Concurrent chemoradiotherapy in patients with nasopharyngeal cancer: prognostic significance of low expression of bax*

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A randomized trial has demonstrated that concurrent chemoradiotherapy (CRT) is superior to radiotherapy (RT) alone in locally advanced nasopharyngeal cancer (NPC).

Our study comprise 35 patients with locally advanced NPC (stage I: 1, II: 12, III: 7, IV: 15) with 1 cycle of induction chemotherapy (5-fluorouracil 1,000 mg/m^2/day and cisplatin 20 mg/m^2/day, days 1–4) followed by concurrent CRT starting on day 22. Concurrent CRT consisted of RT (70 Gy/35 fractions for 7 weeks) with cisplatin 20 mg/m^2/day for 4 days on weeks 1, 4, 7 of RT.

Complete response (CR) was achieved in 33 patients (94%). Four-year progression-free survival (PFS) and overall survival (OS) of all patients were 57% and 65%, respectively. In analysis of prognostic factors, low expression of bax was the most significant independent predictor of poor prognosis in both PFS (p=0.002) and OS (p=0.008).

In conclusion, the outcome of patients treated with this combined therapeutical modality appears to be comparable with that of Intergroup 0099 trial with high CR rate. Low expression of bax was significantly associated with poor PFS and OS.

Key words: nasopharyngeal cancer, chemoradiotherapy, bax, low expression, prognosis

Although nasopharyngeal cancer (NPC) is common in Southeast Asia, it is an uncommon type of malignancy in Korea [1–3]. The traditional treatment approach of locally advanced NPC was radical radiotherapy (RT) alone [1, 2, 4–6]. Sequential chemoradiotherapy (CRT) approaches using neoadjuvant or adjuvant chemotherapy have failed to improve survival compared with RT alone [1, 2, 4, 6–10]. However, a phase III randomized study (Intergroup 0099) [7] demonstrated that concurrent chemoradiotherapy (CCRT) followed by adjuvant chemotherapy has been proven to be more effective than RT for locally advanced NPC [11]. Another randomized trial from Taiwan also showed improved survival with CCRT compared to RT [12]. Therefore, CCRT is now considered as a standard treatment for locally advanced NPC [13,14]. Under these backgrounds, we investigated the treatment results of locally advanced NPC patients treated with induction chemotherapy followed by CCRT in our institution with evaluation of prognostic significance of various clinicopathologic characteristics including apoptosis-related proteins, p53, bcl-2, and bax.

Patients and methods

Patients. All patients were required to have the followings to be a candidate of CRT; 1) histologically documented NPC; 2) previously untreated; 3) locally advanced stage II–IV according to the American Joint Committee on Cancer (AJCC) [15]; 4) ECOG performance status 0–2; 5) no other serious disease. Patients with distant metastasis were excluded. Each patient underwent the following staging procedures: physical examination, chest radiograph, MRI with or without CT of the nasopharynx and neck, FDG-positron emission tomography (PET) scan of head and neck, radionuclide bone scan, abdominal ultrasonography, pure tone audiometry (PTA), and hematologic and biochemical profiles. Although this treat-

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ment protocol was not clinical trial, patients were followed and treated prospectively.

Informed consent for the treatment was obtained from each patient before treatment. This research protocol was approved by the Institutional Review Board of the Ajou University Medical Center.

Radiotherapy. Radiotherapy was administered on linear accelerator. Patients were treated 5 days per week in daily radiation dose of 2 Gy with single fraction per day. Total dose to primary tumor was 70 Gy in 7 weeks. Primary tumor was irradiated with 6MV X-ray using conventional bilateral portals or multiple portals of conformal or intensity modulated radiation therapy (IMRT) techniques. Some patients were treated with conventional bilateral portals initially followed by boost dose of 10–16 Gy with conformal or IMRT techniques. When using 3 dimensional radiotherapy techniques such as conformal or IMRT, physician's tumor volume included gross tumor volume and nasopharynx proper with 0.5–1 cm margin. We did not limit the radiation dose to inner ear structure on radiation treatment planning. Forty-four to 50 Gy of radiation was delivered for elective neck treatment.

Chemotherapy. After completion of diagnostic procedures, patients were treated by induction chemotherapy with 5-fluorouracil (5-FU) 1,000 mg/m^2/day and cisplatin 20 mg/m^2/day continuous intravenous infusion days 1–4. CCRT started on day 22 consisting of RT with cisplatin 20 mg/m^2/day continuous infusion for 4 days during weeks 1, 4, and 7 of RT. After completion of CCRT, two cycles of adjuvant chemotherapy with the same regimen as in the induction chemotherapy were given if possible.

Evaluation. The response to treatment was assessed 8 weeks after completion of CCRT using MRI with or without FDG-PET scan as well as physical examination including flexible nasopharyngoscopy. Physical examination was performed monthly up to 1 year, every 3 months for 2 year, and every 6 months thereafter. MRI and FDG-PET scan were done alternatively every 3 months after initial posttreatment evaluation up to 2 years. Then, the patient was evaluated with FDG-PET scan every 6 months for 3 years, and yearly thereafter.

Response to treatment was evaluated according to the World Health Organization criteria [16]. The complete response (CR) was defined as the complete disappearance of all symptoms and signs of tumor for more than 4 weeks. The partial response (PR) was defined as a reduction by 50% or more of the sum of the products of all measurable lesions lasting more than 4 weeks. Stable disease was indicated by a less than 50% reduction or less than 25% increase in tumor size. Progressive disease was defined as an increase of more than 25% in tumor size or the appearance of new lesions. Toxicity was assessed using the World Health Organization criteria [16].

Immunohistochemical staining for apoptosis-related proteins. Immunohistochemical staining of formalin-fixed, paraffin-embedded tumor tissue was performed using mouse anti-human monoclonal antibodies against p53 (DO-7, dilution 1:20, Novocastra Laboratories Ltd, Newcastle upon Tyne, United Kingdom) and bcl-2 (NCL-bcl-2, dilution 1:50, Novocastra Laboratories Ltd), and rabbit anti-human polyclonal antibody against bax (dilution 1:1,000, DAKO, Carpinteria, CA, USA).

Sections were deparaffinized in xylene and rehydrated in graded alcohols and water. Endogenous peroxidase activity was blocked by treatment with 3% hydrogen peroxide for 10 minutes. Sections were treated with protein-blocking solution and then with primary antibodies for overnight at 4 °C. After several rinses in phosphate-buffered saline, sections were incubated in biotinylated secondary antibody. Bound antibodies were detected by the streptavidin-biotin method with a Cap-Plus detection kit (Zymed Laboratories Inc, San Francisco, CA, USA). Slides were rinsed in phosphate-buffered saline, exposed to diaminobenzidine, and counterstained with Mayer's hematoxylin. Negative controls for these proteins were made by the omission of the primary antibody during the process of immunohistochemical staining. For positive controls for bax and bcl-2, lymphocytes in germinal center and interfollicular area of normal lymph nodes were used, respectively. Tissue section of colon adnocarcinoma known to have high expression of p53 was used as a positive control for p53.

The slides were examined independently by two observers (JHH, HJJ) blinded to both clinical and pathologic data. Expression of the apoptosis-related proteins was quantified using a visual grading system based on the extent of staining (percentage of positive tumor cells and graded on a scale of 0 to 4; 0 = none, 1 = 1–25%, 2 = 26–50%, 3 = 51–75%, 4 = >75%) as well as the intensity of staining (graded on a scale of 0 to 3; 0 = no staining, 1 = weak staining, 2 = moderate staining, 3 = strong staining). Because the paraffin blocks for immunohistochemistry were unavailable in 3 patients, they were excluded from analysis for the prognostic significance of the apoptosis-related proteins.

Statistical analysis. Progression-free survival (PFS) and overall survival (OS) were calculated using KAPLAN-MEIER method [17]. PFS was defined as the time from start of treatment to disease progression, or second primary cancer, or death from any other cause. Data on patients who did not have a progression were censored at the last follow-up. OS was defined as the time from start of treatment to death; data on survivors were censored at the last follow-up. The differences between the survival curves were tested by using the log-rank test. Cox proportional-hazards regression model was used to determine the joint effects of several variables on survival [18]. All analyses were performed with SPSS for Windows 12.0 software.

Results

Patient characteristics. Between October 1996 and October 2001, 35 patients were treated with induction chemother-

apy followed by CCRT. Clinicopathologic characteristics of 35 patients were listed in Table 1. One patient in stage I was included in this protocol before the publication of 1997 AJCC staging, because he was in stage II with 1992 AJCC staging.

All patients completed induction chemotherapy and RT, although 2 patients could not receive cisplatin during RT because of severe toxicity during the induction chemotherapy (hyponatremia and vomiting, respectively). Eight patients (23%) completed 3 cycles of cisplatin chemotherapy during radiotherapy, while 21 (60%) and 4 patients (11%) received 2 and 1 cycles, respectively. The main reason for not finishing planned 3 cycles of chemotherapy was side effects of CCRT (oral mucositis and/or leukopenia). Although 2 cycles of adjuvant chemotherapy was recommended after completion of CCRT, only 5 patients (14%) received it. Most of the patients refused the adjuvant chemotherapy due to incomplete recovery from side effects of CCRT or fear of re-experiencing the toxicity.

Toxicity. CCRT following induction of chemotherapy was generally associated with significant side effects. Every patient experienced oral mucositis, which was severe (grade ≥3) in 57% of patients. In addition, radiation dermatitis, xerostomia, and ototoxicity were very frequent side effects, while hematologic toxicity was not so significant. There was 1 treatment-related death from radiation-induced brain necrosis 31 months after completion of RT.

Treatment response. Based on post-treatment imaging, CR was achieved in 33 patients (94%), PR in 1 patient (3%), and 1 patient (3%) demonstrated progressive disease.

Survival and prognostic factor analysis. The median follow-up duration of the survivors was 59 months (range: 39–100 months) and no patient was lost to follow-up. Among 33 patients with CR, 12 patients experienced recurrence of disease, 3 local, 2 regional, 1 locoregional, and 6 distant. Fourteen patients died, 12 from recurrence or disease progression, 1 from radiation-induced brain necrosis, and 1 from unknown cause, but probably recurrence. Two patients with recurrence were alive at the time of analysis.

Four-year PFS and OS of total patients were 57% and 65%, respectively (Fig. 1). In univariate analysis, while advanced stage and squamous cell histology (WHO type I or II) were associated with poor PFS and OS, old age (>median age) predicted poor OS (Tab. 2). Overexpression of p53 or bcl-2 was not associated with poor PFS or OS using any parameters including percentage of positive tumor cells, intensity of staining, and combination of extent and intensity of staining (data not shown). The score for the expression of bax ranged from from 0 to 12, which was obtained by multiplying the grades of extent and intensity of staining. The expression of bax was divided into high bax (>6) (18 patients) and low bax (≤6) (14 patients) (Fig. 2). Four-year PFS (78% vs. 29%, p=0.007) and OS (83% vs. 38%, p=0.005) were significantly inferior in patients with low expression of bax (Fig. 3). In

Table 1. Characteristics of patients

Characteristics	Number (%)
Age (years)	
Median	47
Range	19–64
Gender	
Male	26 (74)
Female	9 (26)
Performance status	
0–1	33 (94)
2–3	2 (6)
Histologic type	
WHO type I	2 (6)
WHO type II	9 (26)
WHO type IIIl	24 (68)
Stage (AJCC, 1997)	
I	1 (3)
II	12 (34)
III	7 (20)
IV	15 (43)

Table 2. Progression-free and overall survival of the patients according to various characteristics

Characteristics	No. of patients	4-year progression-free survival (%)	p value	4-year overall survival (%)	p value
Age			0.211		0.035
≤47*	18	67		83	
>47	17	47		46	
Gender			0.349		0.623
Male	26	54		65	
Female	9	67		67	
Histologic type			0.032		0.044
WHO type I or II	11	36		55	
WHO type III	24	67		70	
Stage			0.003		0.006
IB or II	13	92		92	
III	7	43		57	
IV	15	33		46	
Cisplatin during radiotherapy			0.373		0.583
3 cycles	8	50		63	
0–2 cycles	27	59		66	
Adjuvant chemotherapy			0.847		0.860
Yes	5	60		80	
No	30	57		63	

*median age

multivariate analysis, low expression of bax was the most significant independent predictor of poor prognosis in both PFS and OS (Tab. 3, 4).

Discussion

The potential advantages of CCRT include a possible additive or synergistic effect with RT, no delay in primary RT, no

Table 3. Multivariate analysis of progression-free survival*

Prognostic factors	Hazard ratio	95% CI**	p value
Age			
≤47	1.00		
>47	2.76	0.86–8.79	0.087
Gender			
Male	1.00		
Female	0.24	0.06–1.00	0.050
Histologic type			
WHO type I or II	1.00		
WHO type III	0.73	0.25–2.15	0.564
Stage			
I–III	1.00		
IV	2.31	0.77–6.94	0.137
Bax expression			
High	1.00		
Low	6.98	2.01–24.2	0.002

*three patients without bax staining were excluded from analysis, **Cl – confidence interval

Table 4. Multivariate analysis of overall survival*

Prognostic factors	Hazard ratio	95% CI**	p value
Age			
≤47	1.00		
>47	4.23	1.20–14.9	0.025
Gender			
Male	1.00		
Female	0.30	0.06–1.46	0.136
Histologic type			
WHO type I or II	1.00		
WHO type III	0.75	0.23– 2.50	0.645
Stage			
I–III	1.00		
IV	3.49	1.03–11.9	0.045
Bax expression			
High	1.00		
Low	6.08	1.59–23.2	0.008

*three patients without bax staining were excluded from analysis, **Cl – confidence interval

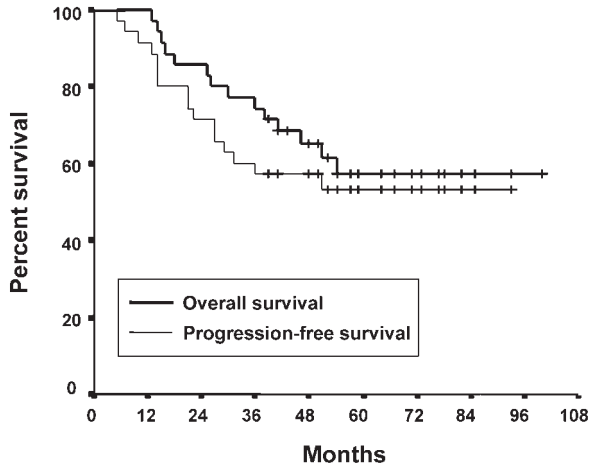

Figure 1. Progression-free and overall survival of NPC patients.

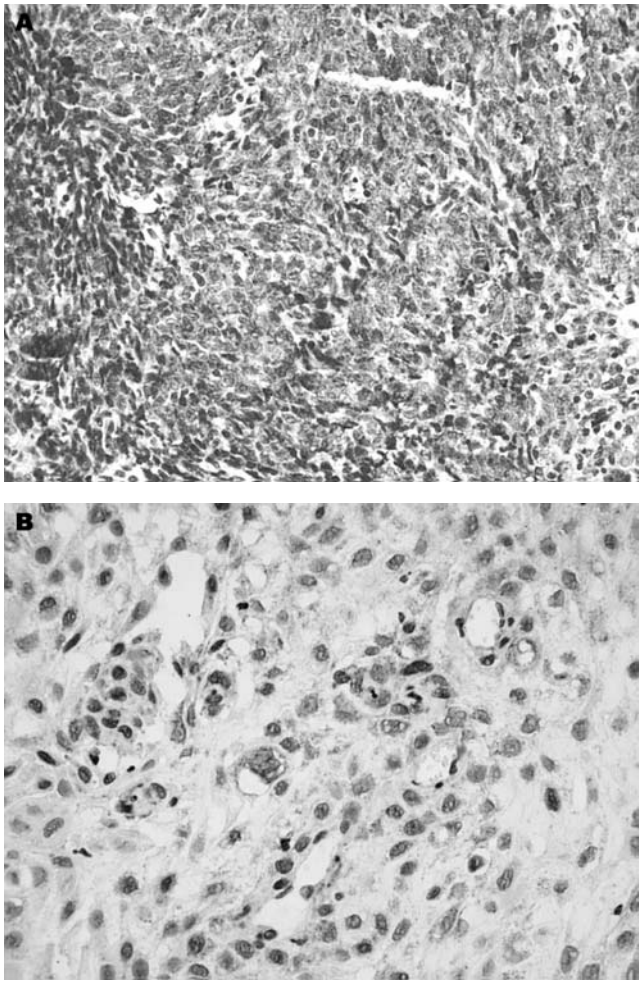

Figure 2. Immunohistochemical staining of bax. (A) High expression of bax: grade 4 in the extent of staining and strong staining (x400). (B) Low expression of bax: grade 2 in the extent of staining and weak staining (x400).

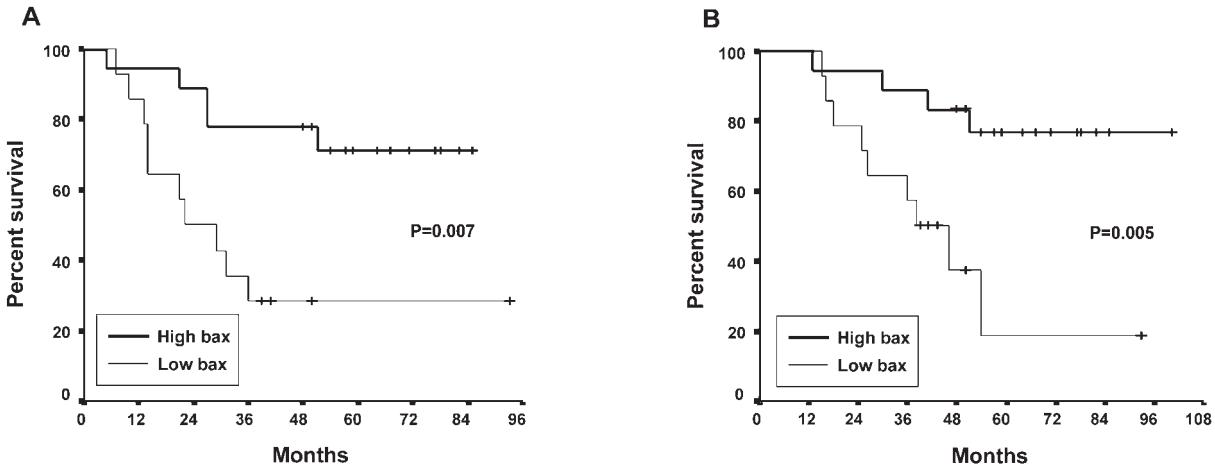

Figure 3. Progression-free survival (A) and overall survival (B) according to the expression of bax.

compromised tumor blood supply leading to an enhanced tumor reoxygenation, with no time for the evolution of cross resistance or accelerated repopulation, triggered by neoadjuvant chemotherapy [12]. Two randomized trials from USA and Taiwan demonstrating the survival advantage of CCRT over RT alone supported these concepts in treatment for locally advanced NPC [11, 12].

In our institution, locally advanced NPC had been treated with CRT protocol similar to that of Intergroup study with some modifications. Unlike Intergroup study, in which cisplatin infusion started concurrently with RT, 1 cycle of 5-FU/cisplatin induction chemotherapy was administered before CCRT. This modification was based on the following practical reason. Significant proportion of patients had to receive pretreatment dental care and sophisticated RT planning, which inevitably required the delay of RT. According to this protocol, patients could undergo these procedures during or after induction of chemotherapy. In addition, we administered cisplatin 80 mg/m^2/cycle by continuous infusion for 96 hours to decrease the severity of emesis.

The 4-year PFS (57%) and OS (65%) in the current study were almost comparable to those of the Intergroup study (60% and 67%, respectively, estimated from the survival curve) [2, 11]. The results of CCRT were also reported as randomized trials or retrospective analysis in Southeast Asian countries where NPC is endemic [12–14, 19, 20]. PFS and/or OS of these studies were somewhat superior compared to those of the current study or Intergroup trial, although direct comparison was difficult because of different median follow-up duration and patient characteristics [11–14, 19, 20]. For example LIN et al [12] demonstrated that 5-year PFS and OS were 71.6% and 72.3%, respectively, in 141 NPC patients treated with CCRT with 5-FU and cisplatin, which was significantly superior to RT alone. A familiarity with NPC in the Southeast Asian groups may be attributable to the difference in the treatment effects [1, 4]. On the other hand, relatively low proportion of undifferentiated carcinomas (68%), which are more sensitive to RT, in the present study compared to that of Southeast Asian series (>90%) could be an alternative explanation [1, 4].

CRT in the present series resulted in very high CR rate (94%) comparable to those (95–98%) of CCRT trials in endemic countries [12–14]. Response evaluation was strict in our cohort because FDG-PET scan was performed to confirm CR if there was suspicious lesion in MRI. Despite of high CR rate, significant proportion of patients experienced recurrence with equal proportion of locoregional and systemic failure. One problem in the present study is that only 14% of patients actually underwent adjuvant chemotherapy after CCRT, although it was recommended as an initial plan. However, the role of adjuvant chemotherapy has been questioned in many studies. First, adjuvant chemotherapy has problems in practical points. In clinical trials of NPC, a large proportion of patients have had difficulties in tolerating adjuvant chemotherapy after CRT or RT [1, 2, 10, 11, 13, 19]. For example, in Intergroup study, 55% of patients received the three planned cycles of adjuvant chemotherapy and 33% never underwent it [11]. Moreover, patients and physicians frequently concern "re-experiencing" mucositis from adjuvant chemotherapy after full-dose RT [2, 10]. Another problem is questionable role of adjuvant chemotherapy for improving survival in addition to CCRT. At present a number of studies have failed to demonstrate any survival benefit for patients receiving post-RT adjuvant chemotherapy compared to those treated with RT alone [1, 2, 10]. Furthermore, in two randomized trials from Southeast Asian countries showing benefit of CCRT over RT in terms of PFS and/or OS with excellent treatment results, adjuvant chemotherapy was not included in the protocols [12, 14]. Therefore, the most of the survival benefits of CCRT plus adjuvant chemotherapy protocols may derive from CCRT rather than from adjuvant chemotherapy. In the presented results, small number of patients with adjuvant chemotherapy did not show any survival advantage. Although a prospective randomized trial is mandatory to

prove the role of adjuvant chemotherapy, adjuvant chemotherapy does not seem to be an essential component in the treatment of NPC in terms of feasibility as well as the treatment results so far.

One important finding of the current study was the prognostic significance of low expression of bax. While the analysis of prognosticators has been seldom performed in CRT trials, advanced stage and old age are known to be independent poor prognostic factors in NPC patients treated with RT alone or CRT [1, 21, 22]. In the presented study, low expression of bax was the most significant poor prognostic factor in terms of PFS and OS in multivariate analysis surpassing advanced stage and old age. Apoptosis is a predominant mechanism of cancer cell death by chemotherapy and plays also an important role in radiation-induced cytotoxicity [23, 24]. Among several proteins involved in apoptosis, the fine interplay between the bcl-2 family anti-apoptotic members and death-promoting members like bax and p53 has been suggested as the most important process [23–25]. Therefore, genetic defect in these proteins may result in resistance to the cytotoxic effects of chemotherapy and RT [26]. In NPC, small number of studies investigated the prognostic significance of apoptosis-related proteins [27–31]. Despite of correlation between overexpression of bcl-2 or p53 and the risk of progression after RT and/or chemotherapy in a few reports, their role as an independent predictor of poor OS was not demonstrated [27–31]. We evaluated the expression of bcl-2, p53, and bax under the assumption that abnormality in these apoptosis-related proteins may be associated with resistance to cisplatin-based CCRT, ultimately leading to poor survival. The role of low expression of bax as a poor prognostic factor has been reported in ovarian cancer patients treated with cisplatin-based chemotherapy [32, 33]. Furthermore, low expression of bax was also associated with adverse prognosis in several malignancies treated with RT such as prostate cancer and head and neck cancer including larynx, tongue, oropharynx, and maxillary sinus cancer [34–38]. Therefore, poor outcome of NPC patients with low expression of bax may have clinical relevance because both RT and cisplatin, which are integral components of treatment for NPC, depend on apoptosis for their cytotoxic effects.

To our knowledge, the current study is the first report investigating the relationship between the expression of bax and outcome in NPC patients. The present result suggests that the prognosis of patients with low expression of bax would be poor by cisplatin-based CCRT, which is now considered as the standard treatment for locally advanced NPC. Although further prospective studies with large cohort is necessary, CCRT or induction chemotherapy followed by CCRT incorporating new chemotherapeutic agents with high activity in head and neck cancer such as taxanes should be considered in patients with low expression of bax [39–41].

In conclusion, the outcome of locally advanced NPC patients treated with CCRT following induction chemotherapy appears to be comparable with that of Intergroup 0099 trial with high CR rate. In addition, low expression of bax was significantly associated with poor PFS and OS.

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