy can be achieved.

Contributors

JC and YH contributed equally to this trial and wrote the article. KL, GC, and CS developed the study concept and protocol. YH, XF, XM and SG assisted in further protocol development. KL supervises the clinical trial and has access to the final trial dataset. All authors contributed to the article and approved the submitted version.

Funding

This trial is funded by Jiangsu Hengrui Medicine, Lianyungang, China (MA-CervC-II-002). The funder had not involvement in the study design, the writing of this article, or the decision to submit it for publication.

Competing interests statement

The authors declare that the research is conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

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Figure legend

Figure 1 The overall trial flow of the study. CPS, combined positive score; MRI, magnetic resonance imaging; Q3W, every 3 weeks; CR, complete response; PR, partial response; SD, stable disease; PD, progression disease; CT, computed tomography; CCRT, concurrent chemoradiotherapy; NACIT, neoadjuvant chemo-immunotherapy; Q3M, every 3 months; Q6M, every 6 months; [1], if there are contraindications for MRI, CT is recommended; [2], in priming phase and NACIT phases, blood samples are collected before (～24h) and after (～24h) each cycle; in patients with surgery, blood samples are collected before and then 7 days after radical surgery, if any, after administration of postoperative adjuvant treatment; in patients with CCRT, blood samples are collected before and immediately after CCRT; [3], in priming phase and NACIT phases, biopsy sample of tumor tissue are collected before each cycle; in patients undergoing surgery, the resected tumor tissue and a paracancer normal tissue will be collected; in patients with CCRT, biopsy sample is collected before CCRT.

Figure 2 Representative image of a multiplex immunohistochemistry of the immune context of cervical cancer patient tumors pre- and post-neoadjuvant chemotherapy (NACT).

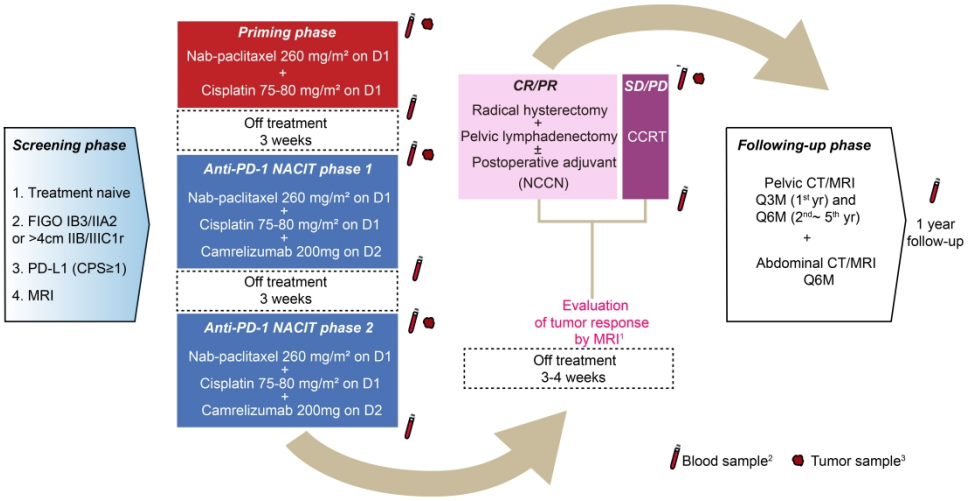

Figure 1 The overall trial flow of the study. CPS, combined positive score; MRI, magnetic resonance imaging; Q3W, every 3 weeks; CR, complete response; PR, partial response; SD, stable disease; PD, progression disease; CT, computed tomography; CCRT, concurrent chemoradiotherapy; NACIT, neoadjuvant chemo-immunotherapy; Q3M, every 3 months; Q6M, every 6 months; 1, if there are contraindications for MRI, CT is recommended; 2, in priming phase and NACIT phases, blood samples are collected before (~24h) and after (~24h) each cycle; in patients with surgery, blood samples are collected before and then 7 days after radical surgery, if any, after administration of postoperative adjuvant treatment; in patients with CCRT, blood samples are collected before and immediately after CCRT; 3, in priming phase and NACIT phases, biopsy sample of tumor tissue are collected before each cycle; in patients undergoing surgery, the resected tumor tissue and a paracancer normal tissue will be collected; in patients with CCRT, biopsy sample is collected before CCRT.

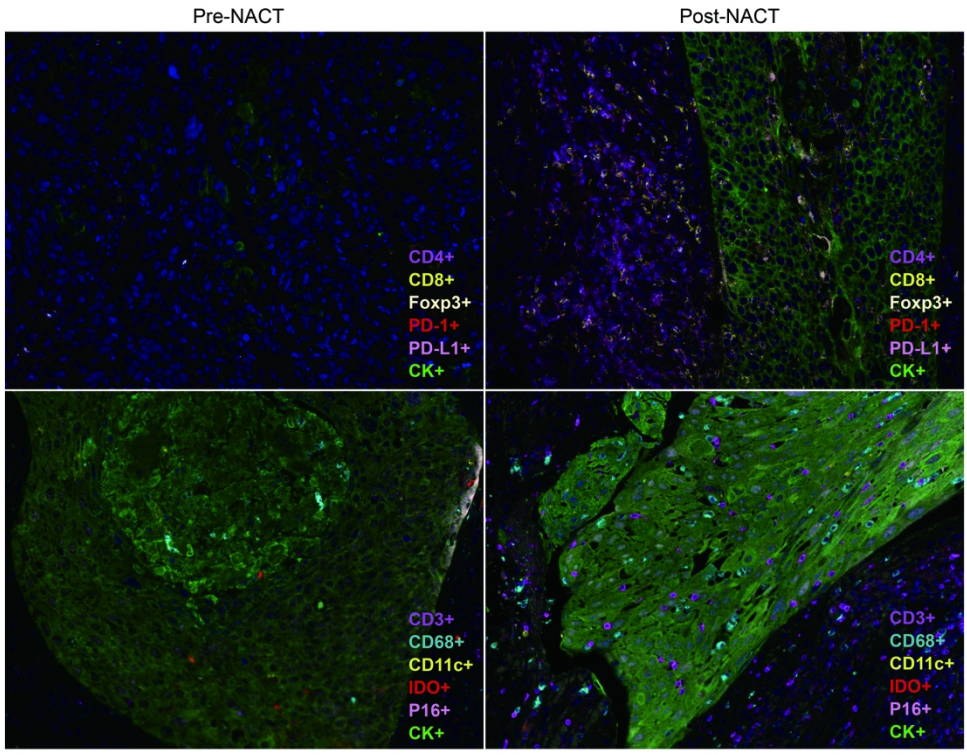

Figure 2 Representative image of a multiplex immunohistochemistry of the immune context of cervical cancer patient tumors pre- and post-neoadjuvant chemotherapy (NACT).

Supplementary Table 1 List of all participating centers

No.	Organization	Principal Investigator
1	Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology	Ding Ma, Kezhen Li, Gang Chen
2	Women's Hospital, School of Medicine, Zhejiang University	Weiguo Lu、Yuanming Shen
3	The Southwest Hospital of Army Medical University	Yanzhou Wang
4	Qilu Hospital of Shandong University	Kun Song
5	Obstetrics & Gynecology Hospital of Fudan University	Xiaojun Chen
6	Anhui Province Cancer Hospital	Bairong Xia
7	Chongqing University Cancer Hospital	Dongling Zou
8	Tianjin Medical University General Hospital	Yingmei Wang

Supplementary Table 2 A plan of collection of the different outcomes.

Item required	Screening		Treatment					Follow-up	
	Within 1 month	Within 7D	NT	P-NACT1	P-NACT2	□Surgery [1]	End of therapy [2]	S1/S3	S2 ~ S12
			D1	D1±7D	D1±7D	□CCRT	±7D	±30D	±30D
Baseline characteristics									
Informed Consent	√								
Medical history [3]	√								
Medication history [4]	√		√	√	√	√		√	√
Lab examination									
Blood routine		√		√	√	√	√	√	
Urine Routine		√							
Blood biochemistry		√		√	√	√	√	√	
Coagulation function		√							
Myocardial enzyme		√							
HBV/HCV/HIV	√								
Tumor marker [5]	√		√	√	√	√	√	√	√
HPV and TCT	√							√	√
Imaging examinations									
Chest X-ray/CT	√		√	√	√	√	√		
Pelvic [6]	MRI		MRI			MRI	MRI	MRI/CT	MRI/CT

Whole Abdomen [7]	MRI		MRI	B/MRI	B/MRI	MRI	B/MRI		MRI/CT
Other examination									
Electrocardiograph		√		√	√	√	√		
Cervical pathology [8]	√					√			
The study drug [9]			√	√	√				
Clinical evaluation									
Physical examination [10]		√		√	√	√	√	√	√
ECOG score		√							
Adverse event [11]			√	√	√	√	√	√	√
Quality of Life		√		√		√	√	√	√
Survival analysis									
Recurrence [12]								√	√
Death [13]								√	√

All examinations and tests are performed according to the study schedule. Unless otherwise specified, all items are recorded as pretreatment results for each cycle.

[1] Choose the treatment according to the neoadjuvant immunotherapy response. Surgical patients with postoperative adjuvant treatment should have their time and dose of radiotherapy, time and dose of chemotherapy administration, adverse events, radiotherapy, or concurrent chemoradiotherapy recorded. The time and dose of CCRT and adverse events should be collected for patients undergoing CCRT.

[2] Definition of end of therapy. In patients undergoing surgery, if they have high-risk factors or meet the Sedlis criteria, it means completion of all postoperative adjuvant therapy. If postoperative risk factors do not meet the NCCN guidelines for treatment, it means that the operation is completed. In patients undergoing CCRT, it means concurrent radiochemotherapy is completed.

[3] Patients during pregnancy and lactation patients are excluded.

[4] Record use of drugs, in addition to the study drugs, is recorded.

[5] Squamous cell carcinoma antigen (SCCA) should be detected for the type of squamous cell carcinoma, CA125 for adenocarcinoma, and both for adenosquamous carcinoma.

[6] If there is contraindicated, contrast-enhanced CT is recommended. If a pelvic MRI shows an enlargement of the retroperitoneal pelvic lymph node (short diameter > 15 mm), an entire abdominal enhanced CT should be performed. MRI/CT means both are allowed. MRI/CT is required at the end of treatment only in the CCRT group

(not in the surgery group), and the CCRT group should be followed up by MRI.
[7] B/MRI and MRI/CT are allowed.
[8] Including vaginal biopsy, histopathological examination, or pathological examination of tumor tissue after surgery.
[9] Including the time and dose of cisplatin, nab-paclitaxel, and camrelizumab.
[10] Including gynecological examinations.
[11] Collecting of intraoperative and postoperative complications in patients undergoing surgery.
[12] Therecurrence, recurrence time, recurrence location, and anti-tumor therapy after recurrence are recorded.
[13] Including the death time and cause of death.
NT, induction chemotherapy cycle; P-NACT; PD-1 inhibitor combined neoadjuvant chemotherapy; D, days; CCRT, concurrent chemoradiotherapy; CT, computed tomography; CT, computed tomography; MRI, magnetic resonance imaging; B, B-ultrasonography; ECOG, Eastern Cooperative Oncology Group.

Supplementary Table 3 List of ongoing trials of chemo-immunotherapies for locally advanced cervical cancer.

Identifier	Study Phase	Study population	Estimated participants	Intervention
NCT05554276	II	Unresectable CSCC	36	NACT combined with PD-1 antibody + RT
NCT05227651	II	IB2-IIA2	30	NACT+AK104+RS
NCT04238988	II	IB2-IIB	45	Neoadjuvant CPP +RS+Adjuvant CPP (high-risk patients)
NCT05013268	II	IB2-IIB	15	Tislelizumab plus TP+RS

CSCC, cervical squamous cell carcinoma; NACT, neoadjuvant chemotherapy; RT, radiotherapy; CPP, Carboplatin-Paclitaxel-Pembrolizumab; TP, paclitaxel/docetaxel+cisplatin/carboplatin regimen.

SPIRIT
STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description
Administrative information		
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym Page 1 Line 1-3
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry Page 2 Line 50
	2b	All items from the World Health Organization Trial Registration Data Set Page 2 Line 50
Protocol version	3	Date and version identifier Page Line 288-291
Funding	4	Sources and types of financial, material, and other support Page 11 Line 434-436
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors Page 11 Line 429-432
	5b	Name and contact information for the trial sponsor Page 1 Line 9-17
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities Page 11 Line 434-436
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee) Page 8 Line 276-284
Introduction		
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention Page 2-4
	6b	Explanation for choice of comparators Page 2-4
Objectives	7	Specific objectives or hypotheses Page 4 Line 130-136

Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory) Page 4 Line 119-128
Methods: Participants, interventions, and outcomes		
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained Supplementary Table 1
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists) Table 1
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered Page 6-7 Line 189-210
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease) Page 7 Line 206-210
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests) Page 7 Line 211-216
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial Page Page 7 Line 206-210
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended Page 4-5 Line 138-162
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure) Figure 1 and Supplementary Table 2
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations Page 7 Line 251-256
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size Page 4 Line 123-125
Methods: Assignment of interventions (for controlled trials) This is a single arm trials		

Allocation:		
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions NA
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned NA
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions NA
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how NA
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial NA
Methods: Data collection, management, and analysis		
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol Page 7 Line 211-216, Supplementary Table 2
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols Page 7 Line 218-229, Supplementary Table 2
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol Page 8 Line 276-284
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol Page 7-8 Line 258-274
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses) Page 7-8 Line 258-274

	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation) Page 7-8 Line 258-274
Methods: Monitoring		
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed Page 8 Line 276-284
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial Page 8 Line 279-282
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct Page 8 Line 276-284
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor Page 8 Line 276-284
Ethics and dissemination		
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval Page 8 Line 289-292
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators) Page 8 Line 276-284
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32) Page 5 Line 169-171, Informed Consent Form
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable Page 7 Line 241-242, Informed Consent Form
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial Page 8 Line 276-284
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site Page 11 Line 438-439

Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators Page 8 Line 276-284
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation Page 8 Line 293-294
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions Page 8 Line 295-299
	31b	Authorship eligibility guidelines and any intended use of professional writers Page 11 Line 429-432
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code NA
Appendices		
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates Informed Consent Form
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable Figure 1

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.