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Original Article

PROGNOSTIC VALUE OF P53 AND THYMIDYLATE SYNTHASE (TS) OVER EXPRESSION IN PATIENTS WITH SQUAMOUS CELL CARCINOMA OF THE HEAD AND NECK TREATED WITH NEOADJUVANT CHEMOTHERAPY

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ABSTRACT

Aim of the Work: Neoadjuvant cisplatin-based chemotherapy has been widely used in the last decade for organ preservation or unresectable disease in advanced stage head and neck cancer. Thymidylate Synthase (TS) and p53 are central molecules in the regulation of cell growth. Differences in the intracellular expression of these proteins by tumor cells may have predictive value for response to chemotherapy and early failure in patients with Squamous Cell Cancer of the Head and Neck (SCCHN). We examined the expression of P53 and TS that have been associated with chemotherapy resistance.

Patients and Methods: Immunohistochemistry was used to assess the tumor cell expression of TS and p53 in pre-therapy biopsies from 65 patients with SCCHN treated with induction chemotherapy.

Results: The overall response rate for cisplatin-based neoadjuvant treatment was 80%. The expression of P53 and TS was associated with resistance to neoadjuvant treatment, but none reached statistical significance. Overall survival (OS) was strongly correlated with the absence of p53 expression. The OS at three years was 80% in the p53-negative group, whereas it was 29% in the p53-positive group for patients treated with neoadjuvant chemotherapy ($P < 0.0001$). Expression of TS was also significantly correlated with decreased OS after neoadjuvant treatment. In the TS-negative group, the three years OS rate was 69% compared with 38% in the TS-positive group ($P = 0.0063$).

Conclusion: Our data showed that p53 and TS may be clinically important predictors of survival in patients receiving neoadjuvant chemotherapy for head and neck cancer.

Key Words: Head and neck cancer, neoadjuvant chemotherapy, P53 and thymidylate synthase.

INTRODUCTION

Cisplatin-based neoadjuvant chemotherapy is widely used in the treatment of head and neck cancer. In untreated patients with head and neck squamous cell carcinoma, overall responses of 70–90% with CRs of ≥30% have been reported when cisplatin is combined with 5-fluorouracil (5-FU)[1].

Neoadjuvant chemotherapy has been used increasingly in the last decade for organ preservation and for unresectable disease in head and neck tumors[2-6]. However, despite the high response rate to neoadjuvant chemotherapy, there is no clear evidence of improved survival for patients treated with cisplatin-based chemotherapy in this setting. Some investigators have concluded that neoadjuvant chemotherapy should not be offered to patients with locally advanced head and neck cancer if improved survival is to be the criteria for selection of treatment[7].

At the present time, there are no accepted prognostic markers that can guide the selection and treatment of patients with head and neck squamous carcinomas or predict the long-term outcome of such treatment. In theory, such markers would be very useful, especially in the neoadjuvant setting because alternative treatments including surgery could be offered to patients who are likely to fail after neoadjuvant treatment.

In head and neck cancer, a number of cellular factors may be important in clinical resistance to both cisplatin and 5-fluorouracil, which is the most widely, used drugs for neoadjuvant treatment. Several cellular products are potentially important in regulating cellular resistance to cisplatin-based chemotherapy in head and neck cancer.

Induction chemotherapy has also been shown to be an effective replacement for surgery in patients with resectable disease without compromising survival, creating the opportunity to preserve laryngeal and swallowing function in these patients[8,9].

Two intracellularly expressed proteins, thymidylate synthase (TS) and p53 may have predictive value for response to chemotherapy. TS is a central enzyme in DNA synthesis and one of the principal targets of 5-fluorouracil (5-FU)-based chemotherapy. TS is the major source of thymidylate, an essential molecule for DNA synthesis. Thymidylate is required for the growth of dividing cells and repair of DNA damage. Increased TS expression has been shown to be a predictor of poor response to 5-FU-based chemotherapy in patients with metastatic colorectal and primary gastric carcinoma[10-12]. In adjuvant trials of breast and rectal carcinoma, high TS expression in tumors is associated with a poorer outcome but improved efficacy of 5-FU-containing adjuvant therapy[13,14]. p53 is a central mediator of cell growth, cell sensitivity to chemotherapy, radiation-induced DNA damage, and programmed cell death[15-18]. Thus, abnormal expression or function of p53 in primary tumors might be expected to predict responsiveness to chemotherapy and radiation. Aberrant, increased expression of p53 has been associated with both increased and decreased response to platinum-based therapies in several tumor systems[16,19,20]. In one study, p53 expression was associated with improved laryngeal preservation after PF chemotherapy without effect on survival or response to chemotherapy[7].

To establish the relative value of tumor cell p53 expression and TS content as predictors of response and locoregional disease control after neoadjuvant chemotherapy, we performed a prospective study of a subset of 65 patients treated with cisplatin/5-FU chemotherapy as neoadjuvant after their initial biopsy either for organ preservation or for unresectable disease.

PATIENTS AND METHODS

Patients: From August 1997 to July 2001, 65 patients with head and neck squamous carcinoma were entered and evaluated for this study. All patients received neoadjuvant chemotherapy after their initial biopsy either for organ preservation or for unresectable disease.

The initial evaluation included a history and physical examination, complete blood cell (CBC) count, routine serum chemistries, creatinine clearance, chest X-ray, C.T. scan or MRI of the head and neck, and bone scan. Local tumor extent and regional metastases were further assessed by triple endoscopy.

All patients received cisplatin, 100mg/m^2, on day one, followed by a continuous infusion of 5-FU at 1000 mg/m^2 per day for five days. Patients were treated every 3 weeks for a minimum of two cycles. Patients treated with neoadjuvant chemotherapy who had a>50% (partial) response at the end of two cycles of chemotherapy had a third chemotherapy treatment and went on to receive definitive radiation therapy. Patients who presented with bulky (N2 or N3) neck disease generally had neck dissection at the completion of radiation therapy.

Immunohistochemistry:

Blocks of paraffin-embedded tissues were obtained from the original primary biopsies in all study patients, and slides were generated from tumor-containing materials for immunohistochemistry. The specimens were then deparaffinized in xylene, dehydrated through graded alcohols and placed in 0.1% hydrogen peroxide to quench endogenous peroxidase activity. Following microwave pretreatment in citrate buffer (pH 6.0) three times for 5min at 750W, these sections were treated with 10% normal rabbit or goat serum for 30min to prevent non-specific binding of the antibody. The slides were then incubated with different antibodies overnight at 4°C. We used an anti-p53 antibody (DO7, 1:100 dilution; Novacastra Laboratories, Newcastle, United Kingdom) and anti-TS mouse monoclonal antibody (1:50 dilution; Novacastra Laboratories, Newcastle, United Kingdom). Tissues were incubated with a biotin-labeled rabbit anti-mouse or goat anti-rabbit secondary antibody [Histofine SAB-PO (M) kit; Nichirei, Tokyo, Japan] for 30min at 37°C followed by reaction with streptavidin-biotin horseradish peroxidase complex [Histofine SAB-PO (M) kit]. The reaction products were visualized by immersing the slides in freshly prepared diaminobenzidine solution for 10min and counterstained with hematoxylin before dehydration and mounting.

P53 staining was graded as present or absent in viable tumor cells in the specimens and was considered positive if staining was observed in >10% of the nuclei of viable tumor cells. TS expression was scored as focal or diffuse staining on the basis of a 25% cut-off of the number of cells stained. Intensity of staining was graded on a 0 to 3+ system, as described by Pestalozzi et al[15].

Evaluation of Response and Survival:

Response to neoadjuvant chemotherapy was evaluated clinically by endoscopic examination before each cycle and radiologically by C.T. scan or MRI performed after the second cycle. Assessment of palpable lymph nodes was done also by clinical examination and palpation.

Tumor responses were defined as complete response (CR), the disappearance of all clinically or radiologically evident tumor; partial response (PR), a>50% reduction in the product of two perpendicular diameters of all measurable tumor but less than CR; and no response (NR), (anything less than the above). DFS and OS were

determined from the date of initial diagnostic biopsy. DFS of last patient contact.

Statistical Analysis:

Univariate analyses for recurrence-free survival and OS included Kaplan-Meier survival estimation (EPI-INFO version 6.1 software package, Atlanta, Georgia, USA), with statistical significance assessed via the log-rank test and the Gehan-Wilcoxon test. Multivariable statistical models were generated using a proportional hazards regression approach[25].

RESULTS

Clinicopathological Parameters and Response:

The clinicopathological characteristics of the 65 patients with SCCHN are shown in table 1. The overall response rate (CR+PR) to neoadjuvant chemotherapy was 80%, 35 patients (53.9%) had complete response to induction chemotherapy (Table 2).

P53 and TS Expression and Response to Neoadjuvant Chemotherapy:

Positive staining (>10% cells) for p53 was observed in the nuclei of the cancer cells in 24 of 68 cases (36.9%) while TS expression was observed in the cytoplasm of the cancer cells in 36 of 65 cases (55.4%). Expression of p53 and TS was not associated with age, gender, site of primary tumor, clinical stage, T stage, and N stage. Table 3 shows the relationship between p53 and TS and clinical response to neoadjuvant chemotherapy. P53 or TS expression was not significantly correlated with response. Other parameters, such as age, gender, clinical stage, T stage, N stage, and tumor differentiation were not significantly correlated with clinical response to chemotherapy.

P53 and TS Expression and Survival:

Table 4 shows the correlation between response to chemotherapy, p53, TS and DFS in the 65 patients analysed. DFS (or PFS in patients who had less than a PR to neoadjuvant chemotherapy and were never free of disease) was significantly correlated with clinical response to neoadjuvant chemotherapy for all patients (Figures 1, 2) (P <0.0001; Hazard ratio, 4.54; Figure 3A). Poor response to treatment (NR, PD) was associated with shorter PFS. At 36 months after diagnosis, the PFS rate was 58% in responders, whereas it was zero in non-responders. For p53, DFS was approximately two times longer for patients who were p53-negative compared with patients who were p53-positive, although the difference did not achieve statistical significance (P = 0.117; Hazard ratio, 1.56; Figure 4A). Expression of TS was not

Table 1: Pretreatment clinicopathological characteristics.

Characteristic	No. of patients	%
Age (yr)		
Median	56	
Range	39-66	
Sex		
Male	52	80
Female	13	20
Clinical stage		
II	5	8
III	20	31
IV	40	61
T		
1	1	1.5
2	17	26.2
3	29	44.6
4	18	27.7
N		
0	21	32.3
1	12	18.5
2	22	33.8
3	10	15.4
Primary site		
Nasopharynx	3	4.6
Oral cavity	18	27.7
Oropharynx	10	15.4
Hypopharynx	15	23.1
Larynx	19	29.2
Pathological grade		
Poor	19	29.2
Moderate	31	47.7
Well	10	15.4
Unknown	5	7.7

Table 2: Response to neoadjuvant chemotherapy.

Response	No. (%)
CR	35 (53.9)
PR	16 (24.6)
NR or PD	14 (21.5)

Table 3: Correlation of P53 and TS with clinical response to neoadjuvant chemotherapy.

	No. (%)	CR+ PR	Response rate (%)	P*
p53				
-ve	41 (63.1)	34	83	0.426
+ve	24 (36.9)	17	71	
TS				
-ve	29 (44.6	25	86	0.413
+ve	36 (55.4)	26	72	

significantly correlated with DFS in patients treated with neoadjuvant chemotherapy. Clinical parameters including age, gender, clinical stage, T stage, site of primary tumor, and tumor differentiation were not correlated with DFS in neoadjuvant cases.

Table 5 shows the correlation between response to neoadjuvant chemotherapy, p53, TS and OS. OS was significantly correlated with clinical response to neoadjuvant chemotherapy (P <0.0001; Hazard ratio, 3.34; Figure 3B). Poor response (NR, PD) was associated with shorter OS. For neoadjuvant treatment, expression of p53 was very significantly correlated with OS (P < 0.0001; Hazard ratio, 6.21; Figure 4B). OS was 80% at 3 years after diagnosis in patients with p53-negative tumors, compared to 29% in patients with p53-positive tumors. Those patients with significant TS expression had an overall poorer survival compared with patient whose tumors did not express TS (P = 0.0063; Hazard ratio, 2.81; Figure 5).

Table 4: Correlation between response to chemotherapy, P53, TS and DFS.

	36 months DFS (%) (SEM)	Hazard ratio	95%confidence interval of hazard ratio	P*
Response				
CR/PR	58 (7)	1.00		
NR/PD	0	4.54	6.12–58	<0.0001
p53				
-ve	53 (8)	1.00		
+ve	35 (10)	1.56	0.87–3.88	0.117
TS				
-ve	54 (12)	1.00		
+ve	35 (10)	1.74	0.80–3.88	0.161

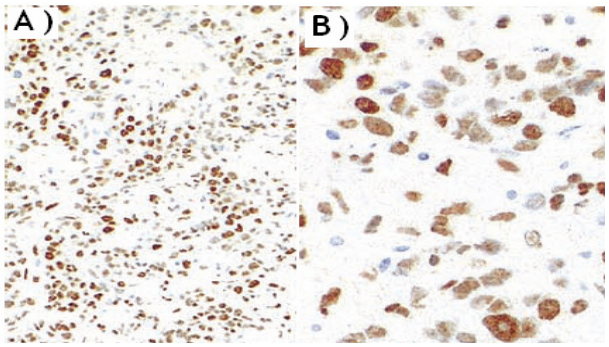

Fig. 1: Immunohistochemical staining for P53 in squamous cell carcinoma of the head and neck. (A X64 and B X170)

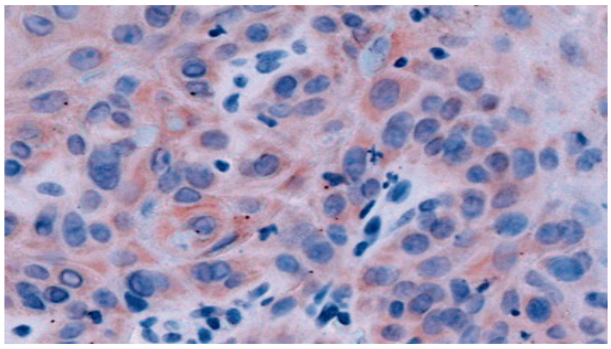

Fig. 2: Immunohistochemical staining for TS in squamous cell carcinoma of the head and neck.

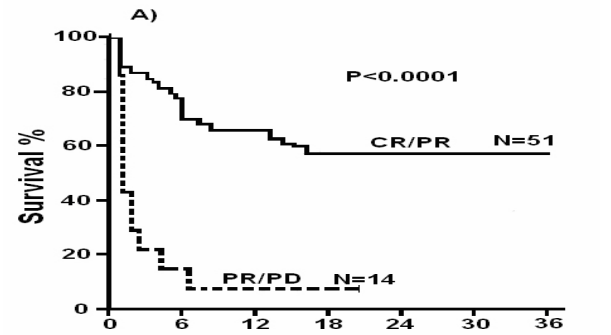

Fig. 3A: Response to chemotherapy and progression free survival in patients receiving neoadjuvant chemotherapy for head and neck tumors

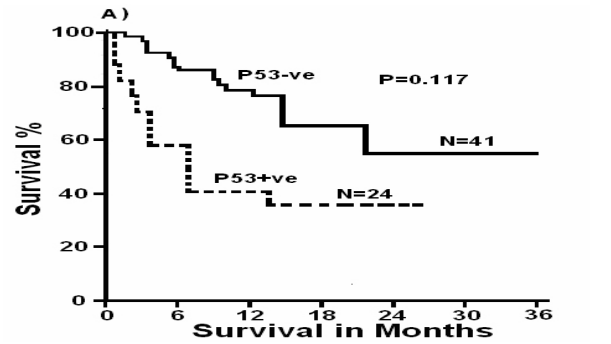

Fig. 4A: Disease free survival according to p53 expression in patients receiving neoadjuvant chemotherapy

Among clinical parameters evaluated, female gender was associated with an improved OS (P = 0.014) and low clinical stage, but other clinicopathological factors were not statistically associated with OS.

Multivariate Analysis:

DFS had no statistically significant correlations with all variables. In the mean time, lymph node metastases, p53 overexpression and TS expression were significant predictors of OS. (Table 6)

Table 5: Correlation between response to chemotherapy, p53, TS and OS.

	36 months OS (%) (SEM)	Hazard ratio	95% confidence interval of hazard ratio	P*
Response				
CR/PR	72 (6.3)	1.00		
PD/NR	30 (11)	3.34	3.14–46.8	<0.0001
p53				
-ve	80 (6.4)	1.00		
+ve	29 (9.3)	6.21	3.42–19.4	<0.0001
TS				
-ve	69 (11)	1.00		
+ve	38 (9.4)	2.81	1.36–7.23	0.0063

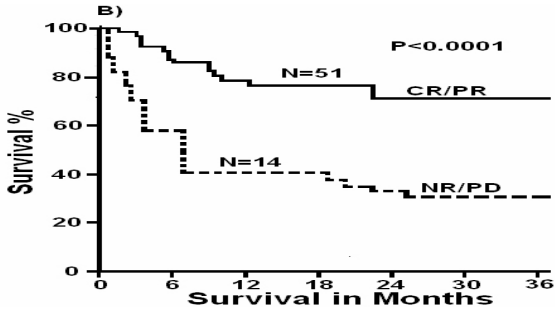

Fig. 3B: Response to chemotherapy and overall survival in patients receiving neoadjuvant chemotherapy for head and neck tumors.

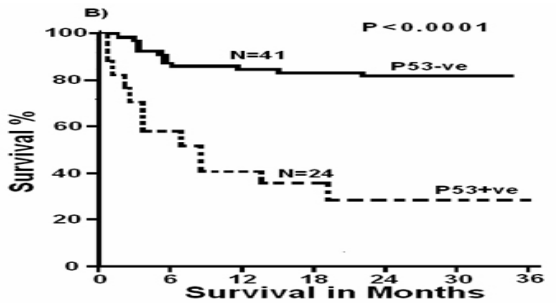

Fig. 4B: Overall survival according to p53 expression in patients receiving neoadjuvant chemotherapy.

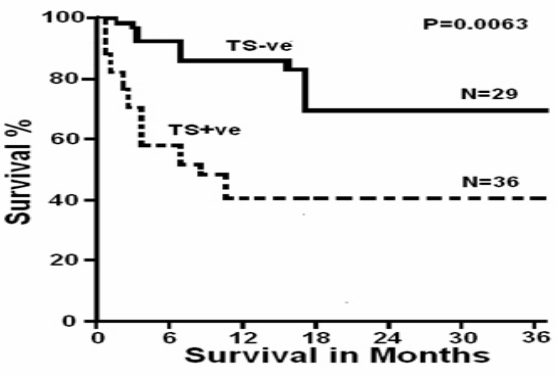

Fig. 5: Overall survival according to TS expression in patients receiving neoadjuvant chemotherapy.

Table 6: Multivariate analysis of over all survival (OS) and other variables.

Variable	Estimated coefficient (SE)	P	Hazard ratio (95% confidence interval)
Lymph Nodes (+ve vs. -ve)	1.45 (0.483	0.001	4.88 (1.75–13.36)
TS 106 (+ve vs. -ve)	1.24 (0.447	0.003	4.45 (1.56–10.68)
p53 (+ve vs. -ve)	0.97 (0.556)	0.002	2.79 (0.67–6.67)

DISCUSSION

To establish the potential value of TS and p53 expression as predictors of response and local control from neoadjuvant chemotherapy in SCCHN, we have evaluated the expression of these two proteins by immunohistochemistry in the tumor cells from primary tumor biopsies of patients treated with induction PF chemotherapy. These two proteins are biologically important mediators of growth, viability, and response to therapy in tumor cells. The relationship of their expression to clinical outcomes may provide insights into their biological role in the continued growth and therapeutic responsiveness of these malignancies. In the present study, we observe that the expression of p53 and TS are associated with poor prognosis.

Loss of wild-type p53 function has been associated with chemotherapy resistance in vitro[21] and in vivo[22,23]. p53 may participate in cellular pathways leading to apoptosis following treatment with DNA-damaging agents such as cisplatin[24,25]. Our results demonstrate that overexpression of p53 protein is a strong indicator of poor prognosis. Similar data have been demonstrated in bladder cancer where p53 overexpression has also been associated with decreased survival[26]. Analysis of the data from the Veterans Affairs larynx preservation trial similarly indicated that p53 was not correlated with response to chemotherapy but with decreased patient survival[27].

TS expression and function have been studied as predictors of response and prognosis in selected malignancies. It has been reported that increased TS biochemical activity or expression in tumor tissue is associated with a reduced responsiveness to 5-FU-based chemotherapy and to a worse prognosis. The results of the present study supports previous studies that suggest that this enzyme may be associated with decreased treatment response and survival in patients with advanced head and neck cancer treated with 5-FU-based chemotherapy[28]. In

that study, TS expression was more common in moderately or well-differentiated tumors. The same association between TS expression and tumor differentiation was also observed in our study. Similar findings have been demonstrated in other malignancies, suggesting that TS expression may in part determine response to 5-FU-based chemotherapy and influence survival in a broad spectrum of tumors[29]. The lack of association between TS expression and treatment response in our series may reflect the relatively small number of cases studied and the high overall response rate to neoadjuvant therapy.

The observation that expression of p53 and TS were more predictive of poor OS than DFS may indicate that these markers reflect the aggressiveness of disease in these patients.

The present study demonstrated that easily measured cellular factors may be important predictors of outcome in patients undergoing neoadjuvant treatment for head and neck malignancies. The magnitude of survival differences seen with p53 and TS suggest that these may ultimately be clinically useful in making treatment decisions for patients being considered for neoadjuvant treatment. For example, an attempt at organ preservation may be less attractive for a patient whose tumor profile predicts that he or she will be unlikely to respond or survive with that treatment strategy. If these data can be confirmed, it may be possible to guide the selection of chemotherapy agents based on the individual profile of gene expression in a given tumor. Patients with high TS expression may be better candidates for other regimens e.g. a taxane-based regimen rather than a 5-FU-containing regimen. Additionally, poor prognosis patients may be identified as candidates for clinical trials involving new agents or dose-intensification schemes.

REFERENCES

1. Lenz HJ, Leichman CG, Danenberg KD, Danenberg PV, Groshen S, Cohen H, et al. Thymidylate synthase mRNA level in adenocarcinoma of the stomach: A predictor for primary tumor response and overall survival. J Clin Oncol 1996 Jan;14(1):176-82.

2. Martin M, Hazan A, Vergnes L, Peytral C, Mazeron JJ, Senechaut JP, et al. Randomized study of 5 fluorouracil and cis platin as neoadjuvant therapy in head and neck cancer: a preliminary report. Int J Radiat Oncol Biol Phys 1990 Oct;19(4):973-75.

3. Tishler RB, Calderwood SK, Coleman CN, Price BD. Increases in sequence specific DNA binding by p53 following treatment with chemotherapeutic and DNA damaging agents. Cancer Res 1993 May 15;53(10 Suppl):2212-16.

4. Nelson WG, Kastan MB. DNA strand breaks: the DNA template alterations that trigger p53-dependent DNA damage response pathways. Mol Cell Biol 1994 Mar;14(3):1815-23.

5. Eastman A. Activation of programmed cell death by anticancer agents: cisplatin as a model system. Cancer Cells 1990 Aug-Sep;2(8-9):275-80

6. Bradford CR, Zhu S, Poore J, Fisher SG, Beals TF, Thoraval D, et al. p53 mutation as a prognostic marker in advanced laryngeal carcinoma. Department of Veterans Affairs Laryngeal Cancer Cooperative Study Group. Arch Otolaryngol Head Neck Surg 1997 Jun;123(6):605-9.

7. Toohill RJ, Anderson T, Byhardt RW, Cox JD, Duncavage JA, Grossman TW, et al. Cisplatin and fluorouracil as neoadjuvant therapy in head and neck cancer. A preliminary report. Arch Otolaryngol Head Neck Surg 1987 Jul;113(7):758-61.

8. Lenz HJ, Hayashi K, Salonga D, Danenberg KD, Danenberg PV, Metzger R, et al. p53 point mutations and thymidylate synthase messenger RNA levels in disseminated colorectal cancer: an analysis of response and survival. Clin Cancer Res 1998 May;4(5):1243-50.

9. Sarkis AS, Bajorin DF, Reuter VE, Herr HW, Netto G, Zhang ZF, et al. Prognostic value of p53 nuclear overexpression in patients with invasive bladder cancer treated with neoadjuvant MVAC. J Clin Oncol 1995 Jun;13(6):1384-90.

10. Johnston PG, Fisher ER, Rockette HE, Fisher B, Wolmark N, Drake JC, et al. The role of thymidylate synthase expression in prognosis and outcome of adjuvant chemotherapy in patients with rectal cancer. J Clin Oncol 1994 Dec;12(12):2640-47.

11. Pestalozzi BC, Peterson HF, Gelber RD, Goldhirsch A, Gusterson BA, Trihia H, et al. Prognostic importance of thymidylate synthase expression in early breast cancer. J Clin Oncol 1997 May;15(5):1923-31.

12. Harris CC. Structure and function of the p53 tumor suppressor gene: Clues for rational cancer therapeutic strategies. J Natl Cancer Inst 1996 Oct 16;88(20):1442-55.

13. Pinedo HM, Peters GF. Fluorouracil: Biochemistry and pharmacology. J Clin Oncol 1988 Oct;6(10):1653-64.

14. Pestalozzi BC, Peterson HF, Gelber RD, Goldhirsch A, Gusterson BA, Trihia H, et al. Prognostic importance of thymidylate synthase expression in early breast cancer. J Clin Oncol 1997 May;15(5):1923-31.

15. Browman GP. Evidence-based recommendations against neoadjuvant chemotherapy for routine management of patients with squamous cell head and neck cancer. Cancer Invest 1994;12(6):662-70.

16. Lowe SW, Ruley HE, Jacks T, Housman DE. P53-Dependent apoptosis modulates the cytotoxicity of anticancer Agents. Cell 1993 Sep 24;74(6):957-67.

17. Lowe SW, Bodis S, McClatchey A, Remington L, Ruley HE,

Fisher DE, et al. P53 status and the efficacy of cancer therapy in Vivo. Science 1994 Nov 4;266(5186):807-10.

18. Oren M. Relationship of p53 to the control of apoptotic cell death. Semin Cancer Biol 1994 Jun;5(3):221-17.

19. Leichman CG, Lenz HJ, Leichman L, Danenberg K, Baranda J, Groshen S, et al. Quantitation of intratumoral thymidylate synthase expression predicts for disseminated colorectal cancer response and resistance to protracted-infusion fluorouracil and weekly leucovorin. J Clin Oncol 1997 Oct;15(10):3223-9.

20. Righetti SC, Della Torre G, Pilotti S, Menard S, Ottone F, Colnaghi MI, et al. A comparative study of p53 gene mutations, protein accumulation, and response to cisplatin-based chemotherapy in advanced ovarian carcinoma. Cancer Res 1996 Feb 15;56(4):689-93.

21. Bradford CR, Zhu S, Wolf GT, Poore J, Fisher SG, Beals T, et al. Overexpression of p53 predicts organ preservation using induction chemotherapy and radiation in patients with advanced laryngeal cancer. Department of Veterans Affairs Laryngeal Cancer Study Group. Otolaryngol Head Neck Surg 1995 Oct;113(4):408-12.

22. Vokes EE, Panje WR, Mick R, Kozloff MF, Moran WJ, Sutton HG, et al. A randomized study comparing two regimens of neoadjuvant and adjuvant chemotherapy in multimodal therapy for locally advanced head and neck cancer. Cancer 1990 Jul 15;66(2):206-13.

23. Perego P, Giarola M, Righetti SC, Supino R, Caserini C, Delia D, et al. Association between cisplatin resistance and mutation of p53 gene and reduced bax expression in ovarian carcinoma cell systems. Cancer Res 1996 Feb 1;56(3):556-62.

24. Rusch V, Klimstra D, Venkatraman E, Oliver J, Martini N, Gralla R, et al. Aberrant p53 expression predicts clinical resistance to cisplatin-based chemotherapy in locally advanced non-small cell lung cancer. Cancer Res 1995 Nov 1;55(21):5038-42.

25. Lee JS, Yoon A, Kalapurakal SK, Ro JY, Lee JJ, Tu N, et al. Expression of p53 oncoprotein in non-small-cell lung cancer: A favorable prognostic factor. J Clin Oncol 1995 Aug;13(8):1893-903.

26. Buttitta F, Marchetti A, Gadducci A, Pellegrini S, Morganti M, Carnicelli V, et al. P53 Alterations are Predictive of Chemoresistance and Aggressiveness in Ovarian Carcinomas: A Molecular and Immunohistochemical Study. Br J Cancer 1997;75(2):230-35.

27. Kish JA, Weaver A, Jacobs J, Cummings G, Al Sarraf M. Cisplatin and 5-fluorouracil infusion in patients with recurrent and disseminated epidermoid cancer of the head and neck. Cancer 1984 May 1;53(9):1819-24.

28. Induction chemotherapy plus radiation compared with surgery plus radiation in patients with advanced laryngeal cancer. The Department of Veterans Affairs Laryngeal Cancer Study Group. N Engl J Med 1991 Jun 13;324(24):1685-90.

29. Lefebvre JL, Chevalier D, Luboinski B, Kirkpatrick A, Collette L, Sahmoud T. Larynx preservation in pyriform sinus cancer: Preliminary results of a European Organization for Research and Treatment of Cancer phase III trial. EORTC Head and Neck Cancer Cooperative Group. J Natl Cancer Inst 1996 Jul 3;88(13):890-99.