Toripalimab plus intensity-modulated radiotherapy for recurrent nasopharyngeal carcinoma: an open-label single-arm, phase II trial

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ABSTRACT

Background Toripalimab is a humanized immunoglobulin G, monoclonal antibody against programmed death 1. We aimed to investigate the efficacy and safety of toripalimab in combination with intensity-modulated radiotherapy (IMRT) for recurrent nasopharyngeal carcinoma (rNPC).

Methods We conducted a single-arm, phase II trial with patients with rNPC who had biopsy-proven disease and were unsuitable for local surgery. Eligible patients received IMRT in combination with toripalimab administered via intravenous infusion of 240 mg once every 3 weeks for a maximum of seven cycles. The primary endpoint was the objective response rate at 3 months post radiotherapy. The secondary endpoints included safety profiles, progression-free survival (PFS).

Results Between May 2019 and January 2020, a total of 25 patients with rNPC were enrolled (18 men (72.0%) and 7 women (28.0%); median (IQR) age, 49.0 (43.5–52.5) years). With a median (IQR) follow-up duration of 14.6 months (13.1–16.2) months, 19 patients (79.2%) achieved an overall response, and disease control was achieved in 23 (95.8%) patients at 3 months post radiotherapy. The 12-month PFS was 91.8% (95% CI 91.7% to 91.9%). The incidences of acute (grade ≥3) blood triglyceride elevation, creatine kinase elevation, skin reaction, and mucositis were 1 (4.0%), 1 (4.0%), 2 (8.0%), and 1 (4.0%), respectively. The incidences of late severe (grade ≥3) nasopharyngeal wall necrosis, nasal bleeding, and trismus were 28.0%, 12.0%, and 4.0%, respectively.

Conclusions Toripalimab combined with IMRT was tolerable and showed promising antitumor activity in patients with rNPC.

Trial registration number NCT03854838.

INTRODUCTION

Nasopharyngeal carcinoma (NPC) has the highest prevalence in Southeastern Asia, with age-standardized rates ranging from 22.2 to 27.2 per 100,000 among men.1 The incidences of local recurrence in endemic NPC range from 10% to 20% after previous radical radiotherapy.2 3 In this subgroup of patients with resectable diseases, surgery is the standard of care and first-line treatment.4 5 However, reirradiation is routinely performed in patients with unresectable lesions. Despite the utilization of intensity-modulated radiotherapy (IMRT) and combined chemotherapy, the response rate was reported to be approximately 50%–65.4%, and the 3-year overall survival (OS) rate was reported to be only 41.8%–68.7%, with high rates of grade 5 treatment-related toxicities of approximately 33.0%.7 8 Therefore, there is an urgent need to develop new therapies combined with IMRT in unresectable recurrent NPC (rNPC) that potentially reduce recurrent toxicity and improve survival benefit.

Immune checkpoint inhibitors against programmed death 1 (PD-1), exemplified by the humanized monoclonal antibodies pembrolizumab and nivolumab, have been approved for the treatment of patients with relapsed squamous cell carcinoma of the head and neck (HNSCC) after previous platinum-based therapy. Recently, the promising antitumor activity and good tolerance of PD-1 therapies including nivolumab, pembrolizumab, toripalimab and camrelizumab were reported in several phase 1/2 trials for recurrent or metastatic NPC; however, the efficacy of PD-1 therapy alone was moderate, with a reported objective response rate of 20%–30%.9–11 Theoretically, radiotherapy can improve the efficacy of tumor immunotherapy by modulating the peptide repertoire and enhancing major histocompatibility complex (MHC) I expression,12–13 which reveals the rationale behind combining radiotherapy and PD-1 therapy. Recently, two
phase 1 studies demonstrated the safety and promising efficacy of pembrolizumab in combination with chemoradiotherapy in locally advanced HNSCC and non-small cell lung cancer. However, there are still no reports of radiotherapy combined with PD-1 therapy in NPC.

We therefore conducted a phase 2, Simon two-stage clinical trial investigating the efficacy and safety of toripalimab, a humanized immunoglobulin G4 monoclonal antibody against PD-1, in combination with IMRT in rNPC.

PATIENTS AND METHODS

Study design

This report presents the analysis of a single-arm, phase 2 trial to assess the preliminary antitumor activity and safety of toripalimab combined with IMRT as the first-line therapy for patients with unresectable locally rNPC. The trial was performed in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines. All participants provided written informed consent. The trial was registered at ClinicalTrials.gov.

Patient selection

The eligibility criteria for this trial included age 18–65 years; histologically confirmed NPC; patients with local recurrence (recurrent II-IVa) after curative radiotherapy who are unsuitable for local surgery; recurrence interval more than 12 months from the end of prior radiotherapy; no previous systemic chemotherapy for recurrent disease; an Eastern Cooperative Oncology Group performance status of 0 or 1; at least one measurable lesion assessed with Response Evaluation Criteria in Solid Tumors V.1.1 (RECIST V.1.1) by the investigators; and adequate organ function. Exclusion criteria included a history of active autoimmune disease; medical conditions requiring the use of immunosuppressive medications; active hepatitis B or C virus infection; uncontrolled hypertension and cardiac disease; previous treatment with anti-PD-1 or anti-programmed death-ligand 1 (PD-L1) antibodies; and patients who were pregnant or breast feeding.

Pretreatment evaluation included a complete medical history and physical examination; hematologic and biochemical analyses; nasopharyngoscopy; and MRI or contrast-enhanced CT if patients had contraindications to MRI of the head and neck. 18F-fluorodeoxyglucose positron emission tomography was utilized to exclude distant metastasis.

Procedures

Toripalimab was administered via intravenous infusion of 240 mg once every 3 weeks for a maximum of seven cycles or until disease progression, death, or dose-limiting toxicities occurred or the patient requested to stop treatment. Off-protocol anticancer drugs were not allowed before the occurrence of protocol-defined disease progression. Dose modifications of toripalimab were not permitted. Details of the interruption and discontinuation of toripalimab are provided in the online supplemental file, Protocol.

Patients received IMRT after the first cycle of toripalimab. IMRT target volumes were delineated according to a previously described institutional treatment protocol. Briefly, the primary NPC tumor (gross tumor volume (GTV)nx) and gross cervical lymph nodes (GTVnd) were delineated. The clinical tumor volume (CTV) was then defined by GTVnx with a 0.5–1.0 cm margin. The prescribed doses were 60 Gy, 60 Gy and 54 Gy in 27 fractions for the planning target volumes derived from GTVnx, GTVnd, and CTV, respectively. Complete details on the radiotherapy planning are provided in the online supplemental appendix.

Tumor response after radiotherapy was based on the RECIST criteria V.1.1 and assessed by nasopharyngoscopy and MRI of the primary site. We defined a complete response (CR) as a complete lack of unequivocal soft tissue mass in the local region and cervical lymph nodes.
that all had a short axis of less than 10 mm according to the RECIST guidelines.16

Patients were followed up after the completion of radiotherapy every 3 months until death to evaluate the efficacy and safety of the treatment. We recorded the survival status and subsequent lines of therapies. Adverse events (AEs) were scored according to the Common Terminology Criteria for Adverse Events V.5.0 and the acute and late radiation morbidity scoring criteria of the Radiation Therapy Oncology Group at each follow-up visit. Acute AEs were defined as those occurred during the radiotherapy or within 3 months post radiotherapy, while late AEs were defined as those occurred over 3 months after the end of radiotherapy.

Study endpoints
The primary endpoint of the study was the objective response rate (ORR) at 3 months after radiotherapy, which was defined as the proportion of patients who had a confirmed objective response (defined as complete or partial response according to RECIST V.1.1). The secondary endpoints included safety profiles, progression-free survival (PFS) (defined as the time from enrollment to locoregional or distant metastasis relapse or death from any cause, whichever occurred first), OS (defined as interval from enrollment to death due to any cause) and biomarker analysis. Imaging results to assess the ORR and PFS were centrally reviewed.

Table 1  Baseline demographics and disease characteristics

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<th>Toripalimab plus IMRT N=25</th>
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<td>Sex</td>
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<td>Female</td>
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<tr>
<td>Male</td>
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<td>Non-smokers</td>
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<td>Histology</td>
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<tr>
<td>Non-keratinizing undifferentiated (type III)</td>
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<td>Non-keratinizing differentiated (type II)</td>
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<tr>
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<tr>
<td>T3</td>
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<tr>
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<tr>
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<tr>
<td>N2</td>
<td>16 (64.0%)</td>
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<tr>
<td>N3</td>
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<tr>
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<tr>
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<tr>
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<td>N0</td>
<td>16 (64.0%)</td>
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Table 1  Continued

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<thead>
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<th>Characteristic</th>
<th>Toripalimab plus IMRT N=25</th>
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<tr>
<td>N1</td>
<td>9 (36.0%)</td>
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<tr>
<td>Recurrent M classification</td>
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<tr>
<td>M0</td>
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</tr>
<tr>
<td>M1</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>Recurrent stage</td>
<td></td>
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<td>II</td>
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<tr>
<td>III</td>
<td>16 (64.0%)</td>
</tr>
<tr>
<td>IVa</td>
<td>7 (28.0%)</td>
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<tr>
<td>PRANCIS prognostic index</td>
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<td>Low risk</td>
<td>17 (68.0%)</td>
</tr>
<tr>
<td>High risk</td>
<td>8 (32.0%)</td>
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<tr>
<td>Disease-free interval, median months (IQR)</td>
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<td></td>
<td>37.0 (21.5–73.0)</td>
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<td>Previous radiotherapy</td>
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</tr>
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<td>0 (0.0%)</td>
</tr>
<tr>
<td>Yes</td>
<td>25 (100.0%)</td>
</tr>
</tbody>
</table>

Data are presented as the median or n (%) unless otherwise stated. EBV, Epstein-Barr virus; IMRT, intensity-modulated radiotherapy; PRANCIS, Predicting Radioresistant Nasopharynx Carcinoma Survival.
were formalin-fixed and paraffin-embedded (FFPE) samples and 11 were fresh frozen tissues. WES was performed using the Sure-Select Human All Exon V6 Kit (Agilent) on tumor biopsies and matched peripheral blood mononuclear cell samples. Genomic alterations including single nucleotide variants, short and long insertions/deletions (indels), copy number variants, and gene fusions were assessed. The tumor mutation burden (TMB), frameshift indels, neoantigen burden, copy number burden—the weighted Genome Integrity Index (wGII) score and microsatellite instability score were determined.

**Statistical planning and analysis**

A Simon two-stage optimal design with a one-sided type I error rate of 5% and power of 80% was utilized. The null hypothesis was an ORR at 3 months post radiotherapy of ≤50%, and the alternate hypothesis was an ORR ≥75%. Consequently, 11 subjects were enrolled in the first stage. If six or fewer responded at the initial stage, the trial would be terminated, and the study would be concluded. If more than 6 patients achieved a partial or CR, then the treatment would be considered worthy of further investigation, and 14 more subjects would be enrolled in the second stage for a total sample size of 25 subjects. If there were more than 16 subjects with partial or CR, then the treatment regimen was considered a success.

All enrolled patients were included in the efficacy and safety analyses. For all patients, the median follow-up time was calculated using the reverse Kaplan-Meier method. The ORR and 95% CIs were calculated using the Clopper-Pearson method. The duration of response, PFS, and OS were analyzed using the Kaplan-Meier method. Summary statistics were provided for clinical and demographic characteristics and for AEs.

In post-hoc analyses, the associations between PD-L1 expression, genome/clinical characteristics and CR and PFS were assessed. For the comparison between patients with CR or not, χ² test or Fisher’s exact test and Wilcoxon rank-sum test were used. Univariate analysis of the effects of these parameters on PFS was conducted by the Cox proportional hazards model to calculate the HRs and 95% CIs. We performed all statistical tests using Stata V.14.2 software (StataCorp). The authenticity of this article has been validated by uploading the key raw data onto the Research Data Deposit public platform (www.researchdata.org.cn), with the approval RDD number RDDA2021851016.

**RESULTS**

**Patients and treatment**

Between May 2019 and January 2020, a total of 25 patients with rNPC were enrolled in the study. All 25 patients’ lesions were recurrent in the irradiation field. All 25 patients were included in the efficacy and safety analysis (figure 1).
The baseline demographics and disease characteristics are listed in table 1. The median age was 49.0 (IQR 43.5–52.5) years. Twenty-three of 25 (92.0%) patients were stage rT3–T4, and 25 of 25 (100.0%) patients were stage rN0–N1. Seventeen of 25 (68.0%) patients were defined as low risk, while 8 of 25 (32.0%) patients were defined as high risk according to Predicting Radioresistant Nasopharynx Carcinoma Survival (PRANCIS) prognostic model.2 Twenty-four of the 25 patients (96.0%) were treated with platinum-based chemotherapy during their primary therapy. Twenty-two of the 25 patients (88.0%) completed all seven cycles of toripalimab; one patient received only five cycles because of death from hemorrhage. Two patients received six cycles (one patient developed disease progression, and one patient discontinued treatment due to AEs). No modifications of the dose of toripalimab were observed. Toripalimab administration was delayed in one patient due to the COVID-19 pandemic. All 25 patients (100.0%) completed the protocol-defined IMRT. The median dose of IMRT was 60.21 Gy (IQR, 60–60.21 Gy), and the median duration of IMRT was 36 days (IQR, 35–38 days) (online supplemental table 1).

**Efficacy**

In the first 11 patients enrolled, confirmed responses were noted in 10 patients. The ORR threshold for the first stage of Simon’s two-stage was reached, and the trial continued to full accrual. The data cut-off date for the analysis was February 2021. Two patients were lost to follow-up.
1 patient died, and 22 patients were alive. The median follow-up time for PFS was 14.6 months (IQR 13.1–16.2) months. Nineteen of the 24 patients (79.2%) achieved an overall response, and disease control was achieved in 23 (95.8%) patients at 3 months post radiotherapy (table 2, online supplemental figure 1). Among the 23 patients with a confirmed objective response within 12 months post radiotherapy, the median time to response was 2.65 months (IQR 1.88–5.27), and the median duration of response was not reached (not reached to not reached) (table 2). The treatment response remained ongoing in 17 of 25 patients (68.0%) at the cut-off point. Overall, all 25 patients (100.0%) with at least one post-baseline tumor assessment had a decrease in the size of their target lesions from baseline. The median change from baseline was −100.0% (IQR −71.3% to −100.0%) (figure 2). Five patients had documented disease progression including three local progressions, one regional relapse, and one death. The median PFS was 18.67 months (95% CI 12.04 to 25.29 months), and the 12-month PFS was 91.8% (95% CI 91.7% to 91.9%) (figure 3). The median follow-up time for OS was 15.7 months (IQR 13.9–18.0) months, the median OS was not reached, and the 12-month OS was 96.0% (95% CI 88.4% to 100.0%).

Adverse events
All 25 patients were included in the safety analysis (table 3). The most common AEs were G1-2 fatigue in 22 (88.0%) patients, G1-2 nausea in 19 (76.0%) patients, G1-2 serum creatinine elevation in 13 (52.0%) patients, G1-2 weight loss in 11 (44.0%) patients, G1-2 blood triglyceride elevation in 11 (44.0%) patients, G1-2 hypothyroidism in 8 (32.0%) patients, G1-2 pruritus in 8 (32.0%) patients, G1-2 blood cholesterol elevation in 6 (24.0%) patients, G1-2 creatine kinase elevation in 4 (16.0%) patients, G1-2 leukopenia in 4 (16.0%) patients and G1-2 myositis in 4 (16.0%) patients. G3 serum triglyceride elevation was observed in 1 (4.0%) patient, G4 creatine kinase elevation was observed in 1 (4.0%) patient, G3 skin reaction was observed in 2 (8.0%) patients, and G3 mucositis was observed in 1 (4.0%) patient. Regarding late AEs, the most common AEs were G1-2 hearing loss in 18 (72.0%) patients, G1-2 dry mouth in 18 (72.0%) patients, G1-2 cranial neuropathy in 9 (36.0%) patients, and G1-2 neck tissue damage in 7 (28.0%) patients. Additionally, we recorded 7 (28.0%) cases of ≥G3 nasopharyngeal wall necrosis, 3 (12.0%) cases of ≥G3 nasal bleeding, and 1 (4.0%) case of G3 trismus. One patient died due to G5 nasal bleeding attributed to artery blowout.

Exploratory studies
Nineteen tumor biopsy samples were obtained for IHC staining of PD-L1. Four PD-L1-negative samples (21.1%) and 15 PD-L1-positive samples (78.9%) were identified. PD-L1-positive status was associated with higher CR rates (86.7% vs 50.0%), and PD-L1-positive patients had a better PFS (HR=0.43); however, these differences were not statistically significant (online supplemental tables 3 and 4).

WES results were obtained from 18 patients with rNPC. TMB was determined by analyzing somatic mutations within the coding region of the human genome. TMB was generally low in this study, with a median TMB of 0.72 (IQR 0.28–1.44) mutations per million base pairs. Patients with a non-CR tended to have a higher copy number burden (wGII score) (median, 0.30 vs 0.12, p=0.056) and TMB (median, 1.25 vs 0.73, p=0.574) than patients with a CR, while patients with a higher wGII score (HR 2.78, 95% CI 1.04 to 6.88, p=0.037) and TMB (HR 2.91, 95% CI 0.18 to 46.68, p=0.451) tended to have reduced PFS (online supplemental figures 2 and 3).

WES identified 4508 genetic alterations, including 370 missense, 30 non-sense, 49 splice site, 52 frameshift mutations, 2131 amplification and 1876 deletions. According to our previous study, the nuclear factor kappa-B (NF-κB), PI3K-AKT, JAK-STAT, and cell cycle pathways were enriched in somatic mutations in rNPC, and the DNA mismatch repair pathway was associated with radiotherapy sensitivity. However, no correlation between somatic mutations or these associated pathway alterations and clinical response or improved PFS was found (online supplemental figure 3).

Additional clinical characteristics analyzed for correlation with clinical efficacy included age, sex, Epstein-Barr virus (EBV) DNA copy number, T stage, N stage, GTV, GTV Dmin (minimum dose), GTV Dmax (maximum dose), GTV D95 (dose covering 95% volume) and GTV D50 (median dose). Among the subgroups, early T stages (T2/3) and smaller GTV were associated with a higher CR rate but without a statistically significant difference, while patients with larger GTV had reduced PFS compared with other patients (HR per cc increase 1.04, 95% CI 1.01 to 1.07, p=0.021). However, pretreatment EBV DNA copy number was not significantly associated with PFS outcome, while both T and N stages were also not significant prognostic factors of PFS in these patients. (online supplemental tables 3 and 4, online supplemental figure 4).

Figure 3 Kaplan-Meier curves of the progression-free survival. Progression-free survival was assessed in the whole population (n=25).
Table 3  Adverse events and grade

<table>
<thead>
<tr>
<th>Event</th>
<th>Toripalimab plus IMRT N=25</th>
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<tbody>
<tr>
<td></td>
<td>All grades</td>
</tr>
<tr>
<td>Acute toxicity</td>
<td></td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td>1 (4.0)</td>
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<tr>
<td>Neutropenia</td>
<td>2 (8.0)</td>
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<tr>
<td>Leukopenia</td>
<td>4 (16.0)</td>
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<td>ALT elevation</td>
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<tr>
<td>AST elevation</td>
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<tr>
<td>Total bilirubin elevation</td>
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<tr>
<td>Direct bilirubin elevation</td>
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<tr>
<td>Blood cholesterol elevation</td>
<td>6 (24.0)</td>
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<tr>
<td>Blood triglyceride elevation</td>
<td>12 (48.0)</td>
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<td>Hyperglycemia</td>
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<td>Serum creatinine elevation</td>
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<td>Creatine kinase elevation</td>
<td>5 (20.0)</td>
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<td>Hypertension</td>
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<tr>
<td>Hyperthyroidism</td>
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<tr>
<td>Nausea</td>
<td>19 (76.0)</td>
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<tr>
<td>Vomiting</td>
<td>2 (8.0)</td>
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<tr>
<td>Diarrhea</td>
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<tr>
<td>Pruritus</td>
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<tr>
<td>Capillary hyperplasia</td>
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<td>Weight loss</td>
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<tr>
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<td>Mucositis</td>
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<td>Dry mouth</td>
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<td>Late toxicity</td>
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<td>Nasopharyngeal wall necrosis</td>
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<td>Massive nasal bleeding</td>
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<td>Eye damage</td>
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<td>Deafness</td>
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<td>Trismus</td>
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<td>Dry mouth</td>
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<tr>
<td>Skin reaction</td>
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<tr>
<td>Neck tissue damage</td>
<td>7 (28.0)</td>
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<tr>
<td>Cranial neuropathy</td>
<td>9 (36.0)</td>
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</tbody>
</table>

All data are presented as No. (%).
ALT, Alanine aminotransferase; AST, Aspartate aminotransferase; IMRT, intensity-modulated radiotherapy.
DISCUSSION

To our knowledge, this study is the first report to evaluate the combination therapy of an anti-PD-1 antibody and IMRT in patients with NPC. Our results revealed that toripalimab plus IMRT showed promising antitumor activity against unresectable locally rNPC, with a favorable response rate, PFS outcome and manageable toxicity profile.

The ORR at 3 months post reirradiation was reported to be only 50% for rNPC, as radiation dose coverage to tumor volumes is often suboptimal due to the close proximity to critical organs at risk. Although the number of studies on combined chemoradiotherapy in rNPC has increased in recent years, such as radiotherapy combined with induction and/or concurrent chemotherapy, the role of combination therapy has also remained undefined, with ORR rates of approximately 60% and 3-year OS rates of 40%–50%, while acute side effects have increased significantly, with severe hematological and non-hematological rates of 11.7%–42.4% and 23.5%–33.3%, respectively. The main problem of second-course radiotherapy for rNPC is the unsatisfactory local control rate and severe radiation-related injury. Immunotherapy, such as the use of PD-1 inhibitors, has mild side effects and continuous efficacy. Radiotherapy not only has direct killing effects on tumor cells but also overcomes an immunosuppressive tumor microenvironment and promotes antigen presentation. Therefore, radiotherapy combined with PD-1 might be theoretically effective and have lower toxicity. The primary purpose of this trial is to improve the local control rate, especially at the routine assessment point of 3 months after the completion of radiotherapy. Therefore, we prescribed the use of seven cycles of PD-1 therapy, which ended exactly 3 months after the completion of radiotherapy. The ORR findings herein meet the prespecified endpoint for this phase 2 trial. Nineteen (79.2%) patients achieved an overall response at 3 months post radiotherapy, which is better than the historical data of 50%–65.4% which all utilized IMRT with standard fractionated scheme of 60 Gy, 27–30 fractions, five fractions per week as the second-course radiation therapy for rNPC with similar rT stages of 92%–100% of rT3–rT4. This suggests that the efficacy of radiotherapy combined with PD-1 monoclonal antibody treatment is promising.

In our study, toripalimab combined with IMRT was well tolerated, with all patients completing IMRT and 88% of patients completing seven cycles of toripalimab. Regarding severe acute toxicities, only 4.0%–8.0% of patients were observed G3-4 blood triglyceride elevation, creatine kinase elevation, skin reaction or mucositis, respectively, showing that combined PD-1 therapy did not significantly increase the side effects of radiotherapy and systemic side effects. Regarding late AEi, we recorded 11 (44.0%) cases of nasopharyngeal wall necrosis, which is similar to the historical data (31.5%–40.6%). However, only 3 (12.0%) patients had G3-4 nasal bleeding because embolization of the affected internal carotid artery was performed for patients with G3-4 nasopharyngeal necrosis, preventing the occurrence of massive nasal bleeding.

Interestingly, copy number burden (wGII score) was observed to be a potential biomarker for PD-1 plus radiotherapy in rNPC, which was consistent with the findings of previous reports on head and neck cancer, prostate cancer, and other cancers demonstrating that copy number burden is a prognostic factor associated with recurrence and death. Finally, we observed that patients with lower GTV had a statistically significant PFS advantage compared with other patients, which also demonstrated that GTV impacted locoregional control in rNPC.

We acknowledge that this study has some limitations. First, this was a single-arm study with no control group for comparison, and thus, selection bias could not be ruled out. Second, the small sample size reduces the certainty of the observed effectiveness.

Our data showed that toripalimab combined with IMRT had promising antitumor activity and manageable toxicity in patients with rNPC. Larger randomized controlled trials are warranted to validate our findings.

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Open access


REFERENCES


Trial protocol

Intensity-modulated radiotherapy (IMRT) combined with toripalimab in unresectable local recurrent nasopharyngeal carcinoma: a single-center and single-arm phase II clinical trial

Version date: 2019.3.30

Principal Investigator: Dr. Ming-Yuan Chen

Sponsor: Sun Yat-sen University Cancer Center (SYSUCC)
1. Abstract

Local recurrence is one of the most challenging issues to arise in the treatment of nasopharyngeal carcinoma, and approximately 10 to 15% of primary nasopharyngeal carcinoma patients will experience local recurrence. Minor recurrent lesions can be treated with salvage surgery, but most lesions still cannot be surgically removed.

The main treatment for unresectable locally recurrent nasopharyngeal carcinoma (rNPC) is still secondary-course radiotherapy. According to the 2018. V2 NCCN guidelines, intensity-modulated radiotherapy (IMRT) is the main treatment for rNPC. However, the efficacy remains poor, even if intensity-modulated radiotherapy (IMRT) is used. The 5-year overall survival rate of rNPC treated with repeated IMRT is approximately 13.2-36%, and there are also limited benefits from combined chemotherapy or molecular-targeted therapy. Thus, there is an urgent need to improve treatment options and their efficacy for patients with unresectable rNPC.

Immunotherapy has emerged as a promising treatment approach in recent years. Immunotherapy has fewer adverse reactions and longer-lasting action than chemotherapy and molecular targeted therapy, which could potentially improve the survival outcomes and quality of life of patients. At present, numerous studies both at home and abroad have shown that PD-1 monoclonal antibodies could significantly prolong the survival time of patients with various cancers, such as malignant melanoma and lung carcinoma. Regarding the research on the application of PD-1 monoclonal antibodies in nasopharyngeal
carcinoma, a number of clinical studies have been carried out in our hospital, among which an open-label phase II clinical trial by Prof. Ruihua Xu’s team initially found that the objective response rate (ORR) of patients with recurrent and metastatic nasopharyngeal carcinoma treated with toripalimab was 30.8%, and the disease control rate (DCR) was 61.5%, which is an inspiring result (https://oncologypro.esmo.org/Meeting-Resources/ESMO-2018-Congress).

Toripalimab is a humanized anti-programmed cell death-1 (PD-1) IgG4K (γ4,κ) monoclonal antibody (mAb) that is a coinhibitory receptor expressed on T cells. By binding to PD-1, it sterically blocks the binding of PD-1 and PD-L1/PD-L2, increasing the activity of T lymphocytes; it can also lower the expression on the cell membrane surface of PD-1 by inducing the endocytosis of PD-1, thereby enhancing the ability of T lymphocytes to react to antigen stimulation and exert antitumor effects. In this study, we intend to carry out a single-arm clinical trial using IMRT combined with toripalimab to treat unresectable rNPC in order to clarify the efficacy and safety of this new combination therapy. Confirmation that IMRT combined with toripalimab is safe and effective for the treatment of rNPC will provide the evidence needed for an expanded phase III clinical trial to improve the therapeutic effect of IMRT plus PD-1 therapy in rNPC patients.

2. Project basis

Nasopharyngeal carcinoma is one of the most common malignant carcinomas in China, with the highest incidence being in South China and
Hong Kong. According to the data released by the National Cancer Center in 2018, the number of new cases of nasopharyngeal carcinoma in China in 2014 was estimated to be 44,600, with 24,200 attributable deaths, and its incidence ranks 20th among malignant carcinomas. Nasopharyngeal carcinoma usually occurs during prime adulthood, and it is likely to cause major impacts on society, economy, labor, and families. Radiotherapy combined with or without chemotherapy is the primary initial treatment of nasopharyngeal carcinoma. Due to advancements in radiotherapy technology, the 5-year overall survival rate for the initial treatment of nasopharyngeal carcinoma is approximately 80% [1-5]. However, 10-15% of patients will have local recurrence [3, 6-8].

Our previous research found that approximately 38% of locally recurrent carcinomas (rT1-2N0M0) can be treated with salvage surgery, and the operable scope includes rT1N0M0, rT2N0M0 (carcinoma confined to the surface of the parapharyngeal space), rT3N0M0 (carcinoma confined to the bottom of the sphenoidal sinus), and rT0N1-3M0 (carcinoma does not infiltrate the cervical spine, brachial plexus, neck muscles, carotid artery), but most of the lesions (62%) still cannot be surgically removed [9]. For inoperable locally recurrent nasopharyngeal carcinoma, although two-course radiotherapy, palliative chemotherapy or combination therapy have proven to be effective treatments for patients with locally recurrent nasopharyngeal carcinoma, based on our research, we have found that second-course radiotherapy is still the most effective and widely applicable treatment method among the various options available for inoperable recurrent nasopharyngeal carcinoma. Due to the development of radiotherapy technology, radiotherapy technologies such as two-dimensional radiotherapy, three-dimensional conformal radiotherapy, post-loading radiotherapy, stereotactic radiotherapy, and intensity-modulated radiotherapy have been incorporated into clinical practice [10-13]. However, our previous research found that intensity-modulated radiotherapy (IMRT) can significantly prolong the survival time of nasopharyngeal carcinoma patients.
compared with traditional two-dimensional radiotherapy [14], and due to the accuracy of the radiation dose and the protection offered to surrounding normal tissues, intensity-modulated radiotherapy (IMRT) has been recommended as the preferred radiotherapy technique for nasopharyngeal carcinoma treatment by the NCCN treatment guidelines [14-16]. Even so, current domestic and foreign reports show that the effect of IMRT in recurrent NPC is still poor, with a 5-year OS rate of only 13.2-36%[16-21]. According to the 2018 V2 NCCN guidelines, radiotherapy is the first choice for the treatment of inoperable and locally recurrent nasopharyngeal carcinoma.

This study is a single-arm, open phase II clinical trial using Simon's two-phase optimal design. The main purpose of the study is to evaluate the effectiveness and safety of intensity-modulated radiotherapy combined with toripalimab for the treatment of locally recurrent inoperable nasopharyngeal carcinoma. Previous retrospective studies have shown that the response rate after radiotherapy for nasopharyngeal carcinoma has a striking correlation with overall survival (OS), failure-free survival (FFS) and distant metastasis-free survival (DMFS). Multivariate analyses have also shown that the response rate is an independent prognostic indicator of OS, FFS, and DMFS [22-24]. Therefore, this study proposes an objective response rate (ORR) of 50% for unresectable nasopharyngeal carcinoma [25] as the reference value for sample size estimation in this study.

Synchronous radiochemotherapy is the standard treatment for advanced nasopharyngeal carcinoma. However, the 5-year OS rate in recurrent locally advanced nasopharyngeal carcinoma is only 34.3% [21], and the toxicity and side reactions are severe, with grade 3-4 toxicities and side reactions occurring in up to 53% of patients [26], indicating poor tolerance. For molecular targeted drug therapy, phase II clinical trials have proven that targeted drugs have limited therapeutic effects in recurrent nasopharyngeal carcinoma, with 2nd- and 3rd-line treatment failure being observed and an efficacy rate of only
11.7%; moreover, drug resistance is inevitable, which limits further improvements in efficacy [27]. Therefore, there is an urgent need for a new method to replace conventional chemotherapy with a treatment option that can assist intensity-modulated radiotherapy and improve the treatment effect in patients with locally recurrent nasopharyngeal carcinoma.

Immunotherapy is an emerging carcinoma treatment method in recent years. Compared with chemotherapy and molecular targeted therapy, immunotherapy has mild adverse reactions and long-lasting effects. Among them, PD-1, an immune checkpoint inhibitor, is an inhibitory molecule of T cells. Its binding with the ligand PD-L1 plays an immunosuppressive role and is an important mechanism for cancer immune escape [28]. Immunotherapy based on blocking the PD-1 and PD-L1 pathways has attracted much attention. Preliminary clinical studies have found that for melanoma, the objective response rate of PD-1 monoclonal antibodies in initial and advanced treatment can reach 37%, with a median overall survival time of 32.3 months [29], which has become a hot topic for researchers. In recent years, because the curative effect of PD-1 antibodies is distinct, the FDA has approved a variety of PD-1 and PD-L1 monoclonal antibodies used in the treatment of melanoma, advanced non-small-cell lung carcinoma, renal cell carcinoma, etc. At the same time, a number of clinical trials of PD-1 and PD-L1 monoclonal antibodies alone or combined with traditional radiotherapy and chemotherapy are in progress; however, the efficacy of PD-1 monoclonal antibodies combined with radiotherapy in recurrent nasopharyngeal carcinoma has not yet been reported.

Studies have confirmed that radiotherapy not only has a direct killing effect on cancer cells but also triggers an immune-mediated anticancer response, especially when combined with immunotherapy [30]. However, it can also cause some damage to immune cells at exposed sites. Therefore, radiotherapy is usually considered to suppress the immune effect of the body.
Recent studies have found that radiotherapy can also activate the anti-carcinoma immune response [31-34]. For example, radiotherapy can release large amounts of cancer-associated antigens by killing cancer cells and cancer stromal cells; at the same time, radiotherapy can promote the immune response by enhancing antigen presentation [32, 34]. Radiotherapy can induce T cell responses, significantly increase the proliferation and activation level of T cells in the cancer lymphatic drainage area, and enhance the anti-carcinoma immune response mediated by CD8+ T cells [33]. In addition, the increase in cancer infiltrating lymphocytes after radiotherapy also proves the activation effect of radiotherapy on the immune system [31]. At present, clinical studies have confirmed that in the treatment of advanced melanoma, the CR rate of PD-1 monoclonal antibody combined with radiotherapy is increased by 19.2% compared with that of PD-1 monotherapy, and the median survival time (MST) is extended by 9 months [35]. Therefore, the above theoretical bases and clinical practice show that the combined application of radiotherapy and immunotherapy is feasible and effective.

In the current clinical trials of immunotherapy combined with radiotherapy, different clinical trials place immunotherapy before, after or at the same time as radiotherapy. However, existing studies have shown that carcinomas can release and expose new antigens after local radiotherapy, which can change the tumor microenvironment and systemic immune response [31-34]. Therefore, using radiotherapy to release new carcinoma antigens and combining immunotherapy can achieve better therapeutic effects. At present, clinical studies have shown that radiotherapy has a positive effect on the immune system [36, 37]; clinical studies have confirmed that for the treatment of advanced melanoma, the CR rate is increased by 19.2%, and the median survival time is extended by 9 months with combination treatment compared to treatment with PD-1 inhibitor alone [34]. For malignant gliomas, the combination of stereotactic radiotherapy and checkpoint inhibitors also
improved overall survival by 15% [38]. For adjuvant immunotherapy after radiotherapy, the PACIFIC trial proved that, in locally advanced non-small-cell lung carcinomas (NSCLCs) that have not progressed after concomitant radiochemotherapy with platinum-based regimens, administration of PD-1 monoclonal antibody after radiotherapy can prolong the PFS of patients to 16.8 months, and the PFS of the placebo group was only 5.6 months. The experimental group had a 2-year overall survival rate that was 10.7% higher than that of the placebo group [39, 40]. These studies provide valuable evidence for the timing of combined radiotherapy with PD-1/PD-L1 monoclonal antibodies. Considering the fatal and subfatal injuries induced by radiotherapy, apoptotic carcinoma cells will continuously release cancer antigens; therefore, combined immunotherapy during and after radiotherapy may be the most appropriate combination.

Regarding the application of PD-1/PD-L1 inhibitors in nasopharyngeal carcinoma, our hospital has carried out a number of clinical studies and obtained inspiring results. Two clinical trials by Prof. Zhang Li explored the safety and efficacy of camrelizumab and combined therapy of camrelizumab+gemcitabine+cisplatinum in advanced or recurrent NPC. In advanced or recurrent NPC, the response rate of camrelizumab as a single drug is 34%, the severe adverse reaction rate is only 16%, and the response rate of combined therapy is as high as 91%. Camrelizumab and combined therapy show good safety and significant efficacy in NPC [41]. An open phase II clinical study conducted by Professor Ruihua Xu's team found that the objective response rate (ORR) and disease control rate (DCR) of patients with recurrent and metastatic nasopharyngeal carcinoma treated with toripalimab reached 30.8% and 61.5%, respectively (https://oncologypro.esmo.org/Meeting-Resources/ESMO-2018-Congress/Recombinant-Humanized-Anti-PD-1-Monoclonal-Antibody-JS001-in-patients-with-refractory-metastatic-nasopharyngeal-carcinoma-Preliminary-results-of-an-open-label-t
he phase-II-a clinical study). These studies provide a feasibility basis for the application of PD-1/PD-L1 inhibitors in nasopharyngeal carcinoma. However, how to combine immunotherapy for recurrent nasopharyngeal carcinoma is still unknown.

Toripalimab is a humanized anti-human programmed cell death-1 (PD-1) IgG4K (γ4,κ) monoclonal antibody (mAb) that is a coinhibitory receptor expressed on T cells. Toripalimab introduces a serine to proline 228 and minimizes replacement of the Fab chain. Similar to other anti-PD-1 antibodies, it has a high affinity and blocks the binding of PD-1 and its ligand (programmed cell death ligand 1 (PD-L1;B7-h1 or CD274)). However, it differs from other ligands in that it binds to PD-1 for a longer period of time (measured by the dissociation constant).

In this study, we intend to use IMRT combined with toripalimab in the treatment of unresectable locally recurrent nasopharyngeal carcinoma through a single-arm clinical trial to clarify the safety and efficacy of this new treatment. Once IMRT combined with toripalimab can be proven to be safe and effective for the treatment of locally recurrent nasopharyngeal carcinoma, it will be able to fill the need for radiotherapy combined with immunotherapy in nasopharyngeal carcinoma, provide evidence-based medical evidence to support an expanded phase III clinical trial, and improve the treatment efficacy of patients with unresectable locally recurrent nasopharyngeal carcinoma.

3. References


4. Research content

1. Ethical considerations

Advantages and disadvantages of toripalimab combined with intensity modulation radiation therapy (IMRT) for the treatment of unresectable local recurrent nasopharyngeal carcinoma:

1) Advantages:
   a) Toripalimab combined with intensity-modulated therapy can fully mobilize the patient's autoimmunity, which is expected to improve the local control rate and the overall survival of the patient.
   b) IMRT can enhance the efficacy of immunotherapy by killing cancer cells and releasing tumor antigens.

2) Disadvantages:
   a) The price of toripalimab would increase the financial burden of patients;
   b) Toripalimab combined with intensity-modulated therapy may cause more
adverse reactions than radiotherapy alone.

Although the use of toripalimab increases the economic burden and toxicity reactions in the early stage of treatment to a certain extent, the local control and overall survival rates are expected to improve after combined immunotherapy. Therefore, based on comprehensive consideration, for patients with inoperable local recurrent nasopharyngeal carcinoma, the advantages of toripalimab combined with modulation therapy outweigh the disadvantages.

2. Sample size estimation

Simon's two-stage optimal design is adopted in this trial. The main objective of this study is to investigate the objective response rate (PR+CR) of PD-1 combined with radiotherapy for the treatment of unresectable rNPC. According to previous studies, if the effective rate of PD-1 combined with radiotherapy is ≤50%, it is considered invalid. The effective rate of PD-1 combined with radiotherapy is expected to be ≥75%, α=0.05, β=0.2, and the optimal two-stage design is 6/11 (16/25). In the first stage, 11 patients will be enrolled. If the number of patients with objective response (CR+PR) is ≤6, the trial will be terminated. Otherwise, 14 patients will be enrolled in the second stage; i.e., a total of 25 patients will be enrolled. If the number of patients with an objective response (CR+PR) exceeds 16 among all patients in the two stages, the combination of PD-1 and IMRT will be considered a success.

3. Estimated time for case collection

The enrollment of 25 patients will be completed at Sun Yat-sen University Cancer Canter, and the eligible patients will account for approximately 5-10% of the total recurrent nasopharyngeal carcinoma in the hospital annually. It is estimated that 50% of the eligible patients refuse to enter the trial; thus, 25 cases will be collected within half a year.

4. Grouping method
This clinical trial employs an open-label single-arm design.

5. Screening period inspection

During the screening period of this study, the following laboratory examination samples will be sent to the laboratory of our center for analysis.

5.1 Examinations to be completed within 2 weeks before treatment

1) Personal data.

2) Physical examination, including height, weight and vital signs.

3) Nasopharyngoscopy and physical examination of the head and neck, including cervical lymph nodes.

4) Thoracoabdominal and general physical examination.

5) Routine blood, urine, and biochemical marker panels, assessment of blood coagulation function and thyroid function, evaluation of HBV serology, HCV serology, HIV testing, and EB virus serology, in blood samples.

5.2 Examinations to be completed within 1 month before treatment

1) Electrocardiogram.

2) Enhanced MRI of the nasopharynx + neck (enhanced CT is used instead for patients who are unable to undergo MRI examination).

3) PET/CT examination.

5) Histopathological examination of biopsies.

6) Confirmation of eligibility and signing of informed consent.

5.3 Tumor tissue specimens

Samples of tumor tissue (archived or freshly collected) before treatment will be obtained before enrollment, and a pathological report will be completed.
Paraffin-embedded (preferred) or fresh nasopharyngeal malignant tumor specimens will be used for exploratory biomarker analysis (including immune-related or NPC biologically related markers, such as PD-L1 expression, genomic characteristics including tumor mutation burden, neoantigen burden, copy number burden, etc.).

5.4 Selection of subjects

5.4.1 Inclusion criteria

1) A recurrence time of more than 12 months from the end of the initial radiotherapy.

2) Histological and/or cytological diagnosis of recurrent nasopharyngeal carcinoma (differentiated or undifferentiated type, WHO classification type II or III).

3) Radiotherapy site having at least one measurable lesion by MRI examination (according to the RECIST v1.1 standard).

4) Clinical stage: rT0-4N1-3M0 or rT2-4N0M0, stage II-IVA (AJCC 8th edition).

5) Age: 18-65 years old.

6) ECOG status of 0 or 1.

7) Good organ function as defined by the following:

   ① Hematology: Leukocytes ≥ 4000/μL, neutrophils ≥ 2.000/μL, hemoglobin ≥ 9 g/dL, platelets ≥ 100,000/μL;

   ② Liver function: Bilirubin ≤ 1.5 times the upper limit of normal (ULN) (patients with Gilbert's disease and a serum bilirubin level ≤ 3 times the ULN may be enrolled), AST and ALT ≤ 3 times, and alkaline phosphatase ≤ 3 times the ULN; albumin ≥ 3 g/dL;

   ③ International normalized ratio (INR), prothrombin time (PT) or activated partial thromboplastin time (aPTT) ≤ 1.5 times; and
Renal function: Serum creatinine \( \leq 1.5 \) times ULN or creatinine clearance \( \geq 60 \) mL/min according to the Cockcroft-Gault formula.

8) Expected survival \( \geq 3 \) months.

9) Agree to sign an informed consent form and willing to comply with the scheduled visits, treatment plan, laboratory tests and other study procedures.

10) Fertile women will be required have a negative urine or serum pregnancy test within 7 days of enrollment and agree to conduct effective contraception during the study period and at least 60 days after the last administration, including chemotherapy and toripalimab.

11) Male subjects who have a female partner who is still fertile will be required to agree to use effective contraception during the study period and for at least 120 days after the last administration.

5.4.2 Exclusion criteria

1) Resectable recurrent nasopharyngeal carcinoma:
   a. rT1N0M0
   b. rT2N0M0 (confined to the surface of the parapharyngeal space and more than 0.5 cm away from the internal carotid artery)
   c. rT3N0M0 (confined to the wall of the sphenoid sinus and more than 0.5 cm away from the internal carotid artery and the cavernous sinus)
   d. rT0N1-3M0 (without invasion into the cervical spine, brachial plexus, neck muscles, carotid artery)

2) A history of severe hypersensitivity to any component of other monoclonal antibodies or PD-1 monoclonal antibodies.

3) Patients with other malignant tumors.

4) Patients with a known or suspected autoimmune disease, including dementia and seizures.

5) Co-occuring serious mental illness.

6) Patients with nasopharyngeal necrosis, radiation-induced brain injury,
severe neck fibrosis, etc., who are not suitable for radiotherapy assessed by the PI.

7) Severe heart disease, lung dysfunction, heart function, or lung function lower than grade 3 (including grade 3).

8) The laboratory test values within 7 days before enrollment do not meet relevant standards.

9) Received systemic or local glucocorticoid therapy within 4 weeks before enrollment.

10) Complications requiring long-term use of immunosuppressive drugs.

11) Patients with active tuberculosis (TB) who are receiving anti-TB treatment or who have received anti-TB treatment within 1 year prior to screening.

12) Patients who use traditional antitumor herbs within 4 weeks before enrollment.

13) Prior use of anti-PD-1/PD-L1 antibodies or anti-CTLA-4 antibodies (or any other antibodies acting on T-cell co-stimulation or checkpoint pathways) and anti-angiogenic agents.

14) Subjects with any active autoimmune disease or a history of autoimmune disease (including interstitial pneumonia, uveitis, enteritis, hepatitis, hypophysitis, nephritis, hyperthyroidism, and hypothyroidism; patients with vitiligo and patients who achieved complete remission of asthma in childhood and needed no intervention after adulthood were enrolled, but patients with asthma requiring medical intervention with bronchodilators were not included).

15) HIV positivity.

16) HBsAg positivity and HBV DNA copy number positivity (quantitative detection ≥ 1000 cps/ml); chronic hepatitis C with blood screening positivity (HCV antibody positive).

17) The receipt of any anti-infection vaccines (such as influenza vaccine, varicella vaccine, etc.) within 4 weeks before enrollment.
18) Pregnant women of childbearing age and lactating women.

6. Protocol, Course of Treatment and Dose

6.1 Treatment plan

Total radiotherapy dose: PTVnx 60 Gy/27 F, PTVnd 60 Gy/27 F, PTV1 54 Gy/27 F, once a day, 5 days a week.

Toripalimab administration with the first day of radiotherapy: 240 mg, intravenous injection for more than 60 minutes, Q3W, lasting until 3 months after the end of radiotherapy (maximum of 7 cycles).

Criteria of Treatment Termination
1. Intolerable toxicity;
2. Occurrence of disease progression (according to RECIST criteria);
3. Any concomitant treatments during the trial have a significant impact on the safety and efficacy of the experimental drugs;
4. Pregnancy during the study period;
5. Other conditions for which continued administration is not considered appropriate by the investigator.

6.2 Criteria of Radiotherapy Suspension
1) Hematologic examination of the patients revealing leukocytes < 2×10^9/L or absolute values of neutrophils < 1.0×10^9/L and platelets < 50×10^9/L.

2) Severe gastrointestinal reactions caused by radiotherapy, such as anorexia, nausea and vomiting, that do not improve after general clinical treatment.

3) Increase in body temperature to 38.5°C.

4) Severe acute radiotherapy reactions such as severe throat erosion or oral ulcers.

6.3 Criteria for withdrawal from the test
5) Severe treatment complications:
   A) Grade IV neutropenia
   B) Grade IV acute mucositis
6) The desire to withdraw from the study at any time
7) Disease progression during treatment
8) Other diseases that occur during treatment that significantly affect the general condition of the patient and lead to the treatment to be stopped.

### 6.4 Design and dose of radiotherapy plan:

Table 1. IMRT and important organ delineation principles for nasopharyngeal carcinoma.

<table>
<thead>
<tr>
<th>Name</th>
<th>Definition</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>GTVnx</td>
<td>All gross nasopharyngeal lesions confirmed by clinical and imaging examinations.</td>
<td>Primary nasopharyngeal lesion</td>
</tr>
<tr>
<td>GTVnd</td>
<td>Positive lymph nodes touchable or visible on imaging examinations (Imaging diagnostic criteria: ① Short diameter of the largest cross-section ≥1 cm; ② Necrotic foci in the center; ③ Extracapsular invasion; ④ Short diameter of clusters ≥0.8 cm; ⑤ PET-CT showing positive lymph nodes).</td>
<td>Metastatic cervical lymph nodes</td>
</tr>
<tr>
<td>CTV</td>
<td>Encompass the GTVnx with a radial margin of 0.5-1 cm; expansion distance can be determined according to the characteristics of the adjacent tissue structure.</td>
<td>High-risk micro-infiltration area</td>
</tr>
<tr>
<td>PTV</td>
<td>PTVnx, PTVnd, and PTV1 are the external expansion of GTVnx, GTVnd, and CTV by a certain distance, generally 0.3 cm in the forward, up, down, left, and right directions, and 0.1–0.3 cm in the backward direction.</td>
<td></td>
</tr>
</tbody>
</table>
### Table 2. Normal tissue dose constraints used for plan optimization

<table>
<thead>
<tr>
<th>Organ at risk</th>
<th>Dose constraints</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain stem, temporal lobe, lens, eyeball, optic nerve, optic chiasm, pituitary gland, parotid gland, temporomandibular joint, mandible, larynx, oral cavity, submandibular gland, inner ear, middle ear.</td>
<td>Items can be increased or decreased as appropriate according to the tumor situation</td>
</tr>
</tbody>
</table>

- **Brain stem** \( D1^* \leq 64 \text{ Gy} \)
- **Spinal cord** \( D1^* \leq 50 \text{ Gy} \)
- **Optic nerves** \( D1^* \leq 60 \text{ Gy} \)
- **Optic chiasm** \( D1^* \leq 60 \text{ Gy} \)
- **Temporal lobe** \( D1^* \leq 64 \text{ Gy} \)
- **Inner ear** \( D_{\text{mean}^*} \leq 50 \text{ Gy} \)
- **Pituitary** \( D1^* \leq 60 \text{ Gy} \)
- **Mandible** \( D1^* \leq 70 \text{ Gy} \)
- **Temporomandibular Joint** \( D1^* \leq 70 \text{ Gy} \)
- **Parotid** \( D_{\text{mean}} \leq 26 \text{ Gy} \)
- **Larynx** \( D_{\text{mean}} \leq 45 \text{ Gy} \)
- **Oral cavity** \( D_{\text{mean}} \leq 45 \text{ Gy} \)

The following table describes the normal tissue dose constraints for reirradiation to the patients whose tumor recurrence occurs more than 3 years after initial radiotherapy; if the recurrence occurred within 3 years after initial radiotherapy, the normal tissue dose constraints for reirradiation should be 2/3 of the following table describes.

- **PRV** = planning organ at risk volume.
- * Dose received by 1% of the target volume
- ^ Dose received by 1 cc of the target volume

### 7. Additional treatment

Patients with tumor progression after treatment may receive salvage treatment, including salvage surgery and chemotherapy as appropriate, but no
third course of radiotherapy.

8. Observation and evaluation of the trial

8.1 Initial Screening Period

As all patients are under standardized management for NPC, they need to undergo a series of examinations as well as provide relevant information to confirm their pathologic diagnosis and clinical stage before being admitted to the trial, including the following:

1) Medical history review
2) Personal data collection
3) Review of present medications and treatment
4) Body examinations, including height, weight, and vital signs
5) Physical examination of the head and neck region, including the nasopharyngeal and cervical LNs
6) Physical examination of the nervous system
7) Nasal endoscopy and lesion biopsy
8) Biopsy
9) Routine blood panel
10) Urine routine
11) Blood biochemistry
12) Thyroid function test
13) Myocardial enzyme assay
14) Adrenal gland and pituitary hormone test
15) Imaging test of the tumor (enhanced MR or enhanced CT of the head and neck (CT was indicated only in patients with contraindications to MRI))
16) PET/CT is compulsorily required during the initial screening period*

*Patients who underwent PET/CT examinations do not need chest X-rays, abdominal ultrasonography, or ECT bone scans.

8.2 During Treatment

The following aspects need to be assessed from the start to the end of
treatment.

a. MRI and/or CT of the primary tumor, which will be performed after treatment, and CR, PR, SD, or PD will be evaluated according to the RECIST version 1.1 criteria. Chest films and abdominal ultrasonography will be reexamined after treatment. PET-CT and ECT bone scans will be performed as clinically indicated. We define complete response (CR) as a complete lack of unequivocal soft tissue mass in the local region and cervical lymph nodes that all had a short axis of less than 10 mm according to the RECIST guidelines. Imaging results to assess the ORR and progression-free survival are centrally reviewed.

b. General conditions
c. Acute and late toxicity assessment (NCI-CTC, version 5.0), including hematological toxicity, gastrointestinal reactions, hepatotoxicity, nephrotoxicity, mucositis, neurotoxicity, ototoxicity, thyroid function, myocardial enzymes, adrenal glands and pituitary hormones.
d. Peripheral neuropathy
e. Laboratory tests: Routine blood tests and blood biochemistry are required within 1 week prior to each cycle of immunotherapy and once per week during treatment. Thyroid function, myocardial enzymes, the adrenal gland and pituitary hormones are required once per 2 cycles of immunotherapy.

8.3 Follow-Up and Recording of Events

After completing radiotherapy, the patients are followed up every 3 months until disease progression or death to evaluate the patients’ recent and long-term efficacy and safety profiles.

Follow-up method: Record of the patient's examination data, a doctor's letter with signature to document the visit, or a doctor's follow-up records collected by telephone.

Follow-up content: Routine examination of the nasopharyngeal lesions and LNs every 3 months and abdominal B-mode ultrasound and chest X-ray.
every 6 months. PET/CT or bone scintigraphy are performed when clinically indicated. The treatment responses were also evaluated according to the RECIST criteria. The earliest date of detecting symptomatic late toxicities and the eventual maximum grade according to the Late Radiation Morbidity Scoring Criteria of the Radiation Therapy Oncology Group (RTOG) and European Organization for Research and Treatment of Cancer (EORTC) are recorded.

9 Risks Associated with Toripalimab

The PD-L1/PD-1 pathway is involved in peripheral immune tolerance; therefore, such therapies may increase the risk of immune-mediated adverse events, particularly the induction or exacerbation of autoimmune diseases. Potential immune-mediated adverse events, including interstitial lung disease, hypothyroidism and hyperthyroidism, liver dysfunction, pancreatitis, hyperglycemia and adrenal insufficiency, have been observed in clinical studies of the safety and efficacy of toripalimab injection (JS001) in the treatment of solid tumors. For more information on clinical safety, see the Tripletrumab Injection (JS001) Investigator's Manual.

10 Overall plan for managing security issues

10.1 Monitoring

In this study, safety will be assessed by monitoring all serious and nonserious adverse events (adverse events will be defined and graded according to NCI CTCAE version 5.0 criteria). Laboratory values must be reviewed before each infusion.

General safety assessments include serial interval histories, physical examinations, and specific laboratory studies, including serum chemistries and blood cell counts.

Patients will be closely monitored for signs and symptoms of autoimmune diseases and infections during the study.

After the final administration of the study drug, patients will be followed for
a safety period of 60 days.

After completion of the study or withdrawal from the study, patients who still have an adverse event associated with the study treatment will be followed until the event has resolved to baseline grade, the event is assessed by the investigator as stable, new anticancer therapy is initiated, the patient is lost to follow-up, or the patient withdraws consent, or it has been determined that study treatment or participation in the study does not result in an adverse event.

**10.2 Management** of toxicity associated with or possibly related to toripalimab should be in accordance with standard medical practice. Other tests, such as autoimmune serologic tests or tissue biopsies, should be used to determine the possible immunogenic cause.

Although most immune-mediated adverse events observed after immunomodulator use are mild and self-limiting, they should be detected early and treated promptly to avoid potentially serious complications. Discontinuation of toripalimab may not produce a direct therapeutic effect, and in severe cases, immune-mediated toxicities may require urgent treatment with topical corticosteroids, systemic corticosteroids, mycophenolate mofetil, or TNF-α inhibitors.

Prior to subsequent administration of toripalimab, researchers should consider the benefit-risk balance for each patient. Toripalimab should be discontinued permanently in patients with life-threatening immune-mediated adverse events.

The most common irAEs included fatigue, pruritus, diarrhea, decreased appetite, rash, pyrexia, cough, dyspnea, musculoskeletal pain, constipation, and nausea.

Checkpoint inhibitors should be discontinued in patients with grade 2 (moderate) immune-mediated toxicities and should not be restarted until symptoms or toxicities resolve to grade 1 or less. If symptoms do not resolve within one week, treatment with corticosteroids (prednisone ≤ 10 mg/day or
Patients who develop grade 3 or 4 (severe or life-threatening) immune-mediated toxicities should be permanently discontinued from checkpoint inhibitor therapy. Large doses of corticosteroids (prednisone 1-2 mg/kg/day or equivalent) should be administered. When symptoms resolve to grade 1 or less, the steroid dose may be tapered over at least 1 month.

Infliximab (5 mg/kg) may be administered if symptoms do not improve significantly after 3 days of intravenous steroid administration.

11. Security Measures and Quality Control

1) Provide a systemic learning program for every member in the research group.

2) Make a monitoring plan for adverse effects and an emergency plan.

3) Research plan is approved by the ethics committee.

4) Develop various standard operation procedures related to this study

5) Establish a standardized evaluation system to unify the diagnostic criteria, curative effect judging criteria, etc.

6) Establish professional statistical plans

7) Arrange a quality controller to create a quality control plan and regularly check on the study

8) Set up a coordination committee, curative effect judging group and follow-up team

11. Research endpoint

11.1 Primary endpoint: overall response rate (ORR)

The objective response rate (ORR) is defined as the proportion of patients whose tumor shrinks to achieve a complete or partial response and remains for a certain time. Clinical and imaging examinations are performed to observe and record the regression of nasopharyngeal and neck lesions. The evaluation indicators were complete response (CR), partial response (PR), stable disease
(SD), or progressive disease (PD), and the tumor response rate is calculated. We defined a complete response (CR) as a complete lack of unequivocal soft tissue mass in the local region and cervical lymph nodes that all had a short axis of less than 10 mm according to the RECIST guidelines.

11.2 Secondary Study Endpoints

1) Progression-free survival (PFS) is defined as the time from enrollment to the date of tumor progression or death for any reason or the last follow-up if there is no tumor progression.

2) Overall survival (OS) is defined as the time from enrollment to death from any cause or the last follow-up if there is no death.

3) Safety indicators are evaluated by NCI-CTC5.0 standards. Acute toxicity is defined as hematological toxicity, mucositis, allergic reactions, neurotoxicity, gastrointestinal reactions, or other adverse events and serious adverse events.

11.3 Data management

All data from the registered patients meeting the enrollment criteria are sent to the center for management. All databases are managed by specially assigned persons. The data platform will remain available for double input and verification.

11.4 Case report form

A case report form should be designed before the start of the study. The form should be able to record the disease and treatment follow-up comprehensively to facilitate filling and entry into the computer database.

11.5 Statistical analysis

All patients enrolled are included in the efficacy and safety analyses. For all patients, the median follow-up time is calculated using the reverse Kaplan-Meier method. The ORR and 95% CIs are calculated using the Clopper-Pearson method. The duration of response, PFS, and OS are analyzed using the Kaplan-Meier method.
The associations between PD-L1 expression, genome/clinical characteristics and objective response, complete response and PFS are assessed via post hoc analyses. For the comparison between subgroups, χ² test or Fisher’s exact test and Wilcoxon rank sum test are used to determine complete response. Univariate analysis of the effects of these parameters on PFS is conducted by the Cox proportional hazards model to calculate the hazard ratios and 95% confidence intervals.
12. SCHEMATIC

- rT0-4N1-3M0 or rT2-4N0M0, Phase II-IVa (AJCC eighth edition)
- Registration, screening, and signing of informed consent
- Toripalimab combined with IMRT
- Follow-up, short-term efficacy and acute toxicity evaluation
- Analyze, summarize, publish summary reports and papers
- Evaluate the effectiveness and safety of IMRT combined with immunotherapy for unresectable locally recurrent nasopharyngeal carcinoma and analyze the prognostic biomarker of IMRT plus PD-1 therapy in rNPC.
Supplementary Appendix
Intensity-modulated radiation therapy (IMRT) planning protocol in this trial

All patients were immobilized in the supine position using a thermoplastic mask that covered the head, neck, and shoulder regions. Both nonenhanced CT (for dose calculation) and contrast-enhanced CT (for target delineation) images were obtained from the vertex to 2 cm below the sternoclavicular joint at a 3 mm slice thickness. The gross tumor volume (GTV), clinical target volume (CTV), cervical lymph node tumor volume (GTVnd) and organs at risk (OAR) were contoured slice by slice on computed tomography images. The GTV encompassed the extent of the tumors defined by the computed tomography/MRI imaging studies. Only one CTV was delineated, which included the GTV plus a 0.5-1.0 cm margin. The planning target volume (PTV) was delineated, including PTVnx, PTVnd, and PTV1, which were the external expansions of GTVnx, GTVnd, and CTV by a certain distance, generally 0.3 cm in the forward, up, down, left, and right directions and 0.1~0.3 cm in the backward direction. The OARs surrounding the CTV included the brainstem, spinal cord, optic nerves, optic chiasm, pituitary gland, lens, temporal lobes, parotid glands, temporomandibular joints, mandible, etc.

The prescribed doses were 60 Gy, 60 Gy and 54 Gy in 27 fractions for the PTVs derived from GTVnx, GTVnd and CTV, respectively. The threshold of doses to the OARs for reirradiation of recurrent NPC is listed in the Protocol1.

Genomic DNA preparation and next-generation sequencing

Genomic DNA from frozen tissues and FFPE samples was extracted using the
DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA) and QIAamp DNA FFPE Tissue Kit (Qiagen, Germany), respectively, following the manufacturer’s protocol. Degradation and contamination were monitored on a 1% agarose gel, and the concentration was measured by using the Qubit® DNA Assay Kit in a Qubit® 2.0 fluorometer (Life Technologies, CA, USA).

For whole-exome sequencing (WES), qualified genomic DNA from tumors and matched peripheral blood from 18 rNPC patients was fragmented by Covaris with resultant library fragments of 180-280 bp, and then adapters were ligated to both ends of the fragments. Extracted DNA was then amplified by ligation-mediated PCR (LM-PCR), purified, and hybridized to the Agilent SureSelect Human Exome V6 for enrichment; nonhybridized fragments were subsequently washed out. Both uncaptured and captured LM-PCR products were subjected to real-time PCR to estimate the magnitude of enrichment. Each captured library was then pooled and sequenced on a Illumina HiSeq X platform with 150-bp paired-end reads.

**Sequence data quality control**

The original fluorescence image files obtained from the HiSeq platform were transformed to short reads (raw data) by base calling and recorded in FASTQ format, which contained sequence information and corresponding sequencing quality information. After excluding reads containing adapter contamination and low-quality/unrecognizable nucleotides, clean data were used for downstream bioinformatics analyses. At the same time, the number of total reads, sequencing
error rate, percentage of reads with average quality >20 and with average quality >30, and GC content distribution were calculated.

**Read mapping and somatic alteration detection**

Valid sequencing data were mapped to the human reference genome (UCSC hg38) by Burrows-Wheeler Aligner (BWA) software to obtain the original mapping results stored in BAM format. Then, Samblaster and Sambamba were used to sort BAM files and perform duplicate marking to generate a final BAM file for computing the sequence coverage and depth.

To call somatic single nucleotide variations (SNVs) and small insertions and deletions (InDels) from paired tumor-normal samples, MuTect and Strelka were used, respectively. Subsequently, the VCF (variant call format) file was annotated by ANNOVAR.

**Copy number burden (chromosomal instability)**

Chromosomal instability was estimated by the Weighted Genome Integrity Index (wGII) score, which was computed as follows: the ploidy of the sample was first determined as the weighted median integer copy number, with weights equal to the lengths of the copy number segments. For each of the 22 autosomal chromosomes, the percentage of gained and lost genomic material was calculated relative to the ploidy of the sample. The wGII score of a sample was defined as the average of this percentage value over the 22 autosomal chromosomes.
**Neoantigen prediction**

To identify neoantigens, we used the NetMHC, NetMHCpan to predict neoepitopes from 8mer to 11mer. Neoepitopes with a binding affinity < 500 nM and less than wild-peptide were predicted as neoantigens.

**MSI calling**

MSIsensor was used to estimate the mircosatellite instability (MSI) status for each patient. For each sample, we note the total number of sites with sufficient data (at least 20 spanning reads in both normal and tumor) and the number of somatic sites. The percentage of somatic sites is the MSI score.

**References**


Table S1. Treatment exposure to toripalimab combined with intensity-modulated radiotherapy.

<table>
<thead>
<tr>
<th></th>
<th>Number (%) or time (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Toripalimab</strong></td>
<td></td>
</tr>
<tr>
<td>7 cycles</td>
<td>22 (88.0%)</td>
</tr>
<tr>
<td>6 cycles</td>
<td>2 (8.0%)</td>
</tr>
<tr>
<td>5 cycles</td>
<td>1 (4.0%)</td>
</tr>
<tr>
<td><strong>Patients who received IMRT, no. (%)</strong></td>
<td>25 (100.0%)</td>
</tr>
<tr>
<td><strong>Patient who completed definitive IMRT, no. (%)</strong></td>
<td>25 (100.0%)</td>
</tr>
<tr>
<td><strong>Median (IQR) dose of IMRT (Gy)</strong></td>
<td>60.21 (60 - 60.21)</td>
</tr>
<tr>
<td><strong>Median (IQR) dose per fraction (Gy)</strong></td>
<td>2.23 (2.22-2.23)</td>
</tr>
<tr>
<td><strong>Median (IQR) duration of IMRT (days)</strong></td>
<td>36 (35-38)</td>
</tr>
</tbody>
</table>

Data are n(%) unless otherwise specified.
Table S2. Dosimetric data (average and range) of 25 patients who completed radiotherapy.

<table>
<thead>
<tr>
<th></th>
<th>rT2-3 (N = 18)</th>
<th>rT4 (N = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GTV Volume (cc)</td>
<td>35.0 (19.5 – 50.7)</td>
<td>52.0 (41.0 – 93.9)</td>
</tr>
<tr>
<td>GTV D_{95} (in BED)</td>
<td>75.8 (75.5 – 76.5)</td>
<td>75.4 (74.9 – 76.0)</td>
</tr>
<tr>
<td>GTV D_{50} (in BED)</td>
<td>77.9 (77.4 – 78.8)</td>
<td>77.8 (77.5 – 78.1)</td>
</tr>
<tr>
<td>GTV D_{min} (in BED)</td>
<td>73.8 (72.6 – 74.9)</td>
<td>73.0 (71.1 – 73.9)</td>
</tr>
<tr>
<td>GTV D_{max} (in BED)</td>
<td>80.3 (79.6 – 81.6)</td>
<td>81.5 (81.0 – 82.9)</td>
</tr>
</tbody>
</table>

Data are median (IQR) unless otherwise specified. BED = biologic effective dose (calculated at $\alpha/\beta = 10$ Gy; $BED = D \times (1 + d/\alpha/\beta)$), where $D =$ total dose and $d =$ fractional dose.
Table S3. Association among PD-L1 expression, clinical characteristics and treatment activity.

<table>
<thead>
<tr>
<th></th>
<th>No. of pts</th>
<th>Response</th>
<th></th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>CR</td>
<td>PR+SD</td>
<td></td>
</tr>
<tr>
<td>PD-L1 expression</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>15</td>
<td>13 (86.7%)</td>
<td>2 (13.3%)</td>
<td>0.178</td>
</tr>
<tr>
<td>Negative</td>
<td>4</td>
<td>2 (50.0%)</td>
<td>2 (50.0%)</td>
<td></td>
</tr>
<tr>
<td>T stage</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T4</td>
<td>7</td>
<td>4 (57.1%)</td>
<td>3 (42.9%)</td>
<td>0.298</td>
</tr>
<tr>
<td>T2/3</td>
<td>18</td>
<td>15 (83.3%)</td>
<td>3 (16.7%)</td>
<td></td>
</tr>
<tr>
<td>N stage</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N1</td>
<td>9</td>
<td>7 (77.8%)</td>
<td>2 (22.2%)</td>
<td>1.000</td>
</tr>
<tr>
<td>N0</td>
<td>16</td>
<td>12 (75.0%)</td>
<td>4 (25.0%)</td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>18</td>
<td>15 (83.3%)</td>
<td>3 (16.7%)</td>
<td>0.298</td>
</tr>
<tr>
<td>Female</td>
<td>7</td>
<td>4 (57.1%)</td>
<td>3 (42.9%)</td>
<td></td>
</tr>
<tr>
<td>Table S4. Hazard ratios for selected prognostic factors of progression-free survival in patients who completed radiotherapy.</td>
<td>Hazard ratio (95% CI)</td>
<td>P value</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>PD-L1 positive (Yes vs No)</td>
<td>0.43 (0.04 - 4.75)</td>
<td>0.488</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (per year increase)</td>
<td>1.02 (0.91 - 1.14)</td>
<td>0.780</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex (female vs male)</td>
<td>0.44 (0.06 - 3.14)</td>
<td>0.413</td>
<td></td>
<td></td>
</tr>
<tr>
<td>rT category (rT4 vs rT2-3)</td>
<td>3.07 (0.50 - 18.84)</td>
<td>0.226</td>
<td></td>
<td></td>
</tr>
<tr>
<td>rN category (rN1 vs rN0)</td>
<td>1.65 (0.25 - 10.75)</td>
<td>0.602</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EBV DNA copy number/mL (≥1500 vs &lt;1500)</td>
<td>0.81 (0.08 – 7.88)</td>
<td>0.859</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GTV (per cc increase)</td>
<td>1.04 (1.01 - 1.07)</td>
<td>0.021</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GTV $D_{50}$ (in BED) (per Gy increase)</td>
<td>0.40 (0.07 - 2.01)</td>
<td>0.275</td>
<td></td>
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</tr>
<tr>
<td>GTV $D_{50}$ (in BED) (per Gy increase)</td>
<td>0.47 (0.11 - 1.98)</td>
<td>0.306</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GTV $D_{\text{min}}$ (in BED) (per Gy increase)</td>
<td>0.94 (0.59 - 1.52)</td>
<td>0.809</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tumor mutation burden (≥30% percent vs &lt;30% percent)</td>
<td>2.91 (0.18 - 46.68)</td>
<td>0.451</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neoantigen burden (per burden increase)</td>
<td>0.70 (0.29 - 1.71)</td>
<td>0.436</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frameshift indels (per indel increase)</td>
<td>1.35 (0.73 - 2.50)</td>
<td>0.334</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Copy number burden (wGII) (≥30% percent vs &lt;30% percent)</td>
<td>2.78 (1.04 – 6.88)</td>
<td>0.037</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MSI score</td>
<td>0.64 (0.06 - 6.39)</td>
<td>0.702</td>
<td></td>
<td></td>
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<tr>
<td>(≥30% percent vs &lt;30% percent)</td>
<td></td>
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</tbody>
</table>

Abbreviations: PFS – progression-free survival, GTV – gross tumor volume, BED – biologic effective dose (calculated at $\alpha/\beta = 10$ Gy; $\text{BED} = D \left(1 + \frac{d}{(\alpha/\beta)}\right)$), where $D$ = total dose and $d$ = fractional dose.
Figure S1. Part of medical imaging evaluated as complete response.
Figure S2. Association between genome characteristics and treatment activity.

A

P = 0.574

B

P = 0.804

C

P = 0.056

D

P = 0.399

E

P = 0.029
Figure S3. Genetic alterations and frequencies identified by whole exome sequencing (WES) from 18 available patients. Patients were grouped by clinical responses and progression.
Figure S4. Association between clinical characteristics and treatment activity.

- **A**: Age
  - P = 0.924

- **B**: GTV volume
  - P = 0.176

- **C**: GTV D95 (BED)
  - P = 0.775

- **D**: GTV D50 (BED)
  - P = 0.824

- **E**: GTV D100 (BED)
  - P = 0.849

- **F**: GTV Dmax (BED)
  - P = 0.324