

# Prognostic analysis of patients with locally advanced nasopharyngeal carcinoma following intensity modulated radiation therapy

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**Abstract.** The present study retrospectively analyzed the prognostic factors of 135 patients with locally advanced nasopharyngeal carcinoma (NPC) who received intensity modulated radiation therapy between August 2008 and January 2012 at Xiangya Hospital of Central South University. Patients were staged from III-IVA according to the 7th American Joint Committee on Cancer staging system. Using Statistical Analysis System 9.3 software, the present study demonstrated that, among these 135 patients, the 5-year overall survival, the 5-year local relapse-free survival, and the 5-year disease metastasis-free survival were 84, 82, and 78%, respectively. Multivariate Cox regression analysis identified that targeted treatment [hazard ratio (95% confidence interval), 2.642 (1.001, 6.972);  $P=0.0497$ ] served as an independent negative prognostic factor in locally advanced NPC. The results of immunostaining revealed that the staining intensity of the radiation-resistant group was increased compared with that of the radiation-sensitive group. These results demonstrate that a high expression of EGFR may be associated with radiation resistance, and targeted treatment may not be effective in patients with locally advanced nasopharyngeal carcinoma with low expression of EGFR.

## Introduction

Nasopharyngeal carcinoma (NPC) is a head and neck malignant tumor that is common to multiple provinces in southeastern China and Asia, and northern Africa (1). Radiotherapy

targeting the nasopharyngeal tumor and draining lymph node echelons remains a common treatment for NPC (2,3). Although early stage NPC may be effectively treated by radiotherapy alone, treatments of regional advanced NPC (Union for International Cancer Control Stage III/IV) require improvements; the incidence rate of a local residual tumor following radical radiotherapy is 7.0-13.0% and the local recurrent rate ranges from 16.8 to 23.0% (4-8). To reduce the rate of local recurrence and distant metastasis, combination treatments, including chemoradiotherapy and molecular targeted therapy are particularly important for cases of locally advanced head and neck squamous-cell carcinoma (HNSCC) (9). Potential therapeutic targets for HNSCC, including epidermal growth factor receptor (EGFR), have been researched over the past decade. High EGFR expression is associated with poor prognosis in head and neck squamous-cell carcinoma and with resistance to radiotherapy (10,11). A previous study demonstrated that nimotuzumab combined with radiotherapy or chemotherapy was associated with improved locoregional tumor control and survival compared with standard chemoradiotherapy in patients with locally advanced HNSCC (12). Cetuximab has been used with radiotherapy or in combination with platinum 5-fluorouracil in advanced or recurrent patients with HNSCC (13-16). However, the use of molecular targeted drugs has been hindered: The molecular targeted therapy causes skin toxicity, and the rates of treatment sensitivity require improvement. In addition, the effect of the drugs when combined with chemotherapy or radiotherapy remains incompletely understood. Pfister *et al* (12) revealed that combining cetuximab with radiotherapy or chemoradiotherapy resulted in drug-associated toxicities in HNSCC; the early trial was subsequently suspended. Numerous other anti-EGFR agents are currently being assessed in phases II and III clinical trials in different HNSCC therapeutic settings (17). None of these molecule-targeting drugs have yet been approved by the Food and Drug Administration for use in patients with HNSCC due to the limited improvement on survival with which they are associated.

The present study assessed which factors influenced the survival of 135 patients with locally advanced NPC following

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radiotherapy. The present study revealed that targeted treatment functions were an independent negative prognostic factor in patients with locally advanced NPC.

## Materials and methods

**Patient characteristics.** The population of the present study comprised 135 patients (99 males, 36 females; 76≥45 years, 59<45 years) with locally advanced NPC who received radical radiotherapy in the Department of Radiation Oncology, Xiangya Hospital, Central South University (Changsha, China) between August 2008 and January 2012. The inclusion criteria were as follows: i) Patients exhibited pathologically proved poorly differentiated squamous-cell carcinoma; ii) patients were staged in III-IVA according to the 7th American Joint Committee on Cancer (AJCC) staging system (18) with no evidence of distant metastasis at the time of diagnosis; and iii) patients did not receive any other anticancer treatments prior to primary radiotherapy or undergo an operation following radiotherapy. The duration of overall survival was calculated from the date of radiotherapy completion to the date of mortality in the patient or the date of the last follow-up. The duration of local relapse-free survival was calculated from the date of radiotherapy completion to the date of tumor local recurrence. The duration of disease metastasis-free survival was calculated from the date of radiotherapy completion to the date of tumor distant metastasis (19,20).

**Intensity modulated radiation therapy.** In accordance with the International Commission on Radiation Units and Measurements (ICRU) reports nos. 50 and 62 (21,22), the primary tumor was named GTVnx, the positive lymph nodes were named GTVnd, and the retropharyngeal lymph nodes were included under the name GTVnx. Clinical target volume 1 (CTV1) included a 5-10 mm extension around the GTVnx and other high-risk regions, including the parapharyngeal space, the inferior part of sphenoid sinus, the posterior 1/3 of the nasal cavity, the posterior 1/3 of the maxillary sinus, the skull base, the clivus, the oval foramen, the lacerated foramen, and high-risk lymphatic drainage areas, including the retropharyngeal lymph nodes, and levels II, III, and Va of the upper cervical lymph nodes. CTV2 included the low-risk lymphatic drainage levels IV and Vb. The corresponding planning target volume (PTV) included a 3 mm margin around the CTV. A total dose of 62.72-80.64 Gy in 33 fractions to the PGTVnx (GTVnx + 3 mm margin), 69.96-72.6 Gy in 33 fractions to the PGTVnd (GTVnd + 3 mm margin), 59.4-64.0 Gy in 33 fractions to the PTV1 and 50.0-54.0 Gy in 28 fractions to the PTV2 were prescribed. All patients were treated with 1 fraction daily, 5 days/week, for 5-6 weeks (23,24). Dose limits for the critical tissue structures and the plan evaluation were designed according to the Radiation Therapy Oncology Group 0225 (25). The patients were re-examined by magnetic resonance imaging (MRI) following either the completion of radiotherapy or the radiation dose reaching ~70 Gy (26). All patients were exposed to 6MV beams and 9 fixed-gantry (0, 40, 80, 120, 160, 200, 240, 280 and 320°) angles were designated. Plans were all normalized so that 95% of the target received ≥100% prescription dose.

**Chemotherapy.** To inhibit NPC progression during treatment planning, 131 of the patients received a platinum-based chemotherapy regimen. The chemotherapy regimens included Taxol (120 mg/m<sup>2</sup> on day 1 + 80 mg/m<sup>2</sup> Cislatin/Nedaplatinon day 2; for 3 weeks/cycle), Gemcitaine (1 g/m<sup>2</sup> on day 1 and 8+80 mg/m<sup>2</sup> Cislatin/Nedaplatinon day 1, for 3 weeks/cycle) or 5-Fu [4 g/m<sup>2</sup> continuous infusion (CI) >96 h + 80 mg/m<sup>2</sup> Cislatin/Nedaplatinon day 1, for 3 weeks/cycle], for 2-6 cycles. The remaining 4 patients did not undergo chemotherapy since they were unwilling or unable. Neoadjuvant chemotherapy was administered when the waiting time for radiotherapy >1 week or to decrease the size of large tumors. Following the completion of radiotherapy, adjuvant chemotherapy was administered to the patients at stage N2/N3 and those with existing residual disease, as detected using MRI or physical examination.

**Sensitization treatment.** Intravenously, 76 patients received 750 mg sodium glycididazole/m<sup>2</sup>/fraction, 3-5 fractions/week for 5-6 weeks, within a 1 or 2 h window, to ensure that the drug remained active during administration.

**Target treatment.** A monoclonal antibody treatment of EGFR was administered to 20 of the patients who were able to purchase the drug. Of these, 16 received 100-200 mg nimotuzumab/week for 6-8 weeks, while 4 received 400 mg cetuximab/m<sup>2</sup> as an initial dose and 250 mg cetuximab/m<sup>2</sup>/week for 6-8 weeks, the duration of treatment was determined by the patient response to the drug.

**Immunohistochemical analysis.** Biopsy specimens from the 20 patients with NPC who had been receiving targeted drugs were 10% formalin-fixed and paraffin-embedded. Samples were fixed in 10% formalin at room temperature for 48 h. For the immunohistochemical detection of EGFR, 4 μm tissue sections were deparaffinized in xylene and subsequently exposed to microwaves in a microwave oven (750 W) at 60-70°C for 15 min in EDTA buffer (pH 9.0, G1202). Following cooling for 30 min and washing in PBS, endogenous peroxidase was blocked with 3% hydrogen peroxide for 25 min. Specimens were subsequently incubated at room temperature in a humidified box with PBS containing 10% normal goat serum for 30 min. Specimens were incubated overnight at 4°C with the anti-EGFR antibody (cat. no. bs-0165R, 1:200; BIOSS, Beijing, China). A horseradish peroxidase-labeled goat anti-mouse IgG secondary antibody (dilution 1:1,000, cat. no. K5007; Dako; Agilent Technologies, Inc., Santa Clara, CA, USA) was then incubated with the tissue for 30 min at 37°C. Immunostaining was detected using the ChemMate kit (cat. no. K5007, ready-to-use; Dako; Agilent Technologies, Inc.) with 3,3'-diaminobenzidine as the chromogen. For the negative control, the primary antibody was replaced with non-immune isotypic antibodies. The protocol was repeated in triplicate.

**Evaluation of staining.** The stained sections were viewed separately by two pathologists (Department of Pathology, Xiangya Hospital, Central South University, Changsha, China) who were blinded to the clinical or clinicopathological status of the cases. EGFR expression was evaluated by scanning the entire tissue specimen under low power magnification (x40) and subsequently confirmed under high power magnification

(x400). The result (positive or negative) was diagnosed through stereological cell counts. The absence of positive cells resulted in a negative diagnosis. When <25% of the cells were positive the diagnosis was weakly positive. A moderately positive diagnosis was made when the proportion of positive cells was 25-50%. A strongly positive diagnosis was made when >50% of the cells were positive. According to this method of assessment, staining scores of negative and weakly positive were regarded as representing tumors with decreased EGFR expression, while staining scores of moderately and strongly positive were regarded as representing tumors with increased EGFR expression.

**Follow-up.** The follow-up methods included direct telephone calls to the patients or their families, or hospital visits for the patients. Follow-up was measured from the first day of treatment to the last follow-up date or the date of patient mortality. Following radiotherapy, follow-up examinations were conducted once every 3 months in the first 2 years, once every 6 months in years 2 to 5, and once annually following this. MRI of the nasopharynx and neck region was performed once annually for patients with no residual tumors and once every 3-6 months for patients with residual tumors, as described previously (27). Recurrence was defined as regrowth of the tumor following disappearance for  $\geq 1$  month. The follow-up began at August 2008 and ended in September 2015. The median follow-up for survivors was 48 months (range, 2-75 months). In addition, only 129 of the 135 patients were eligible for survival analysis due the loss of follow-up for 6 patients who were treated as mortalities. The follow-up rate was 95.6%.

**Statistical analysis.** All statistical analysis was performed using Statistical Analysis System 9.3 software. Survival curves were calculated using Kaplan-Meier estimates and differences were compared using the log-rank test. Univariate and multivariate survival analysis was performed according to the Cox proportional hazards model. For all statistical tests,  $P \leq 0.05$  was considered to indicate a statistically significant difference.

## Results

**Clinical characteristics of patients.** The clinical characteristics of 135 patients with locally advanced NPC were presented in Table I. Of these patients, 59 were <45 years and 76 were  $\geq 45$  years; 19 exhibited AJCC (7th) stage III NPC and 116 exhibited AJCC (7th) stage IVA NPC. Histologically, all patients were classified as exhibiting poorly differentiated squamous-cell carcinoma according to the World Health Organization classification (28). Of the 135 patients enrolled in the present study, 20 received EGFR monoclonal antibody treatment and the remainder did not.

Only 129 of the 135 patients underwent survival analysis. In total, 4/129 patients developed local recurrence (3.1%), 20/129 patients developed distant metastasis (15.5%), and 1/129 patients developed recurrence and distant metastasis (0.8%). Mortality was recorded in 22/129 patients (17.1%); 17 of these mortality cases were due to tumor recurrence and metastasis, 3 were due to tumor-associated complications (nasopharyngeal hemorrhage, 2 cases; septic shock, 1 case),

Table I. Characteristics and Kaplan-Meier analysis of 135 patients with locally advanced nasopharyngeal carcinoma.

Parameters	n	$\chi^2$	P-value
Sex		0.0704	0.7907
Male	99		
Female	36		
Age (years)		5.3076	0.0212
<45	59		
$\geq 45$	76		
AJCC (7th) stage		0.3251	0.5685
III	19		
IVA	116		
Chemotherapy (platinum-based)		0.2491	0.6177
Yes	131		
No	4		
Prescribed dose, Gy		0.8668	0.3518
$\leq 73.92$	114		
>73.92	21		
Targeted treatment		4.9193	0.0266
Yes	20		
No	115		
Sensitization treatment		0.5548	0.4564
Yes	76		
No	59		

AJCC, American Joint Committee on Cancer; n, number of patients.

1 was due to malnutrition systemic failure and 1 was due to unknown causes.

**Univariate and multivariate Coxregression analyses.** Univariate analyses revealed that patient age ( $\chi^2=5.3076$ ,  $P=0.0212$ ) and targeted treatment ( $\chi^2=4.9193$ ,  $P=0.0266$ ) were negative prognostic predictors of overall survival in patients with locally advanced NPC (Fig. 1A and B). Multivariate Cox regression analysis indicated that targeted treatment [hazard ratio (95% confidence interval), 2.642 (1.001, 6.972);  $P=0.0497$ ] was a negative prognostic predictor of overall survival in patients with locally advanced NPC (Table II).

**Immunostaining analysis.** To further evaluate the clinical significance of EGFR, the protein expression levels of EGFR in 20 paraffin-embedded NPC biopsy specimens from 20 patients with locally advanced NPC receiving targeted treatment were determined using immunohistochemical analysis. The present study defined patients for which tumor size had decreased by <40% following radiation treatment as radiation-resistant, and those for which tumor size had decreased by >60% as radiation-sensitive (29). Of the 20 patients, 2 belonged to the radiation-resistant group and revealed increased expression of EGFR compared with the radiation-sensitive group; tumor size decreased in these patients by 37.1 and 34.6% compared with initial size. The remaining 18 patients belonged to the radiation-sensitive group, with 17 demonstrating decreased

Table II. Multivariate Cox regression analysis of 135 patients with locally advanced nasopharyngeal carcinoma.

Variables	Subset	Hazard ratio (95% CI)	P-value
Age, years	<45 vs. ≥45	2.836 (0.932, 8.631)	0.0664
Targeted treatment	Yes vs. No	2.642 (1.001, 6.972)	0.0497
Prescribed dose, Gy	≤73.92 vs. >73.92	0.528 (0.117, 2.377)	0.4050

CI, confidence interval.

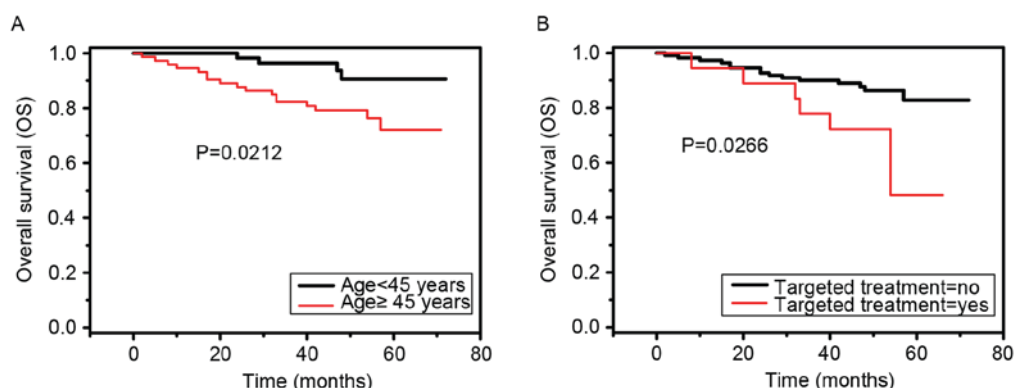


Figure 1. Kaplan-Meier survival analysis for various clinical factors. (A) Age of diagnosis in OS. (B) Targeted treatment in OS. OS, overall survival.

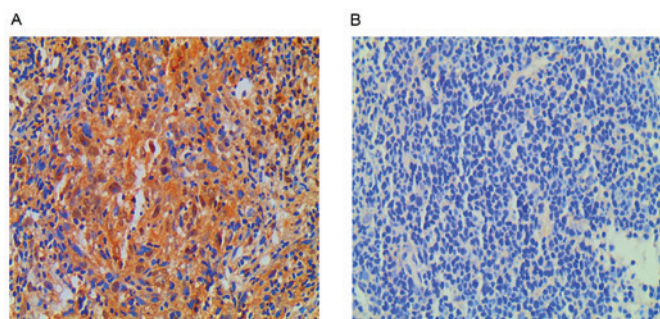


Figure 2. Representative examples of EGFR staining of tumors in the radiation-resistant and radiation-sensitive groups (magnification, x400). (A) Increased EGFR-positive staining intensity in the radiation-resistant group. (B) Decreased EGFR-positive staining in the radiation-sensitive group. Scale bar, 90 μm. EGFR, epidermal growth factor receptor.

expression of EGFR and 1 revealing increased expression of EGFR: Tumor size decreased by 73-100% in the radiation-sensitive group, compared with initial size. EGFR was located in the nasopharyngeal carcinoma cell cytoplasm. There was decreased EGFR expression in 17 of the 20 patients (85%) and increased EGFR expression in the remaining 3 patients (15%). The average staining intensity was increased in the radiation-resistant group compared with the radiation-sensitive group (Fig. 2A and B).

## Discussion

EGFR-specific monoclonal antibodies, including cetuximab and nimotuzumab, have been researched in multiple types of cancer, including sarcoma (30), gliomas (31), and non small

cell lung cancer (NSCLC) (32). These antibodies may inhibit the downstream growth-signaling pathway and may serve as a marker of tumorigenesis in NSCLC and glioma (33). However, few studies have assessed the use of targeted drugs in patients with NPC when combined with chemotherapy or radiation.

The present study enrolled 135 patients with locally advanced NPC, of which 20 received targeted treatment. The present study demonstrated that targeted treatment functions as an independent negative prognostic factor in patients with locally advanced NPC. Immunostaining analysis identified that the staining intensity of the radiation-resistant group was increased compared with that of the radiation-sensitive group. Although 1 patient in the radiation-sensitive group revealed increased expression of EGFR, the staining intensity of the tissue derived from the patient was relatively decreased compared with that from the patients of the radiation-resistant group. This result supported studies by Pan *et al* (34) and Ma *et al* (35), which demonstrated that EGFR overexpression was associated with radiation resistance in head and neck cancer, and prognostically associated with a decreased local control rate and survival. Multiple studies have revealed that ~80% of primary NPC biopsies demonstrate increased expression of EGFR (36,37). In the present study, there were 2/20 patients who received targeted treatment that belonged to radiation-resistant group, these patients exhibited EGFR overexpression. A total of 18/20 who received targeted treatment belonged to the radiation-sensitive group, only 1 patient in this group exhibited EGFR over expression. In the present study, only 15% of the patients demonstrated increased expression of EGFR.

No consensus has yet been reached regarding the effectiveness of an EGFR-targeting treatment in patients with NPC.



Pfister *et al* (12) suggested that combining cetuximab and radiotherapy or chemotherapy may improve loco-regional tumor control and the survival rate compared with conventional chemoradiotherapy in locally advanced HNSCC. Cohen *et al* (17) revealed that cetuximab, which may act as a radiosensitizer, when combined with conventional chemoradiotherapy with cisplatin remained an ineffective treatment for patients with HNSCC. This result may differ from the present study due to EGFR mutation status. Although EGFR mutations are reported to have a decreased prevalence (0-1%) in patients with NPC compared with other NPC-associated genes (38,39), they may serve an important function in tumor development where they do occur; for example, the mitogen-activated protein kinase/extracellular signal-regulated kinase pathway is downstream of EGFR and may lead to cetuximab resistance in patients with NPC (40,41).

Numerous drugs are in the early stages of development for HNSCC treatment, including novel anti-EGFR small-molecule tyrosine kinase inhibitors, EGFR antisense molecules, and multiple add-on therapies to radiation and chemotherapy intended to decrease resistance to anti-EGFR agents (42-45). In addition, numerous other anti-EGFR agents are currently being assessed in phases II and III clinical trials in different HNSCC therapeutic settings (46,47). As the targeted treatment group only had 3/20 patients with high expression of EGFR, the majority of the patients exhibited low expression of EGFR; consequently, those patients were not sensitive to cetuximab and nimotuzumab. Multiple studies have revealed that almost 80% of primary NPC biopsies indicate high expression of EGFR (36,37). EGFR of >25% was associated with a significantly poorer treatment outcome. The 5-year disease-specific survival, relapse-free survival, loco-regional relapse-free, and distant metastasis-free rates in patients with an EGFR of >25% were 48, 36, 60, and 55%, respectively. The corresponding rates in patients with an EGFR of <25% were 86, 80, 93, and 86%, respectively. The differences were all statistically significant, with the exception of distant metastasis. In multivariate analysis, EGFR extent was the only independent factor that predicted disease relapse, loco-regional failure, and mortality due to cancer (48). The present study hypothesized that treatment insensitivity was the primary reason for this unsatisfied curative effect. Collectively, the results obtained from these previously studies were similar to those presented in the present study. Results of the present study demonstrated that <25% EGFR in patients with NPC is associated with reduced overall survival. The prognosis was not only concerned with the positive expression rate of EGFR; however, it also had an association with the amount of EGFR. The possible efficacy of molecular targeted therapy against EGFR is a promising treatment strategy, future studies examining the expression of EGFR prior to treatment in patients with advance-stage NPC are required. Of course, the number of subjects should not be discounted; only 2/20 patients belonged to the radiation resistance group, and therefore the sample size was too small to perform meaningful statistical analysis independently. Validating the results of the present study requires further biopsy collections to assess the expression of EGFR in patients with NPC and whether EGFR expression is associated with radiation resistance. The limitations of the present study were as follows: On one hand, the volume of nasopharyngeal tumor

tissue was small (<0.5 cm<sup>3</sup>), meaning that they were difficult to isolate under the nasopharyngeal microscope. Additionally, targeted treatment was not included as a part of the standard therapy: Only patients with local advanced NPC who could afford the treatment were able to select the targeted drugs. The present study suggested that treatment with cetuximab and nimotuzumab may be associated with the expression of EGFR. However, it may not be effective in patients with low expression of EGFR, despite this EGFR remains an attractive target for treating certain types of cancer.

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