Neoadjuvant chemo-free combination of camrelizumab and apatinib for locally advanced resectable oral squamous cell carcinoma – A pilot study

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Abstract

Novel neoadjuvant therapy regimens are needed to improve the outcomes of patients with locally advanced resectable oral squamous cell carcinoma (OSCC). We conducted a prospective, open-label, single-arm trial (*n* = 21, NCT04393506) to determine the safety and feasibility of neoadjuvant camrelizumab (an anti-PD-1 antibody) plus apatinib (a VEGFR inhibitor) for locally advanced resectable OSCC. The primary endpoints were safety and major pathological response (MPR). Neoadjuvant camrelizumab plus apatinib was well-tolerated and the MPR rate was 40% (8/20), meeting the primary endpoint. All five patients with CPS 10 had MPR. Additionally, patients achieving MPR showed more CD4+ T cell infiltration and a higher CD8+/FoxP3+ ratio than those without MPR ($p < 0.05$), and decreased CD31 and -SMA expression were observed after neoadjuvant therapy. Our findings demonstrate that neoadjuvant therapy with a chemo-free combination of camrelizumab and apatinib is safe and yields a promising MPR rate, supporting further trials.

Background

For patients with locally advanced resectable oral squamous cell carcinoma (OSCC), surgery with adjuvant radiotherapy or chemoradiotherapy has been recommended as the standard treatment[1]. Even after intensive treatments, patients remain at high risk of recurrence or metastasis[2]. In recent years, neoadjuvant therapy before surgery has been shown to reduce the burden of locoregional disease, resulting in improved surgical outcomes; to reduce the risk of distant metastases; and to predict prognosis based on the pathological response in various solid tumors[3]. However, its role remains ambiguous in OSCC. Neoadjuvant chemotherapy using cisplatin plus fluorouracil (PF) or docetaxel plus cisplatin plus fluorouracil (TPF) regimen has been explored in patients with OSCC, but has not demonstrated survival benefits beyond standard treatment[4, 5]. Thus, exploring effective neoadjuvant therapeutic approaches for locally advanced resectable OSCC remains an urgent need.

Immune checkpoint blockade has been demonstrated to have clinically meaningful anti-tumor activity in recurrent/metastatic head and neck squamous cell carcinoma (HNSCC, including OSCC)[6, 7]. Preclinical data suggest that while the tumor is in place, neoadjuvant immunotherapy hypothetically leverages the higher levels of endogenous tumor antigens, thereby enhancing T cell priming and resulting in stronger effects than those of adjuvant therapy[8]. In the neoadjuvant setting, immune checkpoint blockade has shown promising results against many other tumor types[9–12]. Targeted drugs against vascular endothelial growth factor receptor (VEGFR) or angiogenesis have shown to relieve immunosuppression through blood vessel normalization and the oxygen metabolism pathway, thereby having a synergistic effect with anti-programmed cell death-1 (PD-1) immunotherapy and concurrently diminishing the risk of immune-related adverse effects[13–16]. The combination of camrelizumab (an anti-PD-1 antibody) and apatinib (a VEGFR inhibitor) has shown favorable anti-tumor activity and manageable safety in various types of advanced cancers[17–20].

In this study, we conducted a prospective, single-arm trial to assess the safety and pathological efficacy of a chemo-free combination of camrelizumab and apatinib as neoadjuvant therapy in patients with locally advanced resectable OSCC.

Results

Patient information

From April to December 2020, 21 patients were enrolled, and one patient withdrew at the beginning of treatment. The characteristics of the 21 enrolled patients are listed in **Table 1**. Twenty patients received radical surgery, and 18 patients received adjuvant radiotherapy (**Fig. 1**).

Safety

The most common neoadjuvant therapy-related AEs were hyperbilirubinemia (*N* = 8, 40%), thrombocytopenia (*N* = 7, 35%) and proteinuria (*N* = 6, 30%). No neoadjuvant therapy-related grade 3 or above AEs were detected (**Table 2, Supplementary Table S1 and S2 in Additional file 4**). One patient postponed the second cycle of camrelizumab for 14 days because of grade 2 thrombocytopenia, and one patient suspended apatinib for 21 days because of grade 2 hyperbilirubinemia. Surgery-related AEs occurred in four patients, including one each of subcutaneous exudate, post-tracheostomy bleeding, post-flap-reconstruction pharyngeal fistula, and wound infection. The post-tracheostomy bleeding was due to unsecured ligation of the anterior jugular vein, which was detected during the surgical exploration. The other three patients showed no AEs during preoperative laboratory tests, and their surgery-related AEs were all controlled within 2 weeks and were deemed to be unrelated to the neoadjuvant therapy. Two severe AEs occurred: one patient experienced unexplainable cardiac troponin I elevation, resulting in a surgery delay for 7 days, then recovering within 1 week without any corticosteroid treatment; the other patient experienced unexplained shock after radiotherapy and died.

Efficacy

The pathological efficacy indicated that eight (40%) patients achieved MPR including one patient (5%) who achieved pathological complete response (pCR). Among them, all five patients with CPS 10 and all four patients with CPS ≥ 20 achieved MPR (**Fig. 2A, Supplementary Table S3 in Additional file 4**). The number of patients with high CPS between the MPR and non-MPR groups showed significant differences ($P = 0.0005$, Pearson r = 0.7071 for cutoff 10; $P = 0.0041$, Pearson r = 0.6124 for cutoff ≥ 20). One patient with CPS = 90 who achieved radiographic PR was pathologically confirmed pCR (**Fig. 2B**).

Radiographic responses according to RECIST 1.1 were performed on basis of imaging examinations before and after neoadjuvant therapy. The radiographic response indicated three patients with partial response (PR), ten patients with stable disease (SD), and six patients with progressive disease (PD) (**Supplementary Table S4 in Additional file 4**). One superficial gingival lesion was undetectable on radiographic examinations and thus was not evaluated. Interestingly, among the eight patients who achieved MPR, only three showed radiographic PR (**Fig. 2A**). One radiographic PD lesion was further pathologically confirmed MPR. All patients with PD lesions received surgery, and no recurrence was found in primary sites.

For the regional metastatic lymph nodes, pathological response was found in 60% (6/10) of patients, with the characteristics of necrosis, multinucleated giant cells, and calcification. In the patient who achieved pCR in the primary tumor, pCR in one lymph node was also observed (**Supplementary Table S5 in Additional file 4**).

As of September 2021, the median follow-up time was 12 months (range 9–16 months). One patient who did not receive adjuvant radiotherapy had contralateral neck lymph node metastasis, and the 1-year locoregional recurrence rate was 5% (95% CI: 1.2%–31.7%). One patient died, and the 1-year overall survival rate was 95% (95% CI: 75.1%–99.9%).

Pathological response characteristics

We systematically reviewed the pathological features in resected specimens and proposed immune-related pathological response criteria (irPRC) for neoadjuvant therapy in OSCC. We observed the following characteristics of the immune-related pathological regression bed in OSCC: multinucleated giant cell infiltration, dystrophic calcification, tumor-infiltrating lymphocytes, foamy macrophages, neovascularization, proliferative fibrosis, tertiary lymphoid structure, and dense plasma cells (**Additional file 2**). In two patients who achieved MPR, the tertiary lymphoid structure was found in the tumors after neoadjuvant therapy (**Additional file 2**).

No significant change in the degree of TIL infiltration was found over the neoadjuvant therapy course (**Supplementary Fig. S1 in Additional file 4**). The characteristics of TIL infiltration in surgically resected tumors between two groups were further compared, and the MPR group showed more CD4+ T cell infiltration and a higher CD8+/FoxP3+ ratio than the non-MPR group (**Fig. 3**). One patient from the non-MPR group was found to have tumor progression on radiographic evaluation, with a change in growth kinetics exceeding 50% and new neck lymph node metastasis, and was confirmed to have disease hyperprogression (HPD, **Fig. 4A, B**). Interestingly, for this patient, high levels of infiltration for CD8+ and CD163+ cells were found in the biopsy, and CD8+ was diminished while CD163+ was significantly elevated in the surgical sample (**Fig. 4C, D, Supplementary Fig. S1 in Additional file 4**).

For angiogenesis evaluation, decreased CD31 (a marker of vascular endothelial cells) and -SMA (a marker of pericytes) expression were found after neoadjuvant therapy, thus confirming the anti-angiogenesis effect in tumors (**Fig. 5A and B**). No significant difference in CD31 or -SMA expression was found between the MPR and non-MPR groups (**Fig. 5C**).

Discussion

Our results provided the first evidence that neoadjuvant therapy using a chemo-free combination of camrelizumab and apatinib was well tolerated in patients with OSCC, with an MPR of 40%.

The safety profile of camrelizumab and apatinib in the neoadjuvant setting was mostly consistent with the previously reported in advanced cancers[17, 18, 21], We observed no grade 3–4 neoadjuvant therapy-related AEs, which might be due to a short course of administration of camrelizumab and apatinib. Furthermore, the safety in our trial seems to be superior to that of other neoadjuvant chemotherapy regimens in OSCC trials, such as neoadjuvant chemotherapy with TPF regimen (9–38% grade 3–4 therapy-related AEs)[4, 22, 23], or targeted therapies (25–61.6% grade 3–4 therapy-related AEs)[24, 25]. Recent neoadjuvant immunotherapy studies in head and neck cancer showing favorable side effects further supported the superior safety of neoadjuvant immunotherapy[26–28]. Given that previous studies reported anti-VEGF(R) therapies could increase the risk of bleeding[29, 30], we set a time interval of 5 days between apatinib administration and surgery in order to reduce the risk of complications in subsequent surgery. Although surgery-related AEs observed in our study were considered unrelated to neoadjuvant therapy, trials with larger sample sizes are necessary to definitively indicate the effects of neoadjuvant anti-PD-1 plus anti-VEGFR therapy on surgery.

In addition to safety, the pathological efficacy including MPR is a crucial criterion for proposing neoadjuvant therapy in OSCC or HNSCC. The combination of camrelizumab and apatinib showed a promising MPR (40%), as compared with chemotherapy with PF (33%) or TPF (27.7%) in OSCC[4, 23], pembrolizumab monotherapy (5.6%) and nivolumab monotherapy (5.9%) in HPV-unrelated HNSCC[27, 31], and nivolumab monotherapy (8%) or nivolumab combined with ipilimumab (20%) in OSCC, although cross-study comparisons should be made with caution.[28] In another neoadjuvant therapy trial in HNSCC, the cisplatin/docetaxel/durvalumab/tremelimumab combination in a neoadjuvant setting showed a superior pathological response in terms of pCR (48%) but also had a higher rate of grade 3–4 AEs (68%)[32]. One trial using immunoradiotherapy in a neoadjuvant setting achieved a high MPR rate (86%) in HNSCC, thus supporting further evaluation of the addition of stereotactic body radiation therapy to neoadjuvant immunotherapy[33]. Results from this study suggested that CPS > 10 might have 100% sensitivity in detecting MPR from neoadjuvant therapy. Owing to the limited sample size, this predictive value of CPS for anti-PD-1 plus anti-VEGFR therapy must be validated in a larger study. The concept of using CPS to guide the choice of neoadjuvant immunotherapy was consistent with principles suggested for recurrent/metastatic HNSCC[34].

In all these reported neoadjuvant immunotherapy trials in OSCC or HNSCC, the pathological response varied in terms of MPR ratios, and the assessment procedures also varied. Unlike non-small-cell lung carcinoma and melanoma[35, 36], in OSCC or HNSCC, irPRC has not been well defined. In previous neoadjuvant immunotherapy trials, the pathological response has been described as “visible regressed tumor, inflammation, giant cell reaction and acellular keratin” and quantified as “a percentage of the overall tumor bed (area of pathological response/area of pathological response plus viable tumor)”[27, 28]. Regardless of the assessment method being used, the definition of the tumor regression bed after neoadjuvant therapy is key, especially in significantly shrunk tumors. On basis of the criteria of determining the range of the immunotherapy-induced tumor regression bed proposed in lung cancer[36]. We systematically evaluated the features in tumors from different oral cavity sites in this trial, thus providing a reference of irPRC for following neoadjuvant immunotherapy in OSCC.

The irPRC we proposed for OSCC did not include the pathological response characteristics for neck lymph node metastasis. We found only one lymph node with confirmed tumor regression, whereas a different reaction occurred in another lymph node from the same patient. As described in a previous lung cancer study, the limitations in nodal disease assessment have been attributed to sampling issues.[36] In agreement with findings from the study using nivolumab, a similar response has been found between primary sites and lymph nodes[37]. Nodal upstaging occurred in four patients in our study, and all the corresponding primary tumors did not achieve MPR; therefore, lymph nodes are suggested to be monitored more frequently during neoadjuvant treatment.

Because of the short period of 2 weeks between the last neoadjuvant immunotherapy and surgery, radiographic re-evaluation based on modified RECIST1.1 criteria for immune based therapeutics (iRECIST) could not be performed in this study[38]. In pathological re-evaluation of the resected lesions, the RECIST 1.1 criteria did not show sufficient sensitivity in response assessment in our trial. Among the eight patients who achieved MPR, only three showed PR on radiographic scans. One radiographic PD lesion in our trial was further pathologically confirmed MPR, thus indicating the importance of re-evaluation on progression assessment after immunotherapy. This finding was consistent with the radiographic response analysis of neoadjuvant immunotherapy in lung cancer, in which 30% of patients with SD virtually achieved pCR[39]. In future neoadjuvant therapy trials, other modified methods for radiographic response evaluation should be proposed, such as the criteria used in the window of opportunity, in which a size reduction 10%, rather than 30%, might be defined as indicating a “radiographic responder”[37].

In agreement with findings from other neoadjuvant immunotherapy trials in OSCC or HNSCC[27, 28], the degree and the features of baseline TIL infiltration did not predict pathological response in our study. However, we observed more CD4+ T cell infiltration and a higher CD8+/FoxP3+ ratio in resected tumors that achieved MPR. In addition, an abnormal increase of CD163+ infiltration was observed in HPD tumors. Because higher levels of CD4+ and CD8+/FoxP3+ ratios were associated with better outcomes of neoadjuvant therapy, and M2 macrophages in the tumor microenvironment have been reported to be

associated with the occurrence of HPD[40–42], further analyses of our tumor samples are urgently needed to explore the underlying mechanisms. Angiogenesis markers for apatinib treatment, including CD31 and -SMA, were inhibited by neoadjuvant therapy in this trial, similarly to previously reported preclinical results[43, 44]. The expression of angiogenesis markers showed no differences between the MPR and non-MPR groups, whereas patients with high CPS tended to have a higher rate of MPR. Therefore, in this trial, we speculated that the pathologic response might be mainly due to the anti-PD-1 effect rather than anti-angiogenesis. However, crosstalk between tumor vascular normalization and immune reprogramming exists and plays a vital role in pathologic response[45]. A deeper understanding of this synergic effect could provide novel strategies to predict responses and to further improve the efficacy of cancer immunotherapy.

In the past decade, MPR and pCR have been considered a candidate early surrogate endpoint for survival in the neoadjuvant setting[35, 46, 47]; however, whether MPR or even pCR might result in long-term survival improvement remains to be confirmed in this and other neoadjuvant immunotherapy trials. For patients with OSCC who achieved MPR or even pCR through neoadjuvant therapy, whether the intensity of surgery and the following adjuvant therapy could be reduced remains controversial. The pathological response characteristics in lymph nodes, as well as their potential correlation with the efficacy of immunotherapy, require further investigations. To investigate the predictive biomarkers for refined neoadjuvant therapy, further in-depth biomarkers in the host, tumor, and blood (such as PET-CT parameters, tumor mutation burden, neoantigens, circulating tumor DNA, and T cell activation and exhaustion) should be evaluated in this and other ongoing neoadjuvant trials[48].

In conclusion, this pilot trial showed that neoadjuvant therapy using a chemo-free combination of camrelizumab and apatinib was well tolerated in patients with OSCC. The MPR rate was promising and CPS might be a signal predictor. These results support further neoadjuvant therapy trials for OSCC using anti-PD-1 plus anti-VEGFR, guided by the CPS.

Methods

Study population and trial design

This single-center, open-label trial was performed at the Ninth People's Hospital, Shanghai Jiao Tong University School of Medicine in Shanghai, China. Eligible patients were aged 18 to 75 years; had histopathological confirmed locally advanced OSCC with a clinical stage of III or Iva (American Joint Committee on Cancer, 8th Edition); and had an Eastern Cooperative Oncology Group performance status (ECOG PS) of 0 to 2. The full eligibility criteria are provided in the trial protocol (**Additional file 1**). The trial followed the ethical guidelines of the Declaration of Helsinki and was approved by the Institutional Ethics Committee, Ninth People's Hospital, Shanghai Jiao Tong University School of Medicine. Each patient provided signed informed consent before participating in this trial. The clinical trial registration number is NCT04393506.

Procedures

The patients received three cycles of intravenous camrelizumab (200 mg) on d1, d15, and d29; and oral apatinib (250 mg) daily, starting on d1 and ending on the 5th day before surgery. Standard radical surgery was planned on d42–45. Adjuvant radiotherapy or chemoradiotherapy was planned within 6 weeks after surgery, according to the pathological stage.

The standard operation procedure of determining the regression bed induced by neoadjuvant immunotherapy in oral cancer was proposed (**Additional file 2**). The percentage of residual viable tumor (RVT) cells was evaluated on resected tumor slides. Radiographic response evaluation was performed according to Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1 criteria.

Study endpoints

The primary endpoints were safety and major pathological response (MPR) rate (MPR, defined as the presence of 10% or fewer RVT cells). The 2-year survival rate (defined as the proportion of patients alive at the 2-year follow-up) and local recurrence rate (defined as the proportion of patients with local recurrence) were the secondary endpoints. Adverse events (AEs) were assessed according to the Common Terminology Criteria for Adverse Events (CTCAE, version 5.0). Neoadjuvant therapy-related AEs were managed mainly according to the American Society of Clinical Oncology Clinical Practice Guidelines[49]. Surgery-related AEs were assessed with the Clavien-Dindo Classification of Surgical Complications[50].

Immunohistochemistry and multiplex immunofluorescence (MIF)

Programmed cell death-ligand 1 (PD-L1) expression was evaluated with a PD-L1 IHC 22C3 pharmDx assay (Dako North America, Carpinteria CA). The combined positive score (CPS) was defined as the total number of PD-L1 staining cells (including tumor cells, tumor-associated lymphocytes, and macrophages) divided by the total viable tumor cells plus 100. MIF was performed for the evaluation of tumor-infiltrating lymphocytes (TILs) and angiogenesis markers in the tumor microenvironment. Detailed information on the MIF procedures is provided in **Additional file 3**.

Statistical analyses

A sample size of 20 evaluable patients was required to achieve 90% power to detect an increase in the MPR rate from 7% (anti-PD-1 monotherapy) to 30%, with a one-sided exact test with a significance level (alpha) of 0.0500.

Overall survival from the date of enrollment until death was evaluated with the Kaplan-Meier method. Based on the different CPS cutoffs, the *P* value of patient number between MPR and non-MPR groups was calculated with the chi-square test. The confidence interval (CI) of the survival rate and local recurrence rate were calculated with the Clopper-Pearson method, and the *P* values of quantified

fluorescence differences between two groups was calculated with Student's *t*-test. The significance level for two-sided *P* values was set at 0.05 in statistical analyses. Statistical analysis was performed in IBM SPSS Statistics and GraphPad Prism software.

Ethics approval

The trial followed the ethical guidelines of the Declaration of Helsinki and was approved by the Institutional Ethics Committee, Ninth People's Hospital, Shanghai Jiao Tong University School of Medicine. Each patient provided signed informed consent before participating in this trial.

Data availability

The trial protocol is available as Additional file 1 in the Supplementary Information file. Any additional datasets used and/or analysed during the current trial are available from the corresponding author on reasonable request.

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Declarations

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Author contributions

LPZ was chief investigator of the trial. LPZ, QZ, JL contributed to conception and design of the trial. WTJ, RHX, DWZ, JL, LZW, SJD, MJD, QS, TCZ, ZHZ, SYL, YYH, YT, QZ contributed to patient enrolment and care, acquisition of data. WTJ, RHX, DWZ contributed to data analysis, data interpretation, drafting of the manuscript. WTJ, RHX, SCW contributed to statistical analysis. LPZ, DWZ, QZ. WTJ, RHX, DWZ contributed equally to this paper. All authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

Tables

Table 1. Baseline Characteristics

Characteristics	*N* (%)
Age, median (range), years	56.4 (30-71)
Gender	
Male	12 (57.1)
Female	9 (42.9)
Smoker	
No	9 (42.9)
Yes	12 (57.1)
ECOG PS	
0	5 (23.8)
1	16 (76.2)
Primary tumor site	
Tongue	6 (28.6)
Buccal	3 (14.3)
Gingiva	5 (23.8)
Floor of mouth	5 (23.8)
Palate	2 (9.5)
Pretreatment clinical T-stage [a]	
T3	19 (90.4)
T4a	2 (9.5
Pretreatment clinical N-stage [a]	
N0	16 (66.7)
N1	3 (14.3)
N2	2 (9.5)

Pretreatment clinical stage [a]	
III	17 (80.9)
IVA	4 (19)
Combined positive score	
≥ 1	14 (66.7)
10	5 (23.8)
≥ 20	4 (19)

[a] American Joint Committee on Cancer, 8th Edition staging

Table 2. Neoadjuvant Therapy-Related Adverse Events (Common Terminology Criteria for Adverse Events Version 5.0), and Surgical-Related Adverse Events (Clavien-Dindo) in the 20 Patients

Adverse event	*N* (%)		
	Grade 1	Grade 2	Grade ≥3
Skin (rash, dryness, dermatitis)	0	1 (5%)	0
Pain (lymph node and oral)	3 (15%)	1 (5%)	0
Colitis/Diarrhea	1 (5%)	1 (5%)	0
Fatigue	3 (15%)	0	0
Proteinuria	3 (15%)	3 (15%)	0
Hypertension	1 (5%)	3 (15%)	0
Hyperbilirubinemia	7 (35%)	1 (5%)	0
Thrombocytopenia	6 (30%)	1 (5%)	0
Leukopenia	2 (10%)	1 (5%)	0
Increased AST level	3 (15%)	0	0
Reactive capillary hemangiomas	3 (15%)	0	0
Surgical toxic effects–Clavien-Dindo scoring	4 (20%)	0	0

Abbreviations: AST, aspartate aminotransferase.

Figures

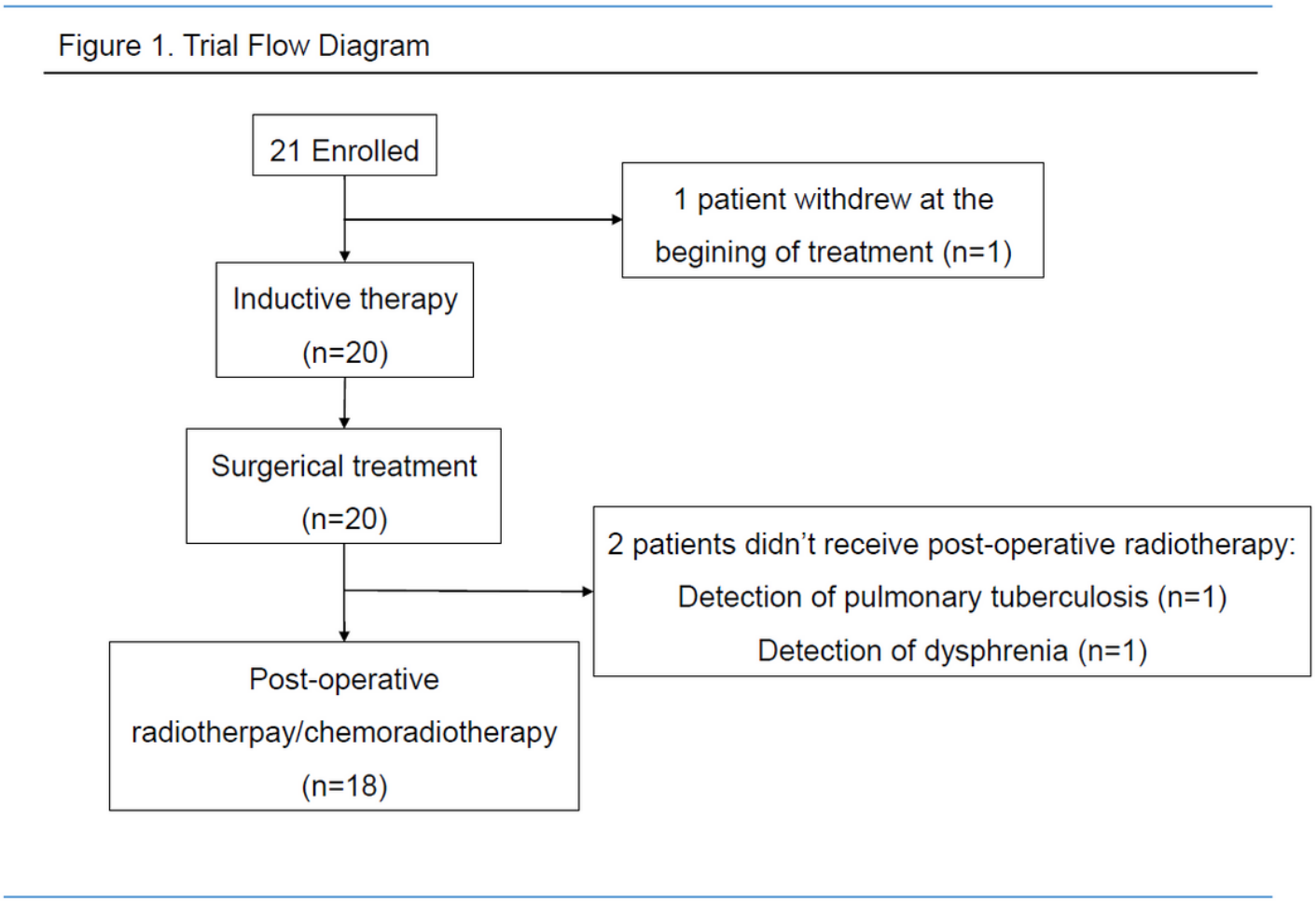

Figure 1

Trial flowchart.

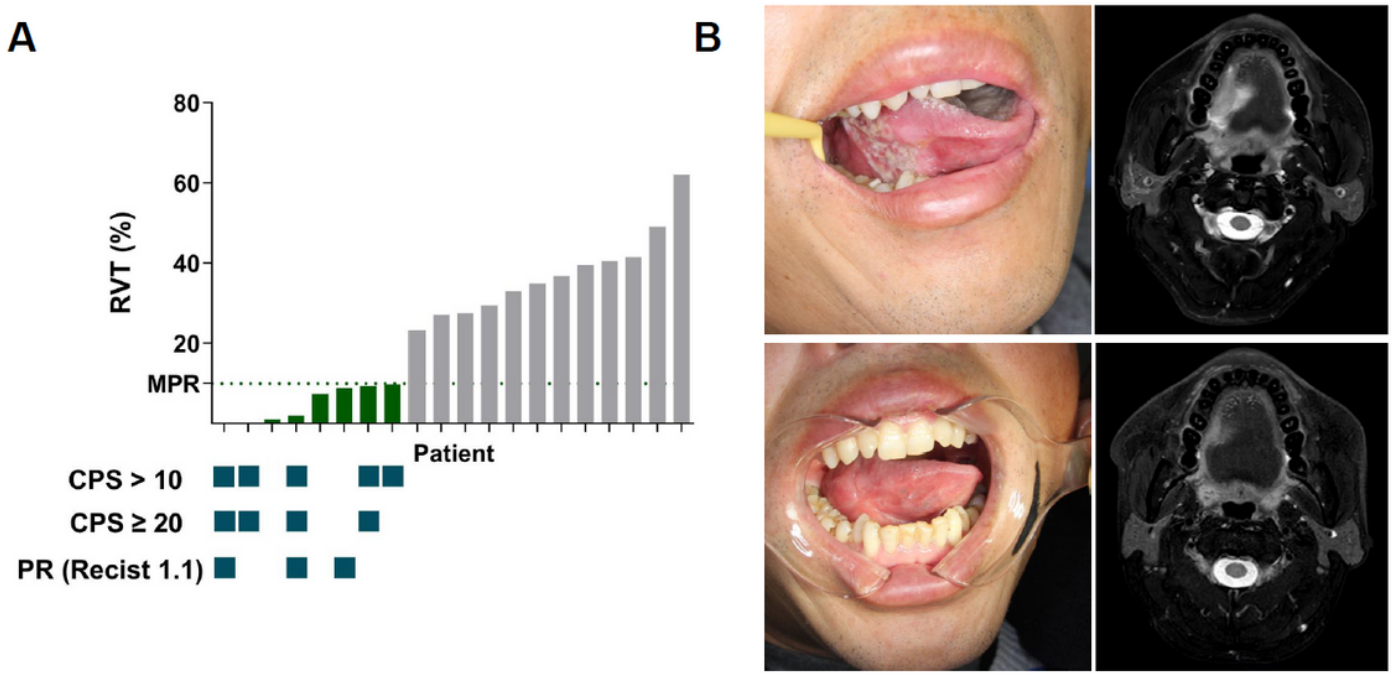

Figure 2

Efficacy of neoadjuvant therapy. (**A**) Residual viable tumor cells (RVT) ratio, combined positive score (CPS), and radiographic partial response (PR) in 20 patients. (**B**) In the patient achieved pathological complete response, images of the oral tongue and magnetic resonance imaging before (upper) and after (lower) neoadjuvant therapy.

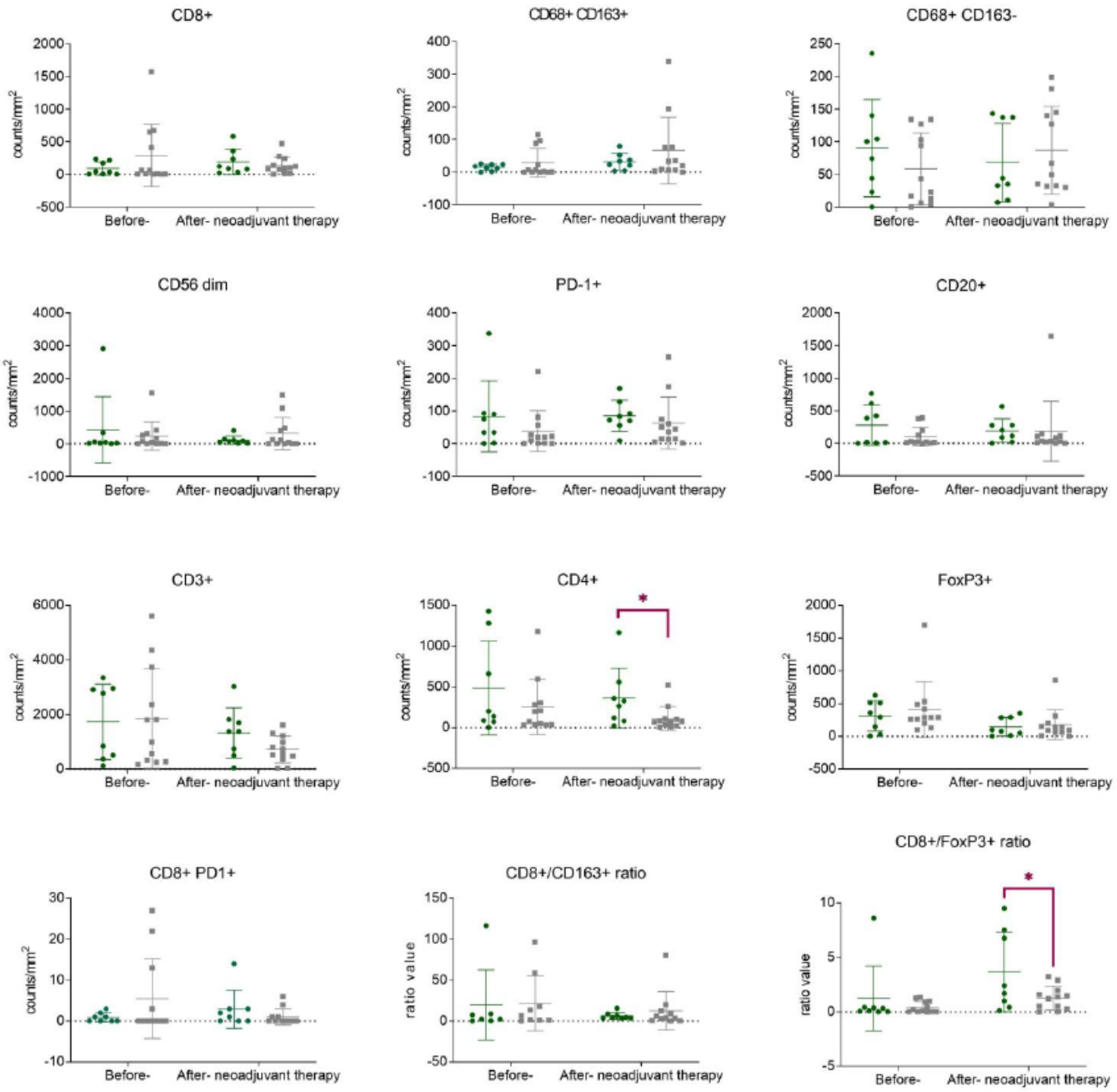

Figure 3

Comparison of TILs between MPR and non-MPR groups. Infiltration density of different lymphocytes subgroups, CD8+/CD163+ ratio, and CD8+/FoxP3 ratio in tumor samples before- and after- neoadjuvant therapy (blue dots for the MPR group, red dots for the non-MPR group). Statistical significance was denoted by *p < 0.05.

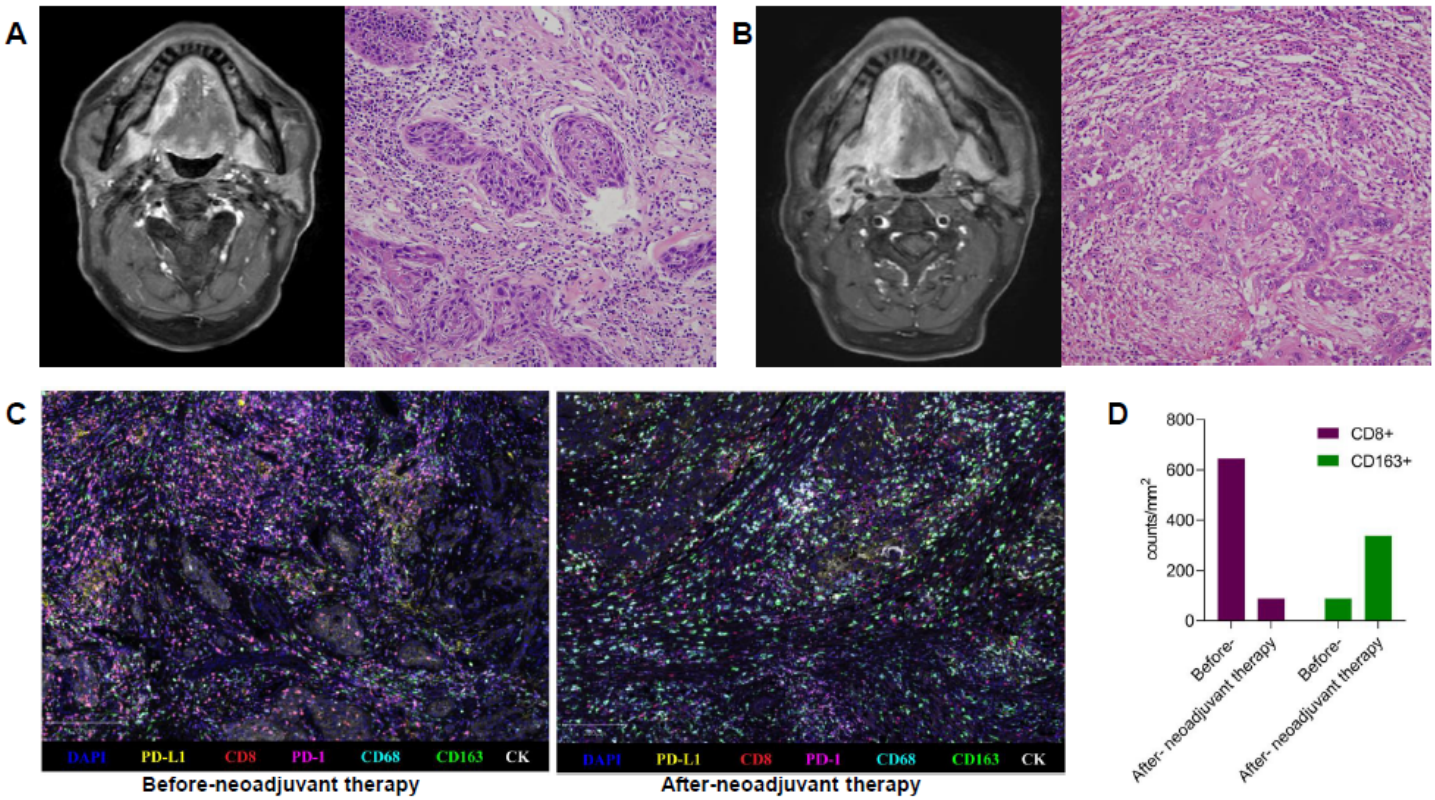

Figure 4

Features of the hyperprogression disease. In the NO.14 patient who showed hyperprogression disease: radiographic and HE staining images of before- (**A**) and after- (**B**) neoadjuvant therapy. (**C**) Multiplex immunofluorescence images of the tumor site before- and after- neoadjuvant therapy. (D) Comparison of CD8+ and CD163+ intensity from C.

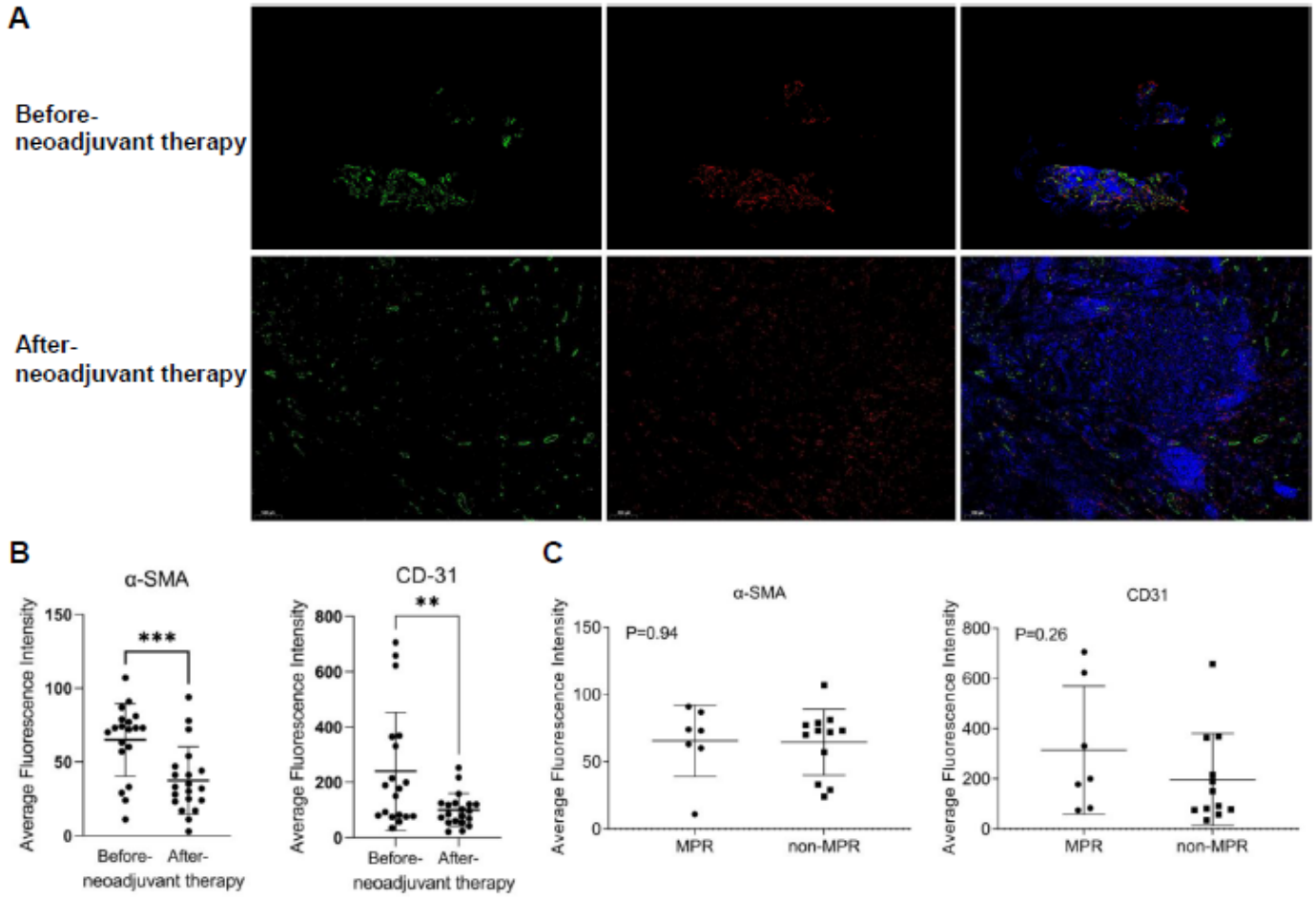

Figure 5

Anti-angiogenesis evaluation. (**A**) Representative immunofluorescence staining images of before- and after- neoadjuvant therapy tumor sections (Case No.7, green for CD31, red for α-SMA, blue for DAPI). (**B**) Fluorescence intensity of CD31 and α-SMA between before- and after- neoadjuvant therapy tumor tissues in all 20 patients. (**C**) Comparison of CD31 and α-SMA fluorescence intensity between MPR and non-MPR groups. Statistical significance was denoted by **p < 0.01, and ***p < 0.001.

Supplementary Files

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