Low Annexin A1 expression is predictive for a benefit from induction chemotherapy in oral cancer patients with moderately/poorly pathologic differentiation grade

Dong-wang Zhu¹*, Ying Liu¹*, Xiao Yang¹, Cheng-zhe Yang¹, Jie Ma¹, Xi Yang¹, Jin-ke Qiao¹, Li-zhen Wang², Jiang Li², Chen-ping Zhang¹, Zhi-yuan Zhang¹, Lai-ping Zhong¹

¹Department of Oral & Maxillofacial-Head & Neck Oncology, Ninth People’s Hospital, College of Stomatodontology, Shanghai Jiao Tong University School of Medicine; ²Department of Oral Pathology, Ninth People’s Hospital, College of Stomatodontology, Shanghai Jiao Tong University School of Medicine

Dong-wang Zhu: zhudw9th@163.com
Ying Liu: liuying9th@163.com
Xiao Yang: yangxiao9th@hotmail.com
Cheng-zhe Yang: yangcz9th@163.com
Jie Ma: majie9th@163.com
Xi Yang: yangxi9th@163.com
Jin-ke Qiao: qiaojk9th@163.com
Li-zhen Wang: wanglizhen9th@hotmail.com
Jiang Li: lij9th@hotmail.com
Chen-ping Zhang: zhangcp9th@hotmail.com
Zhi-yuan Zhang: zhang.z.y@hotmail.com
Lai-ping Zhong: zhonglp@hotmail.com

*Dong-wang Zhu and Ying Liu contributed equally to this paper

Corresponding author: Lai-ping Zhong, PhD, MD; Associate Professor;
Department of Oral & Maxillofacial-Head & Neck Oncology, Ninth People’s
Hospital, Shanghai Jiao Tong University School of Medicine; No.639 Zhizaoju
Road, Shanghai 200011, China; Tel: +86-21-23271699-5160; Fax:
+86-21-63136856; E-mail: zhonglp@hotmail.com
Abstract

**Background:** The benefits of induction chemotherapy in locally advanced oral squamous cell carcinoma (OSCC) remain to be clearly defined. Induction chemotherapy is likely to be effective for biologically distinct subgroups of patients and biomarker development might lead to identification of the patients whose tumors are to respond to a particular treatment. The aim of this study was to investigate Annexin A1 expression in pretreatment biopsies from a cohort of OSCC patients treated with surgery and post-operative radiotherapy or docetaxel, cisplatin and 5-fluorouracil (TPF) induction chemotherapy followed by surgery and post-operative radiotherapy, and the utility of Annexin A1 as a prognostic or predictive biomarker. **Methods:** Immunohistochemical staining for Annexin A1 was performed in pretreatment biopsies from 232 out of 256 clinical stage III/IVA OSCC patients randomized to the clinical trial. Annexin A1 index was estimated as the proportion of tumor cells with Annexin A1 cellular membrane and cytoplasm staining. **Results:** There was a significant correlation between decreased Annexin A1 expression and worse pathologic differentiation grade (P=0.015) in OSCC patients. A low Annexin A1 expression predicted a better survival, especially disease-free survival (P=0.036) and locoregional recurrence-free survival (P=0.031) compared to high Annexin A1 expression. Patients with moderately/poorly pathologic differentiation grade whose tumors had low Annexin A1 expression benefited
from TPF induction chemotherapy on distant metastasis-free survival (P=0.048) and overall survival (P=0.078). **Conclusions:** OSCC patients with moderately/poorly pathologic differentiation grade and low Annexin A1 expression could benefit from the addition of TPF induction chemotherapy to surgery and post-operative radiotherapy. Annexin A1 expression might be used as a predictive biomarker in further validation studies to select OSCC patients with moderately/poorly pathologic differentiation grade that could benefit from TPF induction chemotherapy.

**Keywords:** Annexin A1; Oral squamous cell carcinoma; Induction chemotherapy
Background

Oral squamous cell carcinoma (OSCC) is the most common cancer type of head and neck cancer and the patients with OSCC have a poor clinical outcomes including treatment related organ dysfunction. The 5-year survival rate of OSCC patients is only 50-60\%^{[1,2]}. To improve the clinical management of OSCC patients, it is important to develop methods to determine which patient should be treated more aggressively as well as strategies to be used in the treatment. At current time, for patients with locally advanced and resectable OSCC, the most common treatment is radical surgery followed by post-operative radiotherapy or chemoradiotherapy depending on the presence of high risk features in the surgical specimen. Clinically, only clinical staging and pathologic differentiation grade are used to predict prognosis of OSCC patients^{[3-5]}. Therefore, it is critical to understand biological basis of OSCC and develop novel biomarkers that can distinguish OSCC based on molecular features of tumors to help predict prognosis and likelihood to benefit from a particular treatment strategy.

Induction chemotherapy is regarded as an effective way to reduce or downgrade the locally advanced or aggressive cancers, to improve the chance of eradication of locoregional lesions by radical surgery and/or radiotherapy/chemoradiotherapy, and to preserve the important organs to keep high quality of life. Recently, two randomized phase 3 trials (TAX323 and
TAX324) demonstrate that induction chemotherapy protocol of docetaxel, cisplatin and 5-fluorouracil (TPF) combination followed by radiotherapy or chemoradiotherapy can improve overall survival (OS) compared to cisplatin and 5-fluorouracil (PF)\(^6\)\(^-\)\(^8\). As a result, TPF is suggested as the preferred combination chemotherapy regimen when induction treatment is used for non-surgical management of head and neck squamous cell carcinoma (HNSCC) patients. However, it is still unknown whether TPF induction chemotherapy improves outcomes when given prior to surgery in patients with locally advanced HNSCC, especially OSCC. To address the role of induction TPF in OSCC treated with surgery (as opposed to the non-surgical approach utilized in the DeCIDE\(^9\) and PARADIGM\(^10\) studies), we recently conducted and presented the results of a randomized, phase 3 trial of induction TPF followed by surgical resection versus surgical resection upfront in patients with locally advanced OSCC\(^11\), and we failed to demonstrate a survival advantage for induction chemotherapy in the overall study population. It is possible, however, that induction chemotherapy with TPF might improve outcomes in molecularly defined subgroups of patients, and correlative studies from the aforementioned randomized trials could assist in identifying candidate biomarkers predictive of benefit from induction treatment.

Annexin A1 is an intracellular protein that can bind calcium and phospholipids. It has been suggested to an important role in inflammation response, and potential role in cell proliferation, cell signaling, phagocytosis,
and carcinogenesis\textsuperscript{[12]}. Although there is still controversial on the Annexin A1 expression in different types of cancers, such as breast cancer, pancreatic cancer, hepatic cancer, prostate cancer, urothelial cancer, cervical cancer, head and neck cancer, and so on\textsuperscript{[13-29]}, correlation between Annexin A1 expression and tumor cell differentiation has been reported that low Annexin A1 expression indicating poorly pathologic differentiation grade\textsuperscript{[13-17]}. However, the clinical usefulness of Annexin A1 expression in OSCC is not well understood, it is still unknown if Annexin A1 expression in the pretreatment biopsy from OSCC patients could be used as an indication for choosing induction chemotherapy.

The aim of this study is to evaluate the Annexin A1 expression in the pretreatment biopsy specimens from patients with resectable locally advanced OSCC and had been enrolled in a randomized phase 3 trial of TPF induction chemotherapy followed by surgery and post-operative radiotherapy compared to upfront surgery and post-operative radiotherapy, and to examine the possible prognostic and predictive role of Annexin A1 expression in this patients population. We hypothesize that low Annexin A1 expression will be associated with long survival in patients treated with surgery upfront, and will be predictive of benefit from TPF induction chemotherapy.

\textbf{Patients and Methods}
Patients

256 patients with primary and locally advanced OSCC were enrolled in a prospective, randomized, phase 3 trial at Ninth People’s Hospital, Shanghai Jiao Tong University School of Medicine\(^{[1]}\). The aim of this trial was to test the hypothesis that TPF induction chemotherapy administered prior to surgery and post-operative radiotherapy would improve survival in patients with resectable locally advanced OSCC (trial registration ID: NCT01542931). After eligibility was confirmed with written informed consent, patients were randomly allocated to the control group (surgery followed by post-operative radiotherapy) or experimental group (TPF induction chemotherapy followed by surgery and post-operative radiotherapy).

The TPF induction chemotherapy consisted of docetaxel 75mg/m\(^2\) intravenously on day 1, followed by cisplatin 75mg/m\(^2\) intravenously on day 1, followed by 5-fluorouracil 750mg/m\(^2\)/day as a 120-hour continuous intravenous infusion on days 1 through 5. Induction chemotherapy was given every 3 weeks for 2 cycles. Surgery was performed at least 2 weeks after completion of induction chemotherapy, consisting of radical resection of the primary lesion and full neck dissection with appropriate reconstruction (pedicle or free flap); frozen sections during surgery was performed to confirm adequate margins. Post-operative radiotherapy was initiated 4-6 weeks after surgery, at a dose of 1.8-2Gy/day, 5 days/week for 6 weeks, totally 54-60Gy; in patients with high risk features, such as positive surgical margins, extracapsular nodal spread, or
vascular embolism, a total radiation dose of 66Gy was recommended.

Clinical tumor response was determined by clinical evaluation and imaging studies (performed at baseline and 2 weeks after cycle 2 of induction chemotherapy). Responses were characterized according to the RECIST version 1.0\textsuperscript{30}. Pathologic response to TPF induction chemotherapy was assessed by examination of the resected specimen. A favorable response was defined as absence of any tumor cells or presence of scattered foci of a few tumor cells (minimal residual disease with $<$10\% viable tumor cells), as previously described by Licitra et al\textsuperscript{31}; an unfavorable pathologic response was defined as the presence of $\geq$10\% viable tumor cells in the resected specimen.

After treatment, patients were monitored every three months in the first two years, every six months in the subsequent 3-5 years, and once a year thereafter until death or data censoring.

Detection of Annexin A1 expression using immunohistochemistry

Pretreatment formalin fixed and paraffin embedded biopsy specimens were used for detection of Annexin A1 expression; however, in the control group, if pretreatment biopsy was unavailable, resected surgical specimens were used. Sections of 4\,µm thick were studied using both hematoxylin and eosin (HE) staining and immunohistochemical staining for Annexin A1. The HE sections were reviewed according to the WHO histological criteria\textsuperscript{32}. The
procedure of immunohistochemistry was according to the method as
previously described[33,34]. In brief, after deparaffinization, endogenous
peroxidase block and heat-induced epitope retrieval, primary rabbit polyclonal
antibody to Annexin A1 (product code of BA0640, Boster Biotech Co., Wuhan,
China) at 1:150 dilution was added overnight at 4°C, then visualized using
3,3′-diaminobenzidine (DAB) detection kit (Dako Cytomation, Denmark). The
1:150 dilution was the best dilution compared to 1:50, 1:100, and 1:200.
Negative control was prepared using PBS instead of antibody. Microscopic
examination was performed by two pathologists and all specimens were
blinded. Positive staining for Annexin A1 expression was in the cellular
membrane and cytoplasm. The Annexin A1 expression index was determined
based on the proportion of stained cells on a scale of negative to strong as
follows: negative, absence of stained cells; weak positive, <50% of stained
cells; and strong positive, ≥50% of stained cells. Low Annexin A1 expression
was defined as negative and weak positive Annexin A1 expression, high
Annexin A1 expression was defined as strong positive Annexin A1 expression.
This was based on previous studies demonstrating that the chosen cutoff of
50% was reasonable for prognostic analysis[26].

**Statistical analysis**

Overall survival (OS) was calculated from the date of randomization to the
date of death; disease-free survival (DFS) /locoregional recurrence-free
survival (LRFS) /distant metastasis-free survival (DMFS) were calculated, respectively, from the date of randomization to recurrence/locoregional recurrence/distant metastasis or death from any cause.

For descriptive analysis, categorical data were expressed as number and percentage. Chi-square test was applied to compare the difference between the baseline factors and Annexin A1 expression. The survival analysis was conducted using the Kaplan-Meier method and log-rank test. Hazard ratios (HR) were calculated using the Cox proportional hazards model. Intention-to-treat principle was applied for efficacy analysis.

All hypothesis-generating tests were two-sided at a significance level of 0.05. Data were analyzed with the statistical software SPSS13.0 for Windows (SPSS Inc., USA)

Results

Annexin A1 expression in OSCC patients

From 03/2008 to 12/2010, 256 eligible patients were enrolled in this trial (128 patients in each group), and 232 (91%, 127 patients in the control group, 105 patients in the experimental group) patients were assessed for pretreatment Annexin A1 expression levels in the tumor. Table 1 summarizes their baseline clinical characteristics, with no significant imbalance between two groups. 96 specimens (56 in the control group and 40 in the experimental
group) were low Annexin A1 expression, and 136 specimens (71 in the control group and 65 in the experimental group) were high Annexin A1 expression, with distribution balance of Annexin A1 expression pattern between the two groups (Chi-square test=0.853, P=0.356). No significant difference of Annexin A1 expression was found according to the baseline characteristics with exception of pathologic differentiation grade and alcohol use (Table 1); Annexin A1 expression level was lower in the patients with poorly/moderately pathologic differentiation grade (Figure 1) and positive alcohol use compared to those with well pathologic differentiation grade (Figure 1) and negative alcohol use, respectively. However, there was no significant difference between pathologic differentiation grade and alcohol use (P=0.499).

**Annexin A1 expression and response to induction chemotherapy**

In the experimental group, responses by RECIST in 105 patients with assessment of Annexin A1 that initiated induction chemotherapy were: 78.1% clinical response (4 patients with complete response and 78 patients with partial response) and 18.1% clinical non-response (18 patients with stable disease and 1 patient with progressive disease), 4 patients were unevaluable for response. Favorable and unfavorable pathologic responses were observed in 26.7% (27/101) and 73.3% (74/101) of patients, respectively. Pathologic response could not be evaluated in 4 patients. Annexin A1 expression did not correlate to the clinical response to TPF induction chemotherapy (Chi-square test=1.073, P=0.300), or the pathologic response to induction chemotherapy
(Chi-square test=1.820, P=0.177); even stratified according to alcohol use
(Cochran’s Mantel-Haenszel test=0.313, P=0.576 for clinical response;
Cochran’s Mantel-Haenszel test=0.488, P=0.485 for pathologic response).

**Annexin A1 expression and patients’ outcomes**

No patients were lost to follow-up; the median follow-up time was 30 months among the censored patients. There was no significant difference on OS, DFS, LRFS or DMFS between the patients with and without TPF induction chemotherapy. Locoregional recurrence and distant metastasis occurred in 30.9% and 7.0%, respectively. In general, no significant difference on locoregional recurrence or distant metastasis rates between the patients with and without TPF induction chemotherapy. However, in the experimental group, the patients with low Annexin A1 expression had a significantly lower locoregional recurrence rate, especially a lower local recurrence rate compared to that in the control group *(Table 2)*. Survival analysis using Kaplan-Meier method showed that the patients with low Annexin A1 expression had a better survival, especially the DFS and LRFS *(Figure 2)*. Using the Cox model, the patients less than 60-year old, the patients at cN1/cN2 stage, the patients with moderately/poorly pathologic differentiated grade, and the smokers with low Annexin A1 expression had a better DFS and LRFS compared to those with high Annexin A1 expression; the patients at cN1 stage or clinical stage III with low Annexin expression had a better OS, DFS,
LRFS and DMFS compared to those with high Annexin A1 expression (Figure 3). Univariate Cox model was used to analyze the impact of baseline characteristics on the time-to-event endpoints; Annexin A1 expression (low vs. high), lymph node status (cN0-1 vs. cN2, or cN0 vs. cN1-2), and clinical stage (stage III vs. stage IVA) were risk factors on OS, DFS, LRFS or DMFS. Multivariate Cox model analysis was performed using the risk factors of Annexin A1 expression and clinical stage, lymph node status (cN0-1 vs. cN2 or cN0 vs. cN1-2) was not inputted because the direct correlation between clinical stage and lymph node status. Both the clinical stage (P=0.001) and Annexin A1 expression (P=0.038) were the independent risk factors, because the factor of Annexin A1 expression by clinical stage interaction was not statistically significant (P=0.231).

**Annexin A1 expression, pathologic differentiation grade and patients’ outcomes**

In the patients with well pathologic differentiation grade, there was no significant difference on OS, DFS, LRFS or DMFS between the patients treated with and without TPF induction chemotherapy, regardless of Annexin A1 expression. In the patients with moderately/poorly differentiation grade, the patients with low Annexin A1 expression benefited from TPF induction chemotherapy on OS and DMFS; however, the patients with high Annexin A1 expression did not benefit from TPF induction chemotherapy on OS and DMFS.
Discussion

In this study, we found that Annexin A1 could be used as a prognostic biomarker in locally advanced and resectable OSCC patients, a lower Annexin A1 expression indicating a better survival. The patients with low Annexin A1 expression could benefit from TPF induction chemotherapy compared to those with high Annexin A1 expression on the aspect of rate of local failure or locoregional failure. Annexin A1 expression correlates with pathologic differentiation grade of biopsy specimens from OSCC patients, a lower Annexin A1 expression correlating with a poorer differentiation grade; furthermore, in the OSCC patients with moderately or poorly pathologic differentiation grade, those with low Annexin A1 expression could benefit from TPF induction chemotherapy on the aspect of OS and DMFS, especially DMFS.

Although the precise mechanism of Annexin A1 in cancer development and progression is still not clearly understood, more attention has been paid on this protein in the field of carcinogenesis, cancer diagnosis and cancer treatment. Annexin A1 has been previously linked with varies cancers as a tumor suppressor protein, such as breast cancer, head neck cancer, prostate cancer, cervical cancer, lung cancer\(^1\); however, increased Annexin A1
expression has also been reported in breast cancer, bladder cancer, pancreatic cancer, liver cancer, esophageal cancer, lung cancer. Recently, the prognostic value of Annexin A1 expression has been reported in lung cancer, head neck cancer, bladder cancer and breast cancer, most of which reporting Annexin A1 overexpression indicating a poor prognosis, with the exception of breast cancer reporting Annexin A1 positive expression indicating a better survival. In our study, a high Annexin A1 expression in the biopsy specimens indicates a poor prognosis, suggesting that Annexin A1 could be used as a prognostic biomarker for locally advanced OSCC.

The correlation between Annexin A1 expression and pathologic differentiation grade has also been reported in several kinds of cancers, such as thyroid cancer, cervical cancer and head neck cancer. In this study, we confirm the correlation between Annexin A1 expression and pathologic differentiation grade in OSCC, Annexin A1 expression is lower in moderately/poorly differentiation grade than well differentiation grade. Unfortunately, there is no significant difference between the pathologic differentiation grade and prognosis in both experimental group and control group; radical removal of primary lesion and full neck dissection to eradicate the lesions as much as possible might be an important factor for this result.

Correlation between Annexin A1 expression and response to induction chemotherapy has not been well documented. Absence of Annexin A1 expression coupled with presence of Annexin A2 expression is reported to
correlate with a poor pathological response to induction chemotherapy in breast cancer\textsuperscript{[36]}. Increased Annexin A1 expression is reported to correlate with anti-cancer drug resistance in some tumor cells \textit{in vitro}\textsuperscript{[37]}. In this study, we failed to find a significant correlation between Annexin A1 expression and response to TPF induction chemotherapy in OSCC. Moreover, Annexin A1 was found to have limited utility as a predictive marker of clinical or pathologic response to TPF induction chemotherapy when we looked at the entire cohort of patients that received induction chemotherapy. However, interestingly, a subgroup analysis showed that in the patients with moderately or poorly pathologic differentiation grade, those with low Annexin A1 expression did have an OS and DMFS benefit from TPF induction chemotherapy, which indicates that detection of Annexin A1 expression prior to treatment could be used to guide treatment selection. One could envision a personalized treatment scenario in which OSCC patients with moderately/poorly pathologic differentiation grade and low Annexin A1 expression receive TPF induction chemotherapy prior to surgery while those patients with high Annexin A1 expression, go straight to surgery to avoid the toxicity from chemotherapeutic agents and the delay of definitive treatment. While these results need to be considered exploratory and hypothesis generating, and clearly need to be confirmed in further clinical trials with larger sample sizes.

Conclusions
Out study suggests that Annexin A1 expression could be considered as a prognostic biomarker in patients with resectable locally advanced OSCC, and as a predictive biomarker of response to TPF induction chemotherapy in patients with moderately/poorly pathologic differentiation grade. Annexin A1 could also be used as a marker for determining the pathologic differentiation grade of OSCC, as well as a potential target for treatment.

**Disclosure of potential conflicts of interest:** The authors declare that they have no potential conflicts of interests.

**Acknowledgements:** We thank Dr. Han-bing Fu and Dr. Ting Gu for providing technical support, and Dr. Dong-xia Ye for administrative assistance. This study was supported by research grant 2007BAI18B03 from National Key Technology R&D Program of China; by research grants 30973344 and 81272979 from National Natural Science Foundation of China; by research grant 08QA14056 and 10dz1951300 from Science and Technology Commission of Shanghai Municipality.

**Authors’ contributions:** Lai-ping Zhong was responsible for the study design, interpretation of the data and revision of the manuscript. Dong-wang Zhu and Ying Liu were responsible for data acquisition, analysis of the work presented
and the preparation of the manuscript. Xiao Yang, Cheng-zhe Yang, Jie Ma, Xi Yang, Jin-ke Qiao, Li-zhen Wang, Jiang Li, Chen-ping Zhang and Zhi-yuan Zhang participated in the clinical management of the patients. All authors read and approved the final manuscript.
References


trial: A phase III study comparing sequential therapy (ST) to concurrent chemoradiotherapy (CRT) in locally advanced head and neck cancer (LAHNC) [abstract]. *J Clin Oncol* 2012, 30:356s.


26. Li CF, Shen KH, Huang LC, Huang HY, Wang YH, Wu TF: Annexin-I overexpression is associated with tumour progression and independently predicts inferior disease-specific and metastasis-free survival in urinary bladder urothelial carcinoma. *Pathology* 2010,


31. Licitra L, Grandi C, Guzzo M, Mariani L, Lo Vullo S, Valvo F, Quattrone P,


34. Zhang L, Yang X, Pan HY, Zhou XJ, Li J, Chen WT, Zhong LP, Zhang ZY: **Expression of growth differentiation factor 15 is positively correlated with histopathological malignant grade and in vitro cell proliferation in oral squamous cell carcinoma.** *Oral Oncol* 2009, **45**:627-632.


Figure legends

Figure 1 Immunohistochemical staining for Annexin A1 in the pretreatment biopsy samples from oral squamous cell carcinoma patients. (A) Well differentiated grade, (B) Moderately differentiated grade, (C) Poorly differentiated grade.

Figure 2 Overall survival, disease-free survival, locoregional recurrence-free survival, distant metastasis-free survival in the patients with low and high Annexin A1 expression. A trend of low Annexin A1 expression indicating a better overall survival (A) and distant metastasis-free survival (D) compared to high Annexin A1 expression; however, a low Annexin A1 expression significantly indicating a better disease-free survival (B) and locoregional recurrence-free survival (C) compared to high Annexin A1 expression.

Figure 3 Subgroup analysis of Annexin A1 expression according to the patients’ baseline characteristics.

Figure 4 Overall survival (A, E), disease-free survival (B, F), locoregional recurrence-free survival (C, G), distant metastasis-free survival (D, H) in the patients with moderately/poorly pathologic differentiation grade. The patient with low Annexin A1 expression benefited from TPF induction chemotherapy.
on overall survival (A) and distant metastasis-free survival (D), did not benefit from TPF induction chemotherapy on disease-free survival (B) or locoregional recurrence-free survival (C). The patients with high Annexin A1 expression did not benefit from TPF induction chemotherapy (E-H).
### Table 1 Baseline characteristics and Annexin A1 expression

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Total patients N=256</th>
<th>Annexin A1 expression</th>
<th>P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low N=96</td>
<td>High N=136</td>
<td></td>
</tr>
<tr>
<td></td>
<td>n (%)</td>
<td>n (%)</td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>179 (69.9)</td>
<td>72 (77.2)</td>
<td>88 (61.0)</td>
</tr>
<tr>
<td>Female</td>
<td>77 (30.1)</td>
<td>24 (22.8)</td>
<td>48 (39.0)</td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;60</td>
<td>168 (65.6)</td>
<td>67 (71.1)</td>
<td>90 (64.4)</td>
</tr>
<tr>
<td>≥60</td>
<td>88 (34.4)</td>
<td>29 (29.9)</td>
<td>46 (35.6)</td>
</tr>
<tr>
<td>Site</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tongue</td>
<td>113 (44.1)</td>
<td>42 (42.1)</td>
<td>56 (42.4)</td>
</tr>
<tr>
<td>Buccal</td>
<td>45 (17.6)</td>
<td>15 (17.5)</td>
<td>28 (20.3)</td>
</tr>
<tr>
<td>Gingiva</td>
<td>40 (15.6)</td>
<td>11 (12.3)</td>
<td>27 (21.1)</td>
</tr>
<tr>
<td>Floor of mouth</td>
<td>30 (11.7)</td>
<td>16 (16.7)</td>
<td>13 (8.5)</td>
</tr>
<tr>
<td>Palate</td>
<td>18 (7.0)</td>
<td>7 (6.1)</td>
<td>7 (5.9)</td>
</tr>
<tr>
<td>Retromolar trigone</td>
<td>10 (3.9)</td>
<td>5 (6.1)</td>
<td>5 (2.5)</td>
</tr>
<tr>
<td>Clinical T descriptor</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T1/T2</td>
<td>66 (25.8)</td>
<td>23 (24.6)</td>
<td>38 (28.0)</td>
</tr>
<tr>
<td>T3/T4</td>
<td>190 (74.2)</td>
<td>73 (75.4)</td>
<td>98 (72.0)</td>
</tr>
<tr>
<td>Clinical N descriptor</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N0</td>
<td>110 (43.0)</td>
<td>40 (39.5)</td>
<td>59 (45.8)</td>
</tr>
<tr>
<td>N1</td>
<td>94 (36.7)</td>
<td>33 (39.5)</td>
<td>53 (34.7)</td>
</tr>
<tr>
<td>N2</td>
<td>52 (20.3)</td>
<td>23 (21.0)</td>
<td>24 (19.5)</td>
</tr>
<tr>
<td>Clinical stage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>177 (69.1)</td>
<td>60 (65.8)</td>
<td>100 (72.0)</td>
</tr>
<tr>
<td>IVA</td>
<td>79 (30.9)</td>
<td>36 (34.2)</td>
<td>36 (28.0)</td>
</tr>
<tr>
<td>Pathologic differentiation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Well</td>
<td>80 (31.2)</td>
<td>18 (22.8)</td>
<td>47 (33.1)</td>
</tr>
<tr>
<td>Moderately</td>
<td>165 (64.5)</td>
<td>71 (71.1)</td>
<td>85 (63.6)</td>
</tr>
<tr>
<td>Poorly</td>
<td>11 (4.3)</td>
<td>7 (6.1)</td>
<td>4 (3.4)</td>
</tr>
<tr>
<td>Smoking status**</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current/former</td>
<td>126 (49.2)</td>
<td>52 (56.1)</td>
<td>58 (39.0)</td>
</tr>
<tr>
<td>Never</td>
<td>130 (50.8)</td>
<td>44 (43.9)</td>
<td>78 (61.0)</td>
</tr>
<tr>
<td>Alcohol use***</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>98 (40.6)</td>
<td>44 (48.2)</td>
<td>44 (28.0)</td>
</tr>
<tr>
<td>Negative</td>
<td>158 (59.4)</td>
<td>52 (51.8)</td>
<td>92 (72.0)</td>
</tr>
</tbody>
</table>

*P value from the chi-square test was reported to compare the difference between low and high Annexin A1 expression based on the different baseline factors.

**Former/current smokers defined as at least a one pack-year history of
smoking.
***Positive alcohol use was defined as current alcohol use of more than one drink per day for 1 year (12 ounces of beer with 5% alcohol, or 5 ounces of wine with 12%-15% alcohol, or one ounce of liquor with 45%-60% alcohol). All other patients were classified as negative alcohol use.
**Table 2** Comparison of local/regional/distant failure between low and high Annexin A1 expression in the oral squamous cell carcinoma patients treated with or without TPF induction chemotherapy

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Annexin A1 expression</th>
<th>Chi-square test</th>
<th>Cochran Mantel Haenszel test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low</td>
<td>High</td>
<td>P value</td>
</tr>
<tr>
<td>Surgery+post-operative radiotherapy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No locoregional failure</td>
<td>43</td>
<td>45</td>
<td>0.104</td>
</tr>
<tr>
<td>Locoregional failure</td>
<td>13</td>
<td>26</td>
<td>0.009</td>
</tr>
<tr>
<td>TPF+surgery+post-operative radiotherapy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No locoregional failure</td>
<td>33</td>
<td>41</td>
<td>0.034</td>
</tr>
<tr>
<td>Locoregional failure</td>
<td>7</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>Surgery+post-operative radiotherapy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No local failure</td>
<td>45</td>
<td>52</td>
<td>0.348</td>
</tr>
<tr>
<td>Local failure</td>
<td>11</td>
<td>19</td>
<td>0.020</td>
</tr>
<tr>
<td>TPF+surgery+post-operative radiotherapy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No local failure</td>
<td>37</td>
<td>47</td>
<td>0.012</td>
</tr>
<tr>
<td>Local failure</td>
<td>3</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>Surgery+post-operative radiotherapy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No regional failure</td>
<td>51</td>
<td>60</td>
<td>0.268</td>
</tr>
<tr>
<td>Regional failure</td>
<td>5</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>TPF+surgery+post-operative radiotherapy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No regional failure</td>
<td>34</td>
<td>54</td>
<td>0.795</td>
</tr>
<tr>
<td>Regional failure</td>
<td>6</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>Surgery+post-operative radiotherapy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No distant failure</td>
<td>52</td>
<td>65</td>
<td>0.786</td>
</tr>
<tr>
<td>Distant failure</td>
<td>4</td>
<td>6</td>
<td>0.367</td>
</tr>
<tr>
<td>TPF+surgery+post-operative radiotherapy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No distant failure</td>
<td>40</td>
<td>62</td>
<td>0.168</td>
</tr>
<tr>
<td>Distant failure</td>
<td>0</td>
<td>3</td>
<td></td>
</tr>
</tbody>
</table>
Figure 2

A

Overall survival

P = 0.081

Low Annexin A1 expression
n = 96

High Annexin A1 expression
n = 136

Months

0.0
10.0
20.0
30.0
40.0
50.0

0.4
0.5
0.6
0.7
0.8
0.9
1.0

B

Disease-free survival

P = 0.036

Low Annexin A1 expression
n = 96

High Annexin A1 expression
n = 136

Months

0.0
10.0
20.0
30.0
40.0
50.0

0.4
0.5
0.6
0.7
0.8
0.9
1.0

C

Locoregional recurrence-free survival

P = 0.031

Low Annexin A1 expression
n = 96

High Annexin A1 expression
n = 136

Months

0.0
10.0
20.0
30.0
40.0
50.0

0.4
0.5
0.6
0.7
0.8
0.9
1.0

D

Distant metastasis-free survival

P = 0.069

Low Annexin A1 expression
n = 96

High Annexin A1 expression
n = 136

Months

0.0
10.0
20.0
30.0
40.0
50.0

0.4
0.5
0.6
0.7
0.8
0.9
1.0

Figure 2
<table>
<thead>
<tr>
<th></th>
<th>Overall survival</th>
<th>Disease-free survival</th>
<th>Distant metastasis-free survival</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Low</strong></td>
<td><strong>High</strong></td>
<td><strong>Low</strong></td>
<td><strong>High</strong></td>
</tr>
<tr>
<td>Male</td>
<td>1.656 (0.905 - 3.028)</td>
<td>1.702 (0.976 - 2.968)</td>
<td>1.752 (0.992 - 3.095)</td>
</tr>
<tr>
<td>Female</td>
<td>0.774 (0.318 - 1.888)</td>
<td>1.315 (0.6 - 2.879)</td>
<td>1.315 (0.6 - 2.879)</td>
</tr>
<tr>
<td>Age &lt;60 years</td>
<td>1.7 (0.917 - 3.153)</td>
<td>1.768 (1.021 - 3.081)</td>
<td>1.82 (1.038 - 3.19)</td>
</tr>
<tr>
<td>Age ≥60 years</td>
<td>1.192 (0.51 - 2.788)</td>
<td>1.327 (0.596 - 2.955)</td>
<td>1.327 (0.596 - 2.955)</td>
</tr>
<tr>
<td>Buccal</td>
<td>0.647 (0.3 - 1.392)</td>
<td>0.652 (0.31 - 1.319)</td>
<td>0.623 (0.29 - 1.319)</td>
</tr>
<tr>
<td>Gingiva</td>
<td>0.841 (0.259 - 2.73)</td>
<td>0.826 (0.31 - 2.203)</td>
<td>0.826 (0.31 - 2.203)</td>
</tr>
<tr>
<td>Other sites</td>
<td>0.613 (0.171 - 2.198)</td>
<td>0.462 (0.131 - 1.626)</td>
<td>0.462 (0.131 - 1.626)</td>
</tr>
<tr>
<td>Clinical T1/2</td>
<td>0.547 (0.197 - 1.522)</td>
<td>0.661 (0.254 - 1.721)</td>
<td>0.661 (0.254 - 1.721)</td>
</tr>
<tr>
<td>Clinical T3/4</td>
<td>0.676 (0.28 - 1.2)</td>
<td>0.6 (0.254 - 1.721)</td>
<td>0.6 (0.254 - 1.721)</td>
</tr>
<tr>
<td>Clinical N0</td>
<td>0.729 (0.291 - 1.829)</td>
<td>0.748 (0.345 - 1.62)</td>
<td>0.748 (0.345 - 1.62)</td>
</tr>
<tr>
<td>Clinical N1</td>
<td>0.425 (0.181 - 0.998)</td>
<td>0.375 (0.162 - 0.89)</td>
<td>0.375 (0.162 - 0.89)</td>
</tr>
<tr>
<td>Clinical N2</td>
<td>0.788 (0.331 - 1.873)</td>
<td>0.736 (0.333 - 1.626)</td>
<td>0.736 (0.333 - 1.626)</td>
</tr>
<tr>
<td>Clinical N1+2</td>
<td>0.6 (0.331 - 1.069)</td>
<td>0.551 (0.251 - 1.484)</td>
<td>0.551 (0.251 - 1.484)</td>
</tr>
<tr>
<td>Clinical stage III</td>
<td>0.429 (0.204 - 0.902)</td>
<td>0.428 (0.218 - 0.839)</td>
<td>0.428 (0.218 - 0.839)</td>
</tr>
<tr>
<td>Clinical stage IVA</td>
<td>0.792 (0.385 - 1.526)</td>
<td>0.725 (0.379 - 1.385)</td>
<td>0.725 (0.379 - 1.385)</td>
</tr>
<tr>
<td>Well differentiated</td>
<td>0.754 (0.243 - 2.341)</td>
<td>0.681 (0.251 - 1.848)</td>
<td>0.681 (0.251 - 1.848)</td>
</tr>
<tr>
<td>MP differentiated</td>
<td>0.585 (0.335 - 1.023)</td>
<td>0.592 (0.344 - 0.999)</td>
<td>0.592 (0.344 - 0.999)</td>
</tr>
<tr>
<td>Current/former smokers</td>
<td>0.554 (0.267 - 1.149)</td>
<td>0.474 (0.238 - 0.944)</td>
<td>0.474 (0.238 - 0.944)</td>
</tr>
<tr>
<td>Never smokers</td>
<td>0.746 (0.376 - 1.481)</td>
<td>0.5 (0.249 - 1.46)</td>
<td>0.5 (0.249 - 1.46)</td>
</tr>
<tr>
<td>Positive alcohol use</td>
<td>0.514 (0.218 - 1.215)</td>
<td>0.481 (0.223 - 1.035)</td>
<td>0.481 (0.223 - 1.035)</td>
</tr>
<tr>
<td>Negative alcohol use</td>
<td>0.759 (0.411 - 1.403)</td>
<td>0.755 (0.431 - 1.324)</td>
<td>0.755 (0.431 - 1.324)</td>
</tr>
</tbody>
</table>
Figure 4