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Original Article

Effect of afatinib combined with induction chemotherapy on primary tumor regression and safety of surgical margins in locally advanced oral cancer

Jian Sun[1], Ying Huang[1], Yixi Wang[1], Qiong Zhao[2]

[1]Department of Oral and Maxillofacial Surgery, The Second Hospital of Lanzhou University, No. 82, Cuiyingmen Road, Chengguan District, Lanzhou 730030, Gansu, China; [2]Department of Stomatology, Integrated Traditional Chinese and Western Medicine Hospital of Tianshui City, No. 26, Weibin North Road, Maiji District, Tianshui 741020, Gansu, China

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Abstract: Objective: To explore the impact of afatinib in combination with induction chemotherapy on primary tumor regression, surgical margin security, and long-term prognostic outcomes in patients with locally advanced oral cancer (LAOC). Methods: A retrospective study was performed on 218 LAOC patients admitted to The Second Hospital of Lanzhou University and Integrated Traditional Chinese and Western Medicine Hospital of Tianshui City from 2016 to 2019, assigned to a combination group (afatinib + TPF, n=115) and a control group (TPF alone, n=103). Tumor regression (RECIST 1.1, pathological grading), positive margin rate, tumor markers, adverse reactions, and 5-year survival were analyzed and compared; Logistic and COX regression models were used to analyze risk factors. Results: Combination group had higher ORR (70.43% vs. 57.28%, P=0.043), DCR (93.04% vs. 75.73%, P<0.001) and tumor regression ≥90% (33 vs. 16 cases, P<0.05), lower positive margin rate (14.78% vs. 37.86%, P<0.001), reduced postoperative tumor markers (all P<0.05), and better 5-year OS/DFS (all P<0.001). Afatinib combination was a protective factor for margin and survival, with no significant adverse responses (P>0.05). Conclusion: Afatinib combined with induction chemotherapy enhances tumor regression effect, boosts surgical margin security, and lowers tumor marker levels without elevating adverse event risks, which acts as an effective neoadjuvant strategy for LAOC.

Keywords: Locally advanced oral cancer, afatinib, induction chemotherapy, tumor regression, surgical margin

Introduction

Oral cancer ranks among the malignant tumors posing severe threats to human health globally, and holds a prominent place in the head and neck cancer category. Per the 2020 global cancer statistics issued by the International Agency for Research on Cancer (IARC), oral cancer causes around 377,000 new cases and 177,000 deaths each year, with a morbidity-mortality ratio close to 1:0.47, underscoring the tough challenge posed by its unfavorable prognosis [1]. In China, oral cancer incidence is also notable, ranking 12th among male malignant tumors and exhibiting a year-on-year upward trend [2]. Pathologically, more than 90% of oral cancers are oral squamous cell carcinomas (OSCC), characterized by biological traits of early infiltration and strong metastatic capacity. This results in most patients being diagnosed at the locally advanced stage (Stage III/IV), greatly limiting clinical treatment effectiveness and quality of life [3, 4]. Surgical resection has long served as the cornerstone of radical therapy for locally advanced oral cancer [5]. However, single-modality surgery has inherent limitations. On one side, extensive resection is usually needed to guarantee tumor radicality, causing severe complications like facial deformity, impaired masticatory and swallowing functions, and speech disorders - all of which notably lower postoperative quality of life [6]. On the other side, even with expanded resection, it remains hard to fully avoid positive surgical margins. Clinical researches have indicated

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that the local recurrence rate reaches up to 50-70% in patients with positive margins, while their 5-year survival rate is 30-40% lower than those with negative margins [7]. Thus, how to reduce the surgical scope to the minimum while securing surgical margin safety has become a key problem to solve in the oral cancer treatment field [8].

Along with the advancement of the notion of comprehensive tumor therapy, induction chemotherapy has gradually emerged as a trend for the treatment of locally advanced oral cancer. Induction chemotherapy - also called neoadjuvant chemotherapy - denotes systemic medication given prior to surgery or radiotherapy, with the goals of shrinking tumor volume, downgrading tumors, and enhancing the subsequent surgical resection rate and radicality [9, 10]. Classical induction chemotherapy regimens such as cisplatin combined with 5-fluorouracil (PF regimen) have achieved certain efficacy in clinical practice, enabling 30-50% of patients to achieve objective remission [11]. Further studies have found that the TPF regimen composed of docetaxel, cisplatin, and 5-fluorouracil significantly improves the objective remission rate (56% vs. 36%) and median overall survival (15.2 months vs. 12.8 months) compared with the PF regimen [12, 13]. Nevertheless, induction chemotherapy still faces numerous bottlenecks. First, the problem of drug resistance is prominent, with approximately 40-50% of patients showing primary resistance to traditional chemotherapeutic drugs, failing to achieve effective tumor regression [14]. Second, systemic toxic reactions of chemotherapeutic drugs, including myelosuppression, gastrointestinal reactions, and hepatorenal dysfunction, often lead to treatment interruption or delay, affecting the smooth progress of subsequent therapy [15, 16]. Third, although induction chemotherapy can reduce tumor volume to a certain extent, its inhibition of tumor cell invasion and metastasis is limited, making it difficult to fundamentally reduce the risk of positive surgical margins [17].

Afatinib, as a second-generation irreversible tyrosine kinase inhibitor (TKI), has the ability to irreversibly bind to the tyrosine kinase domains of receptors such as epidermal growth factor receptor (EGFR) and human epidermal growth factor receptor 2 (HER2). It continuously inhibits receptor activation, thereby blocking downstream signaling pathways and exerting an antitumor effect [18, 19]. In the field of non-small cell lung cancer (NSCLC), afatinib has been confirmed to significantly prolong progression-free survival and overall survival, becoming one of the standard therapeutic agents for EGFR-mutated NSCLC [20]. Meanwhile, clinical studies on head and neck squamous cell carcinoma have shown that afatinib monotherapy or combination therapy exhibits antitumor activity, inducing tumor cell apoptosis and inhibiting tumor angiogenesis [21, 22]. However, the application of afatinib in the treatment of locally advanced oral cancer (LAOC) remains in the exploratory stage, and the efficacy and safety of its combination with induction chemotherapy are still unclear [23].

This study aims to investigate the effect of afatinib combined with induction chemotherapy on primary tumor regression and safety of surgical margins in LAOC, providing new theoretical basis and practical guidance for clinical treatment of LAOC, which is expected to bring better therapeutic outcomes and survival benefits to patients.

Research methods

Study subjects

Data of a total of 184 patients with locally advanced oral cancer who were admitted to and treated in The Second Hospital of Lanzhou University and Integrated Traditional Chinese and Western Medicine Hospital of Tianshui City from January 2017 to December 2019 were retrospectively collected. This study has been approved by the Medical Ethics Committee of The Second Hospital of Lanzhou University. Inclusion criteria: (1) Pathologically confirmed oral cancer; (2) Clinically staged as locally advanced Stage III or IV A (according to the TNM staging system of the Union for International Cancer Control); (3) Complete clinical data, including treatment protocols, imaging results, surgical records, pathological reports, and follow-up information. Exclusion criteria: (1) Concurrent other malignant tumors; (2) Severe dysfunction of vital organs (heart, liver, kidney, etc.); (3) Inability to tolerate surgery or chemotherapy; (4) Incomplete treatment as planned or loss to follow-up during the treatment period.

Grouping and treatment protocols

Patients were divided into two groups based on the treatment regimens received: the Combined

Treatment Group (afatinib combined with induction chemotherapy) and the Control Group (single induction chemotherapy). For the Control Group, the commonly used platinum-containing regimen was selected for induction chemotherapy: patients were administered docetaxel injection 75 mg/m^2 (Guoyao Zhunzi H20050879, Beijing Orient Union Pharmaceutical Biotechnology Co., Ltd., Batch No.: 20190901), cisplatin injection 75 mg/m^2 (Guoyao Zhunzi H3702-1358, Qilu Pharmaceutical Co., Ltd., Batch No.: 20181223), and fluorouracil (5-FU) injection 750 mg/m^2 (Guoyao Zhunzi H31020593, Shanghai Xudong Haipu Pharmaceutical Co., Ltd., Batch No.: 20170205). Three weeks were regarded as one course of treatment, and at least 2 courses of induction chemotherapy were given preoperatively. For the Combined Treatment Group, afatinib was added to the induction chemotherapy regimen of the Control Group: afatinib tablets (Boehringer Ingelheim Pharmaceutical Co., Ltd., Shanghai, Guoyao Zhunzi J20170068, Specification: 40 mg/tablet, Batch No.: 20180501) were administered orally at 40 mg once daily, 3 hours after meals. Three weeks constituted one course of treatment, and the total treatment duration was 6 consecutive courses [24, 25].

Surgical procedures

Surgery was performed 2 weeks after chemotherapy for both groups. The surgical standards required standardized treatment by experienced surgeons, including radical resection of the primary lesion and comprehensive cervical lymph node dissection. Postoperative defects were repaired using pedicled or free flaps. The safety margin for radical resection was 1.5 cm, and intraoperative frozen section was used to ensure the safety margin of the surgery.

Observation indicators

(1) Primary lesion regression: 1. Imaging evaluation of induction chemotherapy effect: After the end of the 2nd course of induction chemotherapy, contrast-enhanced CT was used to scan the primary lesion of oral cancer. According to the Response Evaluation Criteria in Solid Tumors (RECIST 1.1), the regression degree of the primary lesion was evaluated [26], which was divided into complete response (CR), partial response (PR), stable disease (SD) and progressive disease (PD), and the response rate (RR = CR + PR) and disease control rate (DCR = CR + PR + SD) were calculated. 2. Postoperative microscopic evaluation: After surgery, the tumor tissue and surgical margin tissue were stained with HE, and the necrosis degree of tumor cells was observed and graded: Grade I, tumor regression ≥90%; Grade II, tumor regression 50-89%; Grade III, tumor regression <50%; Grade IV, no tumor regression or progression.

(2) Tumor marker detection: CEA, SCC-Ag, CYFRA21-1, VEGF, and CA19-9 were detected in patients one month after surgery. CEA, SCC-Ag, CYFRA21-1, and CA19-9 were detected using the ARCHITECT i2000SR automatic immunoassay system (Abbott, USA); VEGF was detected by enzyme-linked immunosorbent assay (ELISA) using a Human VEGF ELISA Kit (Catalogue No.: CSB-E07139h), and a cobas® e602 automatic electrochemiluminescence immunoassay analyzer (Roche Diagnostics Products Shanghai Co., Ltd.) was used. The detection was strictly performed according to the ELISA kit instructions.

(3) Detection of key proteins and related factors in the EGFR/HER2 pathway: Immunohistochemistry (IHC) was used to detect p-EGFR, p-HER2, p-Akt, Snail, E-cadherin, and bFGF. The primary antibodies were as follows: p-EGFR (1:100, Catalog No.: ab10001, Abcam plc, UK), p-HER2 (1:200, Catalog No.: ab20002, Abcam plc, UK), p-Akt (1:150, Catalog No.: ab10002, Abcam plc, UK), Snail (1:100, Catalog No.: ab10003, Abcam plc, UK), E-cadherin (1:100, Catalog No.: ab10004, Abcam plc, UK), and bFGF (1:100, Catalog No.: ab10005, Abcam plc, UK). The secondary antibody was a horseradish peroxidase (HRP)-conjugated goat anti-rabbit/mouse IgG antibody (1:200, Catalog No.: ab10006, Abcam plc, UK).

(4) Safety of surgical margins: Pathological examination of the margin tissues was performed after surgery, combining frozen sections and paraffin sections. If frozen section examination revealed positive margins, the resection range was immediately expanded; paraffin sections further confirmed the margin status, and the positive rate of surgical margins was recorded.

(5) Adverse reactions: Adverse reactions occurring within 2 weeks after surgery were observed and recorded, including hematological toxicity, gastrointestinal reactions, skin toxicity, oral mucositis, hepatorenal dysfunction, hearing loss, etc.

(6) Long-term efficacy: Regular follow-up was conducted, with follow-up once every 3 months for the first 2 years after surgery and once every 6 months for the 3rd to 5th years. The disease-free survival (DFS) and overall survival (OS) of patients were recorded.

(7) Logistic regression was used to analyze independent risk factors for positive surgical margins, construct a nomogram model, and evaluate the model's value through ROC curves and calibration curves.

(8) COX regression was used to analyze independent risk factors for 5-year survival after surgery, and the receiver operating characteristic curve (ROC) was used to detect the prognostic predictive value.

Statistical analysis

Data analysis was performed using SPSS 27.0 and R 4.3.3. For measurement data, the Kolmogorov-Smirnov test was used to assess normal distribution. Data conforming to normal distribution were expressed as mean ± standard deviation, and independent sample t-tests were used for intergroup comparisons; data not conforming to normal distribution were expressed as median [interquartile range], and the Mann-Whitney U test was applied. Enumeration data were expressed as rate (%), and the test method was selected based on frequency for intergroup comparison: chi-square test when all expected frequencies were ≥5; continuity-corrected chi-square test when at least one expected frequency was <5 but ≥1; Fisher's exact test when at least one expected frequency was <1 or data were sparse. Kaplan-Meier (K-M) survival analysis was used to evaluate 5-year OS and DFS after treatment, with log-rank test for analysis. Univariate and multivariate analyses of independent risk factors for positive surgical margins and 5-year mortality were performed using Logistics regression and COX regression, respectively. The predictive value of independent prognostic factors was evaluated using ROC or nomogram models. A $P<0.05$ was considered statistically significant.

The sample size of this study was estimated based on the primary outcome measure "positive surgical margin rate" using PASS 15.0 statistical software. Referring to previous studies on TPF neoadjuvant chemotherapy alone for locally advanced oral cancer [27], the positive surgical margin rate in the control group was preset to approximately 38%. Combining preliminary exploratory data of afatinib in head and neck squamous cell carcinoma [28], the positive surgical margin rate in the afatinib combined with neoadjuvant chemotherapy group was expected to decrease to 15%, indicating an anticipated between-group effect size of 23%. Statistical parameters were set as follows: two-sided significance level $\alpha=0.05$ and power $(1-\beta)=80\%$. The calculation showed that the minimum sample size required for each group was 89 cases. Taking into account potential case data missing, loss to follow-up, and non-adherence to inclusion and exclusion criteria in retrospective studies, a 15% sample size redundancy was incorporated into the calculated value. Eventually, each group was intended to have no fewer than 102 patients. Altogether, 184 eligible patients admitted from January 2017 to December 2019 were recruited for this study, with 103 cases in the control group and 115 cases in the combined treatment group - both satisfying the sample size estimation requirements.

Results

Baseline characteristics of control and combination groups

Baseline data comparison showed that the combination group (n=115) and control group (n=103) were well-balanced in age, gender, smoking history, alcohol consumption history, tumor location, T stage, N stage, AJCC stage, P16 expression, KPS score, pathological differentiation, and diabetes history (all $P>0.05$), indicating comparability between the two groups (**Table 1**).

Tumor regression after induction chemotherapy in both groups

Following the conclusion of induction chemotherapy, the objective response rate (ORR) in the afatinib combination group (70.43%) was notably higher than that in the control group (57.28%, $P=0.043$), indicating a more potent tumor regression effect. The disease control rate (DCR) also showed a significantly higher level in the combination group (93.04%) than the control group (75.73%, $P<0.001$), proving that combination therapy was more capable of controlling disease progression effectively. Furthermore, the proportions of EGFR mutations (51.85 vs. 42.37%) and HER2 amplifica-

Table 1. Comparison of baseline characteristics between the two groups

	Control Group (n=103)	Combination Group (n=115)	χ^2/t	P
Age (years)	61.07±7.90	58.86±9.19	1.891	0.060
Gender			0.303	0.582
Female	49 (47.57%)	59 (51.30%)		
Male	54 (52.43%)	56 (48.70%)		
Smoking History			1.469	0.267
None	45 (43.68%)	41 (35.65%)		
Yes	58 (56.31%)	74 (64.35%)		
Alcohol Consumption			0.534	0.465
None	38 (36.89%)	48 (41.74%)		
Yes	65 (63.11%)	67 (58.26%)		
Tumor Location			0.324	0.851
Tongue	50 (48.54%)	60 (52.17%)		
Floor of Mouth	27 (26.21%)	27 (23.48%)		
Gingiva & Buccal Mucosa	26 (25.24%)	28 (24.35%)		
T Stage			1.563	0.211
T1-2	11 (10.68%)	19 (16.52%)		
T3-4	92 (89.32%)	96 (83.48%)		
N Stage			0.355	0.551
N0	46 (44.66%)	56 (48.70%)		
N1-3	57 (55.34%)	59 (51.30%)		
AJCC Stage			0.662	0.416
Stage III	32 (31.07%)	30 (26.09%)		
Stage IV	71 (68.93%)	85 (73.91%)		
P16 Expression			1.524	0.217
Negative	97 (94.17%)	103 (89.57%)		
Positive	6 (5.83%)	12 (10.43%)		
KPS Score			0.632	0.729
<7	12 (11.65%)	17 (14.78%)		
7-8	51 (49.51%)	52 (45.22%)		
>8	40 (38.83%)	46 (40.00%)		
Pathological Differentiation			1.421	0.233
Poor/Undifferentiated	43 (41.75%)	39 (33.91%)		
Well/Moderately Differentiated	60 (58.25%)	76 (66.09%)		
Diabetes Mellitus			1.035	0.309
No	78 (75.73%)	80 (69.57%)		
Yes	25 (24.27%)	35 (30.43%)		
EGFR Amplification			0.892	0.729
Negative	82 (79.61%)	90 (78.26%)		
Positive	21 (20.39%)	25 (21.74%)		
HER2 amplification			0.567	0.233
Negative	90 (87.38%)	100 (86.96%)		
Positive	13 (12.62%)	15 (13.04%)		

Note: T stage, Tumor Stage; N stage, Node Stage; AJCC, American Joint Committee on Cancer Staging System; P16, Cyclin-dependent kinase inhibitor 2A; KPS, Karnofsky Performance Status; EGFR, Epidermal Growth Factor Receptor; HER2, Human Epidermal Growth Factor Receptor 2.

tions (23.46 vs. 16.95%) among ORR-achieving patients were both greater in the combination group, which aligns with afatinib's mechanism of action. No statistically significant variance

Table 2. Assessment of tumor regression in both groups after combination chemotherapy

	CR	PR	SD	PD	ORR	DCR	Number of EGFR-amplified cases in ORR	Number of HER2 amplification cases in ORR
Control Group (n=103)	5 (4.85)	54 (52.43)	19 (18.45)	20 (19.42)	59 (57.28)	78 (75.73)	25 (42.37)	10 (16.95)
Combination Group (n=115)	24 (20.87)	57 (49.57)	26 (22.61)	12 (10.43)	81 (70.43)	107 (93.04)	42 (51.85)	19 (23.46)
Z/χ^2				2.171	4.091	12.681	1.732	1.926
P				0.003	0.043	<0.001	0.185	0.156

Note: CR, Complete Response; PR, Partial Response; SD, Stable Disease; PD, Progressive Disease; ORR, Objective Response Rate; DCR, Disease Control Rate; EGFR, Epidermal Growth Factor Receptor; HER2, Human Epidermal Growth Factor Receptor 2.

Table 3. Comparison of tumor residue at surgical margins between the two groups

Protocol	R0 Resection Rate (%)	R1 Resection Rate (%)	R2 Resection Rate (%)	Positive Margins
Control Group (n=103)	64 (62.14)	32 (31.07)	7 (6.80)	39 (37.86)
Combination Group (n=115)	98 (85.22)	14 (12.17)	3 (2.61)	17 (14.78)
χ^2				15.164
P				<0.001

was found in the positive proportions between the two groups (P>0.05), as presented in **Table 2**.

Tumor residue at surgical margins in both groups

The positive margin rate in the combination group (14.78%) was significantly lower than that in the control group (37.86%, P<0.001). The combination therapy also showed advantages in R1 resection rate (12.17% vs. 31.07%) and R2 resection rate (2.61% vs. 6.80%) (**Table 3**).

Univariate analysis of risk factors for positive surgical margins

Univariate analysis indicated that positive surgical margins were markedly correlated with age <0.001, history of alcohol consumption (P=0.028), induction chemotherapy <0.001, N stage (*P*=0.035), and pathological differentiation (*P*=0.001), suggesting that these factors might independently affect positive margins. However, gender, smoking history, and T stage exhibited no significant correlation with positive margins (all *P*>0.05) (**Table 4**).

Multivariate analysis of independent risk factors for positive surgical margins

Multivariate Logistic regression analysis revealed that age >60.5 years (OR=11.71, 95% CI: 4.34-31.62, P<0.001), history of alcohol consumption (OR=4.18, 95% CI: 1.53-11.44, P=0.005), and N1-3 stage (OR=4.22, 95% CI: 1.63-10.92, P=0.003) served as independent risk factors for positive surgical margins. On the contrary, induction chemotherapy combined with afatinib (OR=0.27, 95% CI: 0.13-0.58, P=0.001) and well/moderately differentiated pathology (OR=0.18, 95% CI: 0.06-0.53, P=0.002) acted as protective factors, notably lowering the risk of positive margins (**Table 5**).

Construction of nomogram for risk prediction of positive surgical margins

A nomogram was constructed to analyze the score distribution of each variable and its relationship with the total score, identifying strong and weak indicators associated with the risk. Results showed that age and induction chemotherapy regimen were strongly correlated with positive surgical margins, indicating a strong association with the risk of positive margins. Pathological differentiation and alcohol consumption history were moderately associated indicators, while N stage was a weakly associated indicator (**Figure 1**).

Performance validation of the prediction model

By constructing the AUC curve and calibration curve of the model, the ROC curve showed an AUC of 0.813, and the calibration curve indicated a high consistency between the predicted probability and actual probability (chi-square value=2.864, P=0.786), demonstrating

Table 4. Univariate analysis of influencing factors for positive surgical margins

	Positive Margin Group, n=162	Negative Margin Group, n=56	X^2/Z	P
Age (years)	59.00 [52.00, 63.00]	67.00 [63.00, 72.25]	6.588	<0.001
Gender			0.723	0.395
Female	83 (51.23%)	25 (44.64%)		
Male	79 (48.77%)	31 (55.36%)		
Smoking History			0.120	0.729
None	65 (40.12%)	21 (37.50%)		
Yes	97 (59.88%)	35 (62.50%)		
Alcohol Consumption			4.801	0.028
None	57 (35.19%)	29 (51.79%)		
Yes	105 (64.81%)	27 (48.21%)		
Induction Chemotherapy Regimen			15.164	<0.001
Pure Induction Chemotherapy	64 (39.51%)	39 (69.64%)		
Induction Chemotherapy + Afatinib	98 (60.49%)	17 (30.36%)		
T Stage			0.101	0.751
T1-2	23 (14.20%)	7 (12.50%)		
T3-4	139 (85.80%)	49 (87.50%)		
N Stage			4.461	0.035
N0	69 (42.59%)	33 (58.93%)		
N1-3	93 (57.41%)	23 (41.07%)		
Pathological Differentiation			10.109	0.001
Poor/Undifferentiated	51 (31.48%)	31 (55.36%)		
Well/Moderately Differentiated	111 (68.52%)	25 (44.64%)		

Note: T Stage, Tumor Stage; N Stage, Node Stage.

Table 5. Multivariate logistic regression analysis of positive surgical margins

	Variable Assignment	B	*P*-value	Sig	OR	95% CI	
						Lower Limit	Upper Limit
Age	0≤60.5, 1>60.5	2.46	0.507	<.001	11.71	4.336	31.622
Induction Chemotherapy Regimen	0= Pure induction chemotherapy, 1= Induction chemotherapy + Afatinib	-1.306	0.383	0.001	0.271	0.128	0.575
Alcohol Consumption	0= No, 1= Yes	1.43	0.514	0.005	4.18	1.528	11.435
N Stage	1= N0 stage, 2= N1-3 stages	1.44	0.485	0.003	4.223	1.632	10.923
Pathological Differentiation	0= Poorly differentiated and undifferentiated, 1= Well differentiated and moderately differentiated	-1.700	0.54	0.002	0.183	0.063	0.526
Constant		-2.323	0.619	<0.001	0.098		

Note: N Stage, Node Stage.

that the prediction model for positive surgical margins was overall significant and had excellent calibration performance (**Figure 2**).

Results showed that the number of patients in the combination group with postoperative Grade I (tumor regression ≥90%) was significantly higher than that in the control group (33/16, P<0.05), while the number of patients in the combination group with postoperative Grade IV (no tumor regression or progression) was significantly lower than that in the control group (8/21, P<0.05). This indicates that preoperative induction chemotherapy combined with Afatinib resulted in better postoperative tumor regression compared with pure induction chemotherapy (**Figure 3**).

Changes in tumor markers after surgery

The decline in multiple tumor marker levels in the combination group after surgery was sig-

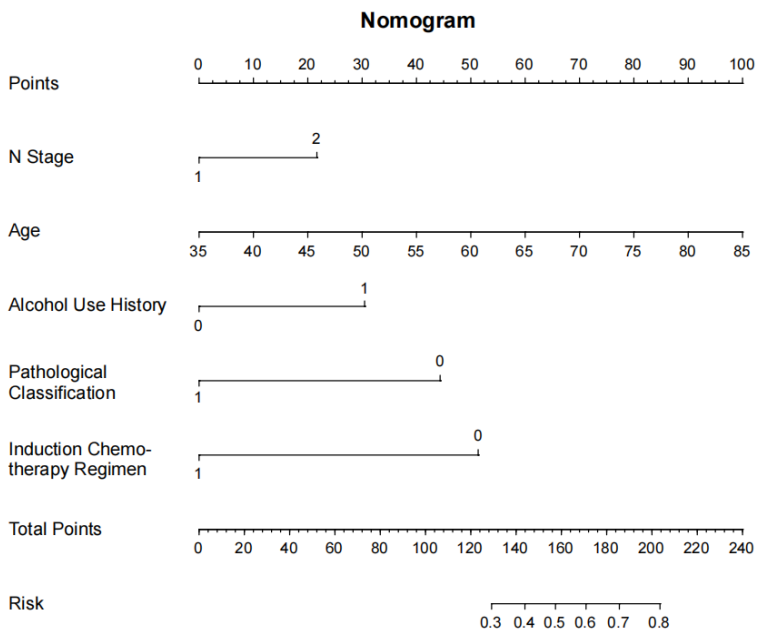

Figure 1. Nomogram for risk assessment of independent risk factors for positive surgical margins. Note: N Stage, Node Stage.

nificantly better than that in the control group. Specifically, the levels of CEA (3.06±0.87 vs. 3.64±0.99, P<0.001), SCC-Ag (1.03±0.22 vs. 1.25±0.31, P<0.001), CYFRA21-1 (1.78±0.40 vs. 1.97±0.50, P=0.002), VEGF (63.24±15.01 vs. 72.07±18.49, P<0.001), and CA19-9 (23.59±6.03 vs. 26.30±5.50, P=0.001) in the combination group were significantly lower than those in the control group (**Table 6**).

Results of key proteins and related factors in the EGFR/HER2 pathway

Postoperative detection of key molecules in the EGFR/HER2 pathway (**Table 7**) showed that in the control group, patients with EGFR mutation or HER2 amplification had significantly higher expressions of p-EGFR, p-HER2, p-Akt, Snail and bFGF, and lower E-cadherin than those with wild-type EGFR or non-amplified HER2 (all P<0.05). In contrast, the combination group exhibited significant downregulation of the aforementioned pro-tumor molecules and upregulation of E-cadherin across all subgroups (all P<0.001 vs. control group), with more prominent pathway inhibition and EMT reversal in patients with EGFR mutation/HER2 amplification (interaction P=0.006-0.012). These results confirm that afatinib combined with induction chemotherapy can inhibit downstream PI3K/Akt signaling and block EMT and angiogenesis by targeting the EGFR/HER2 pathway, with superior efficacy in biomarker-positive patients, providing molecular support for the stratified application of this regimen.

Comparison of adverse reactions between control group and combination group after surgery

Postoperative adverse reaction comparison showed no statistically significant differences in the incidence of hematological toxicity (47.57% vs. 37.39%, P=0.129), gastrointestinal reactions (48.54% vs. 40.87%, P=0.255), skin toxicity (24.27% vs. 26.09%, P=0.758), oral mucositis (21.36% vs. 23.48%, P=0.708), hepatorenal dysfunction (13.59% vs. 17.39%, P=0.440), and hearing loss (7.77% vs. 9.57%, P=0.638) between the two groups (all P>0.05). This suggests that combination therapy did not significantly increase the risk of postoperative adverse reactions, and its safety was comparable to that of the control group (**Table 8**).

Comparison of 5-year survival curves for prognosis

Through 5-year follow-up statistics and K-M curve analysis, the 5-year overall survival (OS) of the control group was significantly lower than that of the combination group (P<0.001), and the disease-free survival (DFS) also showed that the control group was significantly lower than the combination group (P<0.001) (**Figure 4**).

Univariate COX analysis of independent risk factors for 5-year mortality in patients

Univariate COX analysis showed that positive surgical margins (OR=11.72, 95% CI: 7.70-17.86, P<0.001), increased CYFRA21-1 after treatment (OR=12.83, 95% CI: 7.86-20.93, P<0.001), age (OR=1.12, 95% CI: 1.10-1.15, P<0.001), postoperative tumor regression (OR=13.09, 95% CI: 7.94-21.48, P<0.001), and induction chemotherapy combined with Afatinib (OR=0.40, 95% CI: 0.27-0.60, P<0.001) might

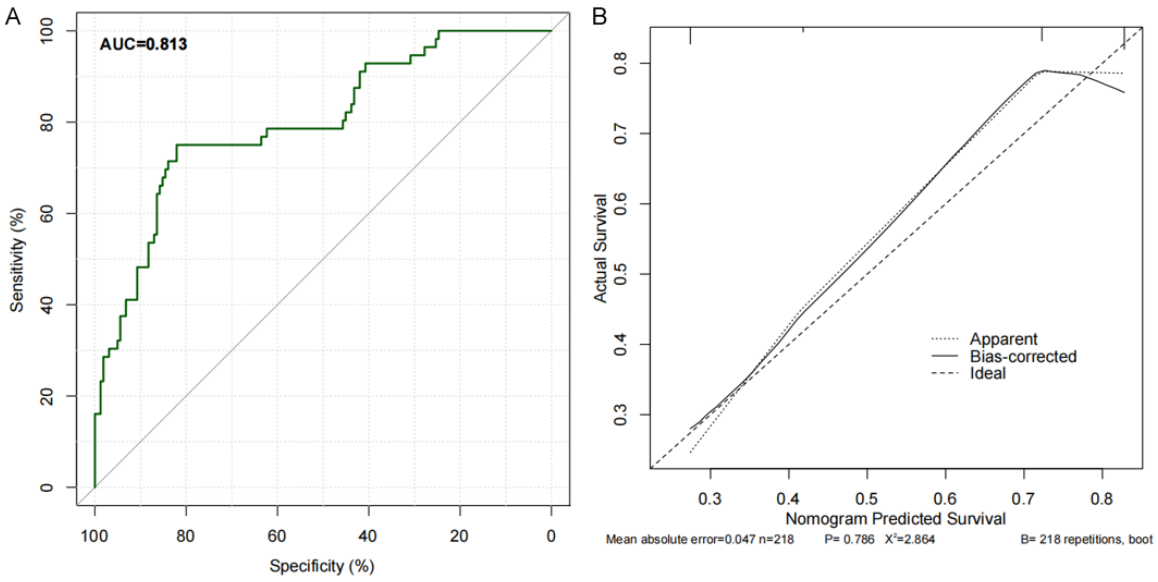

Figure 2. Performance validation of the prediction model. A: ROC curve for validating the performance of the prediction model. B: Calibration curve of the prediction model.

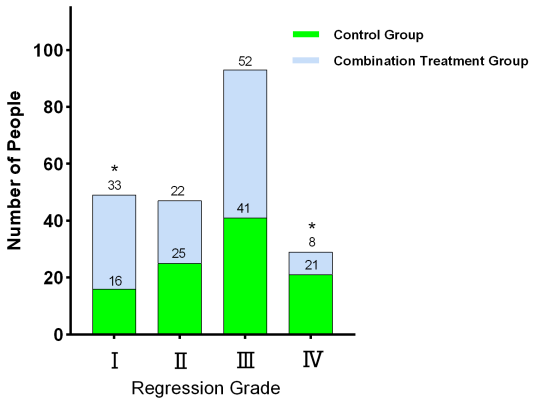

Figure 3. Comparison of tumor regression grades between the two groups after surgery. Note: "*" indicates P<0.05. Grade I: tumor regression ≥90%; Grade II: tumor regression 50-89%; Grade III: tumor regression <50%; Grade IV: no tumor regression or progression.

be independent influencing factors for 5-year mortality. However, CEA, SCC-Ag, VEGF, CA19-9 after treatment, gender, KPS score, and history of diabetes were not significantly associated with the risk of death (all *P*>0.05) (**Table 9**).

Multivariate COX analysis of 5-year survival prognosis

Multivariate COX analysis showed that positive surgical margins (OR=1.93, 95% CI: 1.09-3.41, P=0.024), increased CYFRA21-1 after treatment (OR=3.30, 95% CI: 1.24-8.78, P=0.017), and age growth (OR=1.06, 95% CI: 1.03-1.10, P=0.001) were independent risk factors for 5-year survival. Induction chemotherapy combined with Afatinib (OR=0.41, 95% CI: 0.26-0.65, P<0.001) and postoperative tumor regression ≥50% (OR=0.13, 95% CI: 0.06-0.29, P<0.001) were protective factors (**Table 10**).

Predictive value of independent prognostic factors

By plotting ROC curves to detect the predictive value of independent prognostic factors (surgical margin, induction chemotherapy regimen, CYFRA21-1 after treatment, age, and postoperative tumor regression) for 5-year mortality, the AUC values of surgical margin, induction chemotherapy regimen, CYFRA21-1 after treatment, age, and postoperative tumor regression were 0.727, 0.665, 0.877, 0.805, and 0.855, respectively (**Figure 5**).

Discussion

To explore the mechanism of action and stratified predictive clinical value of the combination therapy, this study detected key molecules in the EGFR/HER2 pathway in tumor tissues. Results showed that the expressions of p-EGFR, p-HER2, and downstream p-Akt in the combination therapy group were significantly lower than those in the control group, directly verifying the specific inhibitory effect of afatinib on this pathway. This is highly consistent with the study by

Table 6. Comparison of postoperative tumor markers between the two groups

	Control Group (n=103)	Combination Group (n=115)	t	P
CEA	3.64±0.99	3.06±0.87	4.604	<0.001
SCC-Ag	1.25±0.31	1.03±0.22	6.089	<0.001
CYFRA21-1	1.97±0.50	1.78±0.40	3.112	0.002
VEGF	72.07±18.49	63.24±15.01	3.887	<0.001
CA19-9	26.30±5.50	23.59±6.03	3.453	0.001

Note: CEA, Carcinoembryonic Antigen; SCC-Ag, Squamous Cell Carcinoma Antigen; CYFRA21-1, Cytokeratin 19 Fragment Antigen 21-1; VEGF, Vascular Endothelial Growth Factor; CA19-9, Carbohydrate Antigen 19-9.

Table 7. Expression levels of key proteins and related factors in the EGFR/HER2 pathway stratified by EGFR mutation/HER2 amplification status at 1 month after treatment

		EGFR Mutant Type	EGFR Wild-Type	HER2 Amplified Type	HER2 Non-Amplified Type	*P* Value (Combination vs. Control)	Interaction *P* Value (Treatment × Biomarker)
p-EGFR	Control Group (n=103)	0.84±0.13	0.66±0.14	0.86±0.12	0.67±0.15	<0.001	0.009
	Combination Group (n=115)	0.27±0.08	0.39±0.10	0.25±0.07	0.40±0.11		
p-HER2	Control Group (n=103)	0.77±0.11	0.58±0.13	0.79±0.10	0.59±0.14	<0.001	0.007
	Combination Group (n=115)	0.23±0.06	0.33±0.09	0.21±0.05	0.34±0.10		
p-Akt	Control Group (n=103)	0.88±0.14	0.70±0.15	0.90±0.13	0.71±0.16	<0.001	0.011
	Combination Group (n=115)	0.29±0.09	0.42±0.11	0.27±0.08	0.43±0.12		
Snail	Control Group (n=103)	0.73±0.10	0.56±0.12	0.75±0.09	0.57±0.13	<0.001	0.008
	Combination Group (n=115)	0.20±0.05	0.30±0.08	0.18±0.04	0.31±0.09		
E-cadherin	Control Group (n=103)	0.21±0.05	0.32±0.07	0.19±0.04	0.33±0.08	<0.001	0.006
	Combination Group (n=115)	0.74±0.13	0.59±0.11	0.77±0.12	0.60±0.12		
bFGF	Control Group (n=103)	35.86±7.52	28.34±7.15	36.92±7.38	28.89±7.22	<0.001	0.012
	Combination Group (n=115)	16.84±5.53	22.56±6.01	16.23±5.41	23.21±6.10		

Note: EGFR, Epidermal Growth Factor Receptor; HER2, Human Epidermal Growth Factor Receptor 2; p-EGFR, Phosphorylated Epidermal Growth Factor Receptor; p-HER2, Phosphorylated Human Epidermal Growth Factor Receptor 2; p-Akt, Phosphorylated Akt Protein Kinase; Snail, Snail Transcription Factor; E-cadherin, Epithelial Cadherin; bFGF, Basic Fibroblast Growth Factor.

Table 8. Incidence of postoperative adverse reactions in both groups

	Control Group (n=103)	Combination Group (n=115)	χ^2	P
Hematological Toxicity	49 (47.57)	43 (37.39)	2.309	0.129
Gastrointestinal Reactions	50 (48.54)	47 (40.87)	1.296	0.255
Skin Toxicity	25 (24.27)	30 (26.09)	0.095	0.758
Oral Mucositis	22 (21.36)	27 (23.48)	0.140	0.708
Hepatorenal Dysfunction	14 (13.59)	20 (17.39)	0.596	0.440
Hearing Loss	8 (7.77)	11 (9.57)	0.221	0.638

Han et al., which demonstrated that afatinib can irreversibly bind to the tyrosine kinase domains of EGFR/HER2, block receptor activation, inhibit the activity of the PI3K/Akt/mTOR pathway, reduce the expression of the anti-apoptotic protein Mcl-1, and enhance the sensitivity of tumor cells to chemotherapy [29]. Meanwhile, the significant downregulation of p-Akt in the combination group echoes the mechanistic finding by Xiang et al. that EGFR pathway inhibition can arrest the tumor cell cycle by downregulating MAPK/ERK phosphorylation [30], suggesting that afatinib may exert a stronger proliferation-inhibitory effect in synergy with chemotherapy by synchronously inhibiting dual pathways.

At the level of EMT regulation and angiogenesis, the combination group showed decreased expression of the pro-metastatic factor Snail and increased expression of the anti-metastatic molecule E-cadherin, revealing the reversal effect of afatinib on the EMT process. A study by Dennis et al. in head and neck squamous

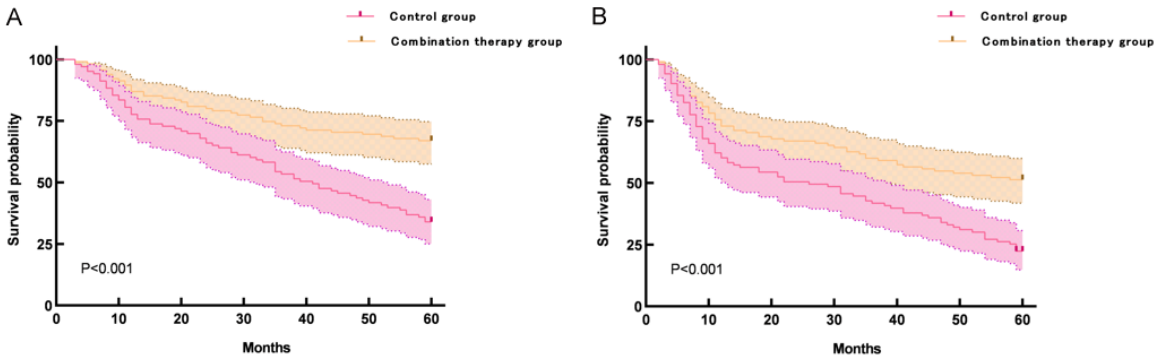

Figure 4. 5-year OS and DFS of patients in both groups after surgery. A: K-M curve of OS in the control group and the combination group. B: K-M curve of DFS in the control group and the combination group. Note: OS, Overall Survival; DFS, Disease-Free Survival.

Table 9. Results of univariate analysis of influencing factors for 5-year mortality

	Survival Group, n=112	Mortality Group, n=106	OR	95% CI	P
Surgical Margin			11.717	7.699-17.857	<0.001
Negative	108 (96.43%)	54 (50.94%)			
Positive	4 (3.57%)	52 (49.06%)			
Induction Chemotherapy Regimen			0.403	0.271-0.600	<0.001
Pure Induction Chemotherapy	35 (31.25%)	68 (64.15%)			
Induction + Afatinib	77 (68.75%)	38 (35.85%)			
CEA after Treatment	3.23 [2.54, 3.82]	3.39 [2.78, 4.01]	1.097	1.902-1.333	0.354
SCC-Ag after Treatment	1.12±0.27	1.14±0.30	1.279	0.842-2.548	0.484
CYFRA21-1 after Treatment	1.58 [1.35, 1.79]	2.14 [1.97, 2.32]	12.828	7.863-20.929	<0.001
VEGF after Treatment	66.57±17.74	68.30±16.98	1.003	0.993-1.014	0.525
CA19-9 after Treatment	24.36±6.43	25.41±5.39	1.023	0.992-1.055	0.154
Age	55.59±8.34	64.46±6.35	1.119	1.099-1.149	<0.001
Gender			0.980	0.670-1.414	0.917
Female	54 (48.21%)	54 (50.94%)			
Male	58 (51.79%)	52 (49.06%)			
KPS Score			0.878	0.668-1.153	0.348
<7	16 (14.29%)	13 (12.26%)			
7-8	47 (41.96%)	56 (52.83%)			
>8	49 (43.75%)	37 (34.91%)			
Diabetes			0.920	0.594-1.424	0.709
No	79 (70.54%)	79 (74.53%)			
Yes	33 (29.46%)	27 (25.47%)			
Postoperative Tumor Regression			13.091	7.943-21.484	<0.001
Regression <50%	24 (21.43%)	98 (92.45%)			
Regression ≥50%	88 (78.57%)	8 (7.55%)			

Note: KPS, Karnofsky Performance Status; CEA, Carcinoembryonic Antigen; SCC-Ag, Squamous Cell Carcinoma Antigen; CYFRA21-1, Cytokeratin 19 Fragment Antigen 21-1; VEGF, Vascular Endothelial Growth Factor; CA19-9, Carbohydrate Antigen 19-9.

cell carcinoma indicated that EGFR/HER2 pathway activation can upregulate the transcriptional activity of Snail and Twist through the Smad2/3-dependent pathway, promoting tumor cells to acquire an invasive phenotype [31]; afatinib can block this signal transduction, re-

Table 10. Results of multivariate analysis of 5-year mortality in patients

	B	*P*-value	Sig.	OR	95% CI	
					Lower Limit	Upper Limit
Surgical Margin	0.656	0.291	0.024	1.928	1.090	3.412
Induction Chemotherapy Regimen	-0.888	0.232	<0.001	0.411	0.261	0.648
CYFRA21-1 after Treatment	1.193	0.500	0.017	3.298	1.238	8.783
Age	0.059	0.018	0.001	1.061	1.025	1.098
Postoperative Tumor Regression	-2.040	0.400	<0.001	0.130	0.059	0.285

Note: CYFRA21-1, Cytokeratin 19 Fragment Antigen 21-1.

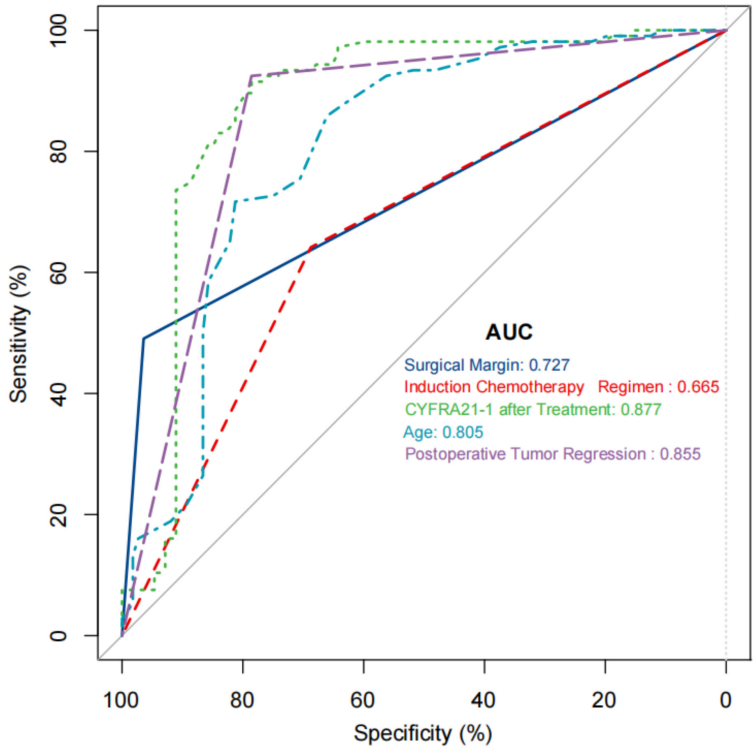

Figure 5. ROC curve for predicting the value of independent risk factors for mortality.

store intercellular adhesion function, and reduce the microscopic infiltration of tumor cells into surgical margins [31], providing a reasonable molecular explanation for the significant reduction in the positive margin rate in the combination group of this study. In addition, the significant downregulation of bFGF in the combination group is consistent with the decreasing trend of VEGF in **Table 6**, confirming the latest research by Jiang et al.: EGFR/HER2 pathway inhibition can downregulate HIF-1α transcriptional activity, synchronously reduce the secretion and receptor binding of VEGF and bFGF, and cut off tumor nutrient supply, explaining the superior tumor regression effect of the combination therapy [32].

Based on the above molecular mechanisms, this study further confirms the therapeutic value of afatinib combined with induction chemotherapy in locally advanced oral cancer, and reveals the value of this combination regimen in tumor control, surgical safety, and long-term prognosis through multi-dimensional comparative analysis.

The degree of tumor regression is a key prognostic indicator for locally advanced oral cancer, closely associated with patients' overall survival, recurrence risk, and surgical margin status, and provides important evidence for formulating individualized treatment strategies [30]. After induction chemotherapy, the combination therapy group demonstrated significant advantages in tumor regression, with both objective response and disease control efficacy superior to those of the monotherapy induction chemotherapy group. Notably, the proportions of EGFR mutations and HER2 amplifications among patients with objective response rate (ORR) were higher in the combination therapy group, which is consistent with the mechanism of afatinib's continuous blocking of the EGFR/HER2 signaling pathway - it not only inhibits the proliferation cycle of tumor cells but also reduces the invasive capacity of tumor cells to surrounding tissues by downregulating the activity of matrix metalloproteinases [33]. This dual mechanism of action enables the combination therapy to shrink the volume of the primary tumor while more effectively inhibiting local tumor invasion,

thereby creating better conditions for subsequent radical surgical resection. However, no statistically significant difference was observed in the positive proportions of these biomarkers among ORR patients between the two groups, which may be related to the sample size and tumor heterogeneity. This result supports the clinical screening of beneficial populations through baseline EGFR/HER2 detection, and further verification of the predictive value of these biomarkers in large-sample studies is required.

The positive surgical margin rate is a core target for prognosis and treatment decision-making in locally advanced oral cancer. Recent five-year studies have confirmed that it is not only strongly associated with recurrence and mortality rates (with the positive rate still as high as 18.1%) but also enables optimized risk stratification via molecular biomarkers such as ITGB1. Even with ultimately negative margins, microscopic tumor cut-through still indicates an unfavorable prognosis. Its status directly guides the development of individualized postoperative intensive treatment regimens, serving as a key link in precision medicine [34]. The significant reduction in the positive rate of surgical margins in the combination therapy group indicates its unique value in improving the radical resection rate of tumors. Multivariate analysis showed that age, N stage, and history of alcohol consumption were independent risk factors for positive margins, while afatinib combination therapy and highly differentiated pathological types constituted protective factors. This result reveals the clinical characteristics of surgical margin risk in locally advanced oral cancer: elderly patients may have tumor residue due to decreased tissue repair ability [35], while the status of regional lymph node metastasis reflects the invasive activity of the tumor. The protective effect of afatinib is closely related to its inhibition of the epithelial-mesenchymal transition (EMT) process in tumor cells. EGFR inhibitors can reverse the migratory phenotype of tumor cells by downregulating transcription factors such as Snail and Twist, thereby reducing microscopic invasion at the surgical margin [36].

Recent five-year studies have also confirmed that the dynamic monitoring of postoperative tumor markers such as CEA, SCC-Ag, and CYFRA21-1 can provide early warning of recurrence, while combined detection can accurately predict prognosis. Their changing trends offer key evidence for the adjustment of individualized treatment regimens in locally advanced oral cancer [37]. Postoperative analysis of tumor markers showed that the combination therapy group significantly outperformed the control group in the decline of indicators such as CEA, SCC-Ag, and CYFRA21-1, which echoed the imaging evaluation of tumor regression and pathological results. Among them, the decrease in CYFRA21-1 levels directly reflected the rebalancing of tumor cell apoptosis and proliferation; the significant reduction in VEGF indicated the potent inhibition of tumor angiogenesis by combination therapy [38]. It is noteworthy that the status of CYFRA21-1 as an independent risk factor in prognostic evaluation suggests its potential as a sensitive indicator for predicting postoperative recurrence.

Five-year overall survival (OS) and disease-free survival (DFS) are core prognostic endpoints for locally advanced oral cancer. Their results directly validate the efficacy of treatment regimens, and they can also stratify risks in combination with tumor regression and surgical margin status, providing key references for the subsequent adjustment of individualized treatment [39]. The 5-year survival analysis showed that the combination therapy group significantly outperformed the control group in both OS and DFS. Multivariate COX analysis showed that postoperative tumor regression ≥50% and afatinib combination therapy constituted independent protective factors. The negative correlation between the degree of tumor regression and the local recurrence rate may be achieved by enhancing dendritic cell activation or inhibiting regulatory T cell function to improve the body's anti-tumor immune response [36, 40]. In addition, age as an independent risk factor suggests that elderly patients may require more intensive combination therapy strategies to overcome their poor cell repair ability and immune function.

The comparison of adverse reactions showed that afatinib combined with induction chemotherapy did not significantly increase the postoperative toxic load, and indicators such as hematological toxicity and gastrointestinal reactions were comparable to those in the pure chemotherapy group. This result provides key evidence for the clinical promotion of this regimen.

The addition of afatinib did not significantly increase irreversible toxicities such as hepatorenal dysfunction or hearing loss, making this combination regimen highly applicable for neoadjuvant therapy of locally advanced oral cancer.

Further stratified analysis based on biomarkers clarified the stratified predictive value of EGFR mutation/HER2 amplification for the combination therapy. In the control group, patients with mutation/amplification showed significantly higher expressions of pro-oncogenic proteins such as p-EGFR and p-HER2 and lower E-cadherin than those with wild-type/non-amplification, reflecting the characteristics of abnormal pathway activation. After combination therapy, the reduction rates of pro-oncogenic proteins in the former (53-68%) were significantly higher than those in the latter (35-45%), with more obvious upregulation of E-cadherin (all interaction $P<0.05$), confirming that these biomarkers can accurately distinguish beneficial populations. Based on this, in clinical practice, positive patients should be prioritized for the afatinib combined with TPF regimen to maximize efficacy due to tumor dependence on the target pathway; for negative patients, the combination regimen offers limited benefits, and chemotherapy combined with immunotherapy is recommended. This stratification not only aligns with the mechanism of targeted therapy but also avoids ineffective treatment.

Although this study has drawn valuable conclusions, several limitations still exist. First, as a retrospective analysis, it may be subject to selection bias. Despite our efforts to control the balance of baseline data, we cannot completely exclude the influence of other potential confounding factors. Second, the sample size of this study was relatively small, which may affect the statistical power of the results. Additionally, molecular markers such as EGFR mutation status were not initially included for stratified analysis, which needs to be further improved in subsequent studies. Therefore, large-sample prospective studies are required for further verification in the future.

Through molecular mechanism and multi-dimensional clinical analysis, this study confirms that afatinib combined with induction chemotherapy can safely and effectively improve tumor regression and surgical margin safety in locally advanced oral cancer, and EGFR mutation/HER2 amplification can serve as predictive biomarkers for efficacy. In the future, it is necessary to integrate the concepts of molecular typing and precision medicine to further optimize treatment strategies and provide more individualized treatment plans for patients.

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Disclosure of conflict of interest

None.

Address correspondence to: Qiong Zhao, Department of Stomatology, Integrated Traditional Chinese and Western Medicine Hospital of Tianshui City, No. 26, Weibin North Road, Maiji District, Tianshui 74-1020, Gansu, China. E-mail: 13893805236@163.com

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