Mcl-1, Vascular Endothelial Growth Factor-R2, and 14-3-3σ Expression Might Predict Primary Response against Radiotherapy and Chemotherapy in Patients with Locally Advanced Squamous Cell Carcinomas of the Head and Neck

Boban M. Erovic,[1] Martina Pelzmann,[2] Matthäus Ch. Grasl,[1] Johannes Pammer,[3] Gabriela Kornek,[4] Werner Brannath,[5] Edgar Selzer,[2,6] and Dietmar Thurnher[1,7]

Abstract

Purpose: This study was done to explore whether the expression of a selected set of proteins could predict primary response to radiotherapy or concomitant radiotherapy and chemotherapy in patients with advanced head and neck cancer.

Experimental Design: Forty-three pretreatment tumor biopsies were taken during diagnostic panendoscopy and examined for Mcl-1, vascular endothelial growth factor (VEGF)-R2, CD9, and 14-3-3σ expression by immunohistochemistry. Forty-three patients underwent primary radiotherapy, of which, 29 patients received concomitant chemotherapy (low dose daily cisplatin, mitomycin C bolus). The primary end-point was locoregional tumor control 6 months after completion of radiotherapy. Mcl-1, VEGF-R2, CD9, and 14-3-3σ expression were correlated with patients' primary response to radiotherapy and chemotherapy and with established clinicopathologic variables.

Results: Thirty complete and 13 partial responses were observed in our patient group. High expression levels of Mcl-1 ($P = 0.021$), VEGF-R2 ($P = 0.032$), and 14-3-3σ ($P = 0.013$), but not of CD9, in tumor biopsies was correlated with complete response. Overexpression of at least two of the three aforementioned proteins in pretreatment biopsies predicted—with a likelihood of 80%—whether a patient would achieve complete response to radiotherapy and chemotherapy. However, if only one of these proteins is overexpressed, there is a likelihood of 84.6% that this patient would not completely respond to therapy.

Conclusion: Determining the expression levels of Mcl-1, VEGF-R2, and 14-3-3σ may be helpful in predicting the early clinical response in head and neck tumor patients receiving primary radiotherapy and chemotherapy and may further allow a pretherapeutic selection of patients.

Despite significant improvements in the treatment of squamous cell carcinomas of head and neck cancer, typical 5-year combined survival rates for all stages of disease are still in the range of 22% to 63% (1). Although the therapeutic management of head and neck cancer has benefited from small and incremental improvements over the past few years, the management of advanced head and neck tumors continues to principally depend on tumor localization and on tumor stage (2). The tumor-node-metastasis stage alone, however, is not sufficient to stratify patients according to their likeliness to respond against different therapies (3, 4). In order to improve treatment outcome, a better understanding of the pathophysiology of head and neck cancer, and the identification of potentially clinically relevant molecular markers for early diagnosis and optimal treatment planning are necessary. Several potential prognostic molecular factors have been described during the past few years (5–25). Proteins which have been implicated in the control of tumor cell survival and which have a prognostic potential are, among others, Mcl-1 (11–18), vascular endothelial growth factor (VEGF)-R2 (19–21), CD9 (22), or 14-3-3σ (23–25). Mcl-1 has been chosen in this study because this antiapoptotic protein is widely expressed (~90%) in squamous cell carcinomas of the head and neck, whereas Bcl-2 is expressed only in 15% of head and neck tumors (26). In addition, in a recent report from our group (27), we have shown that Mcl-1 expression inhibits radiation-induced cell death. Furthermore, our own preliminary data also indicate that down-regulation of Mcl-1 in two head and neck cancer cell lines modulates chemosensitivity.[8]

Authors' Affiliations: Departments of [1]Otorhinolaryngology, Head and Neck Surgery, [2]Radiation Therapy, [3]Clinical Pathology, and [4]Internal Medicine, Division of Oncology and [5]Medical Statistics, [6]Center of Excellence for Clinical and Experimental Oncology, University of Vienna Medical School, Vienna, Austria; and [7]Department of Otorhinolaryngology, Head and Neck Surgery, University of Toronto, Ontario, Canada

Received 5/30/05; revised 9/19/05; accepted 9/28/05.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Requests for reprints: Dietmar Thurnher, Department of Otorhinolaryngology, Head and Neck Surgery, Medical University of Vienna, Währinger Gürtel 18-20, A-1090 Vienna, Austria. Phone: 43-1-40400-3372; Fax: 43-1-40400-3332; E-mail: dietmar.thurnher@meduniwien.ac.at.

doi:10.1158/1078-0432.CCR-05-1170

[8] Manuscript in preparation.

VEGF-R2 and CD9 were included because they were previously described to be expressed in squamous cell carcinomas of the head and neck (19–21). There are several publications strongly supporting a role for the VEGF receptor signaling system in the response to radiotherapy (28–30). CD9—in a recently published study from our group—was shown to be a strong prognostic factor for overall and for disease-free survival in patients with squamous cell carcinomas of the head and neck (22).

14-3-3σ has been found to be expressed in squamous cell carcinomas of the head and neck as well as playing a role in cell death (23–25). Another reason for investigating this factor are unpublished data from our group obtained from proteomics analysis of head and neck tumor samples which indicated that 14-3-3σ might be overexpressed in the malignant phenotype of these tumors.

To the best of our knowledge, a potential correlation between the expression of this set of tumor-associated proteins and the patient's response to radiotherapy in head and neck cancer has thus far not been investigated. The purpose of this article was to analyze the expression pattern of the combination of all four potent predictive factors Mcl-1, VEGF-R2, CD9, and 14-3-3σ in the pretreatment tumor biopsies of 43 patients with advanced head and neck cancer, and to investigate a possible correlation with the early tumor response to radiotherapy alone or radiotherapy in combination with concomitant chemotherapy.

Patients and Methods

Patients. Tumor biopsies of 43 untreated patients (9 females, 34 males), with locally advanced head and neck cancer [T_2 ($n = 1$), T_3 ($n = 11$); T_4 ($n = 31$), N_0 ($n = 2$), N_1 ($n = 2$), N_{2b} ($n = 16$), N_{2c} ($n = 19$), and N_3 ($n = 4$)] were obtained during diagnostic panendoscopy. After panendoscopy, all patients underwent a hyperfractionated and accelerated irradiation schedule (31), with concomitant boost at the Department of Radiation Therapy, Medical University of Vienna, from 1999 to 2002. Twenty-nine of the 43 patients underwent an identical irradiation protocol plus chemotherapy, consisting of low-dose cisplatin (at 6 mg/m^2, days 1-20 of radiotherapy) and mitomycin C (at 12 mg/m^2 on day 21 of radiotherapy). Tumor response was evaluated clinically by the head and neck surgeon and by CT and/or MRI scans 3 and 6 months after the end of radiotherapy. In case of conflicting or unclear results, a biopsy was obtained during control panendoscopy. All patients were seen by the involved physicians (radiologist, radiation oncologist, head and neck surgeon, and/or oncologist) in the tumor board. Patients without evidence of disease or with remaining tumor masses in the primary site and/or locoregional lymph nodes were scored as complete or partial responders, respectively. In the complete responder group, an advanced tumor and lymph node staging (T_{3-4} and N_{2-3}) was diagnosed in 97% and 87% of all patients, respectively. In the partial responder group, 100% of all patients had a T_{3-4} and N_{2-3} staging. Chemotherapy was administered to 21 and 8 patients with complete or partial tumor remission, respectively, and did not affect outcome in a significant manner.

Immunohistochemistry. Dewaxed and microwaved slides were incubated with goat polyclonal anti-Mcl-1 (Dako, Glostrup, Denmark), mouse monoclonal anti-VEGF-R2 (Santa Cruz Biotechnology, Santa Cruz, CA), mouse monoclonal anti-CD9 (Novocastra Laboratories, Newcastle upon Tyne, United Kingdom) and mouse monoclonal anti-14-3-3σ (Research Diagnostics, Inc., Concord, MA) antibodies overnight at room temperature. As a control, slides were exposed to goat preimmune serum (Vector Laboratories, Burlingame, CA) or IgG_1 (Ancell, Bayport, MN) antibody. After washing with TBS thrice, slides were incubated with a multilink antibody (Dako) for 1 hour at room temperature, washed, and again exposed to alkaline phosphatase–conjugated Streptavidin-AP/10% human serum (Dako) for 1 hour at room temperature. Visualization was done with Fast Red TR, 4-chloro-2-methylbenzenediazonium salt (Sigma-Aldrich, St. Louis, MO), counterstained with hemalaun, dehydrated, and mounted. All slides were graded as either positive or negative. The cutoff for positive versus negative immunostaining was set at 33% positive cells per image field. Only a few tumors showed a weak or distinctive protein expression in all cells indicating background staining. The expression pattern of each marker was independently determined in the complete microscopic tumor section on the slide by two investigators (B.M. Erovic and D. Thurnher). Observer bias was minimized by repeating the evaluation without knowledge about clinical data. In 3 out of 43 tumor samples, a difference regarding the interpretation (cutoff of 33%) between the two investigators was found. In these three cases, samples were reanalyzed by an independent reviewer (E. Selzer).

Statistics. To assess the correlation between Mcl-1, VEGF-R2, CD9, and 14-3-3σ expression on one hand, and tumor remission, sex, tumor, and lymph node status on the other hand, univariate and multivariate stepwise logistic analyses were done. Wilcoxon rank-sum test was used to analyze the correlation of lymph node status, chemotherapy, Mcl-1, VEGF-R2, CD9, and 14-3-3σ expression. In order to analyze the quality of possible predictors for remission, we calculated sensitivity, specificity, and the predictive values, assuming a binomial distribution (95% confidence intervals). Confidence intervals were calculated using Clopper-Pearson values. Overall survival curves were calculated using Cox regression analysis and by the Kaplan-Meier method. Statistical significance was assumed if $P < 0.05$. Statistical analyses were done using SAS and SPSS-8.0 statistical software packages.

Results

Immunohistochemistry. Distribution of Mcl-1, VEGF-R2, CD9, and 14-3-3σ expression in 43 pretreatment tumor biopsies is summarized in Table 1 and Fig. 1.

Prediction of patients' primary response. Expression of Mcl-1 ($P = 0.021$), VEGF-R2 ($P = 0.032$), and 14-3-3σ ($P = 0.013$) in tumor biopsies of untreated head and neck cancer patients significantly correlated by univariate logistic regression analysis to patients' response to primary radiotherapy and chemotherapy. No correlation could be found between the expression of CD9 and patients' primary response to radiotherapy and chemotherapy. Multivariate logistic regression analysis showed that 14-3-3σ ($P = 0.02$) and VEGF-R2 ($P = 0.023$) correlated significantly with tumor response (Table 2).

Statistical analyses showed that the combination of Mcl-1, VEGF-R2, and 14-3-3σ expression in each pretreatment biopsy improves the power of the predictive potential of these markers. A pretreatment biopsy which is highly positive for more than two proteins predicts tumor remission with a sensitivity of 80% (confidence intervals, 61.4%, 92.3%) and a specificity of 84.6% (confidence intervals, 54.6%, 98.0%).

Survival data. Six months after therapy, 70% of 43 patients had a complete response, whereas 30% were partial responders; 32% were alive without and 5% with evidence of disease, whereas 63% had died of cancer before the end of the observation period (March 1, 2005). Overall survival and disease-free interval ranged from 3 to 61 months and from 3 to 53 months, respectively. Overall survival did not correlate with Mcl-1, VEGF-R2, CD9, and 14-3-3σ expression in tumor biopsies (data not shown). Median survival time was 22 months.

Table 1. Expression of Mcl-1, VEGF-R2, CD9, and 14-3-3σ in 43 tumor biopsies of untreated patients with advanced head and neck cancer

Expression	Factors							
	Mcl-1		VEGF-R2		CD9		14-3-3σ	
	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative
Complete remission	17	13	24	6	23	7	20	10
Partial remission	2	11	6	7	9	4	3	10

Discussion

We retrospectively compared Mcl-1, VEGF-R2, CD9, and 14-3-3σ expression to patients' clinical response 6 months after radiotherapy alone or in combination with chemotherapy. Taken together, our statistical analysis showed that the expression pattern of Mcl-1, VEGF-R2, and 14-3-3σ in untreated tumor biopsies predicted patients' response to radiotherapy and/or combined radiotherapy and chemotherapy.

To improve patients' survival, particularly in advanced tumor stages, a better understanding of cellular pathways and factors, which could serve as predictive markers, is clearly necessary. Using clinically validated predictors, a stratification of patients into treatment groups, such as surgery or radiotherapy and chemotherapy should be possible. Therefore, intense efforts are under way to identify genes or proteins which are differentially expressed in normal and cancerous tissues or in cancer specimens from patients who did or did not respond to treatment (32–37).

VEGF-R2 was found to be overexpressed in patients who completely responded to therapy. This observation is in agreement with the current scientific literature because recent studies have shown that, particularly in squamous cell carcinomas of the head, increased levels of VEGF, and its receptor VEGF-R2, are surrogate markers for tumor remission after radiotherapy (38, 39).

A key role of 14-3-3σ, also known as stratifin, is its control of the G_2 cell cycle checkpoint. In response to DNA damage, 14-3-3σ is induced in a p53-dependent manner facilitating DNA damage repair before cell cycle progression (23–25). Moreover, an essential role of 14-3-3σ in the response against ionizing radiation-induced cell death could be shown by gene knockout experiments in colorectal carcinoma cell lines (40). We noted significantly increased levels of 14-3-3σ in patients who responded completely to radiotherapy and chemotherapy, whereas decreased levels were observed in the partial responder group.

It is surprising to find overexpression of Mcl-1 in patients with a complete tumor remission. Because Mcl-1 is well known as an antiapoptotic protein, it might be expected that Mcl-1 would be expressed at higher levels in patients with a worse response to radiotherapy and chemotherapy (27, 41).

However, there are examples in the literature linking higher levels of expression of an antiapoptotic protein (Bcl-2) with favorable outcomes, specifically in head and neck cancer patients. Two recent publications reported significantly better responses of Bcl-2-positive tumors of the head and neck region in response to radiotherapy and chemotherapy (37, 42). It is indeed very interesting to find that both high Bcl-2 and Mcl-1 expression levels might be associated with better treatment responses. Moreover, *in vitro* studies with melanoma cell lines could show that Mcl-1 expression is associated with the cell survival response against ionizing radiation (27).

The results of this retrospective study suggest a prospective analysis of Mcl-1, 14-3-3σ, and VEGF-R2 expression in order to confirm these results. In fact, we have now decided to routinely analyze these three proteins, in conjunction with other factors, such as TP53, Bcl-2, and endothelial growth factor receptor expression in pretreatment biopsies of our head and neck patients. We anticipate obtaining sufficient information in the near future to assess the relative importance of the above-mentioned factors as predictors for the response against radiotherapy.

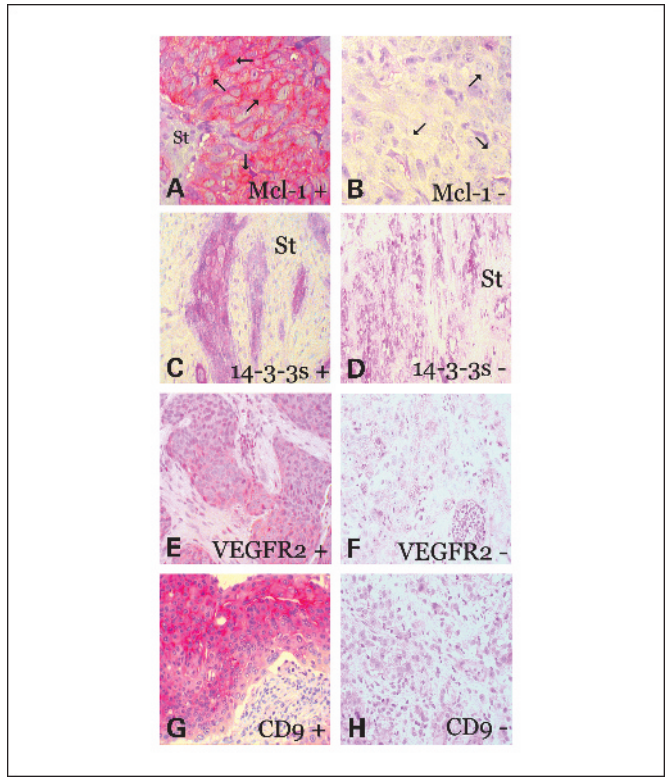

Fig. 1. Arrows, high (*A*) and weak (*B*) Mcl-1 immunoreactivity in the cytoplasm of tumor cells. Peritumoral stroma (*St*) stays immunonegative for Mcl-1. *C*, 14-3-3σ is overexpressed in tumor cells; *D*, low 14-3-3σ levels are observed; *E*, significant overexpression of VEGF-R2; *F*, low expression in tumor cell formations; *G*, high-level expression of CD9; *H*, low-level expression of CD9 (magnification, ×400).

Table 2. Results of the univariate and multivariate logistic regression analyses for tumor remission (complete or partial tumor remission), tumor stage, lymph node stage, chemotherapy, sex and Mcl-1, VEGF-R2, CD9, and 14-3-3σ expression

Factor	Univariate logistic regression		Multivariate logistic regression	
	Odds ratio	*P*	Odds ratio	*P*
Tumor stage	0.36	0.240	not included	—
Lymph node stage	0.75	0.072	not included	—
Chemotherapy	1.46	0.588	not included	—
Sex	0.25	0.074	0.141	0.046
Mcl-1	7.19	0.021	not included	—
VEGF-R2	4.67	0.032	7.78	0.023
CD9	1.46	0.609	not included	—
14-3-3σ	6.67	0.013	7.89	0.020

By analyzing Mcl-1, 14-3-3σ, and VEGF-R2 expression, it might be possible to stratify patients into different treatment groups. Different protein expression patterns may predict resistance to radiotherapy and chemotherapy and thus may be a helpful guide in choosing between therapeutic strategies, such as intensified combined modality therapy versus surgery alone or novel experimental regimen.

It would therefore be of potential clinical relevance if our observation could be further validated with larger patient numbers. In addition, it will also be of interest to investigate potential correlations between the expression of the above-mentioned factors and other proteins, such as the endothelial growth factor receptor, which are potential targets for combination treatments using small molecules and antibodies.

Acknowledgments

We thank Sieglinde Bauer for excellent laboratory assistance and Prof. Dr. Peter Bauer for statistical advice.

References

1. Jemal A, Murray T, Samuels A, Ghafoor A, Ward E, Thun MJ. Cancer statistics, 2003. CA Cancer J Clin 2003;53:5–26.
2. Gath HJ, Brakenhoff RH. Minimal residual disease in head and neck cancer. Cancer Metastasis Rev 1999; 18:109–26.
3. Hannisdal K, Boysen M, Evensen JF. Different prognostic indices in 310 patients with tonsillar carcinomas. Head Neck 2003;25:123–31.
4. Hall SF, Groome PA, Rothwell D, Dixon PF. Using the TNM system to predict survival in squamous cell carcinoma of the head and neck. Anticancer Res 1998;18: 4777–8.
5. Hassan KA, Ang KK, El-Naggar AK, et al. Cyclin B1 overexpression and resistance to radiotherapy in head and neck squamous cell carcinoma. Cancer Res 2002;62:6414–7.
6. Ang KK, Berkey BA, Tu X, et al. Impact of epidermal growth factor receptor expression on survival and pattern of relapse in patients with advanced head and neck carcinoma. Cancer Res 2002;62:7350–6.
7. Smith BD, Haffty BG. Molecular markers as prognostic factors for local recurrence and radioresistance in head and neck squamous cell carcinoma. Radiat Oncol Investig 1999;7:125–44.
8. Salesiotis AN, Cullen KJ. Molecular markers predictive of response and prognosis in the patient with advanced squamous cell carcinoma of the head and neck: evolution of a model beyond TNM staging. Curr Opin Oncol 2000;12:229–39.
9. Carlos de Vicente J, Junquera Gutierrez LM, Zapatero AH, Fresno Forcelledo MF, Hernandez-Vallejo G, Lopez Arranz JS. Prognostic significance of p53 expression in oral squamous cell carcinoma without neck node metastases. Head Neck 2004;26:22–30.
10. Li W, Thompson CH, Cossart YE, et al. The expression of key cell cycle markers and presence of human papillomavirus in squamous cell carcinoma of the tonsil. Head Neck 2004;26:1–9.
11. Wilson GD, Saunders MI, Dische S, Richman PI, Daley FM, Bentzen SM. Bcl-2 expression in head and neck cancer: an enigmatic prognostic marker. Int J Radiat Oncol Biol Phys 2001;49:435–41.
12. Costa A, Licitra L, Veneroni S, et al. Biological markers as indicators of pathological response to primary chemotherapy in oral-cavity cancers. Int J Cancer 1998;79:619–23.
13. Dragovich T, Rudin CM, Thompson CB. Signal transduction pathways that regulate cell survival and cell death. Oncogene 1998;17:3207–13.
14. Boucher MJ, Morisset J, Vachon PH, et al. MEK/ERK signaling pathway regulates the expression of Bcl-2, Bcl-X(L), and Mcl-1 and promotes survival of human pancreatic cancer cells. J Cell Biochem 2000;79: 355–69.
15. Yang T, Buchan HL, Townsend KJ, et al. MCL-1, a member of the BLC-2 family, is induced rapidly in response to signals for cell differentiation or death, but not to signals for cell proliferation. J Cell Physiol 1996;166:523–36.
16. Itoh T, Itoh A, Pleasure D. Bcl-2-related protein family gene expression during oligodendroglial differentiation. J Neurochem 2003;85:1500–12.
17. Craig RW. The Bcl-2 gene family. Semin Cancer Biol 1995;6:35–43.
18. Klampfer L, Zhang J, Nimer SD. GM-CSF rescues T1 cells from growth factor withdrawal-induced, but not differentiation-induced apoptosis: the role of Bcl-2 and Mcl-1. Cytokine 1999;11:849–55.
19. Neuchrist C, Erovic BM, Handisurya A, et al. Vascular endothelial growth factor receptor 2 (VEGFR2) expression in squamous cell carcinomas of the head and neck. Laryngoscope 2001;111:1834–41.
20. Neuchrist C, Quint C, Pammer A, Burian M. Vascular endothelial growth factor (VEGF) and microvessel density in squamous cell carcinomas of the larynx: an immunohistochemical study. Acta Otolaryngol 1999; 119:732–8.
21. Neuchrist C, Erovic BM, Handisurya A, et al. Vascular endothelial growth factor C and vascular endothelial growth factor receptor 3 expression in squamous cell carcinomas of the head and neck. Head Neck 2003; 25:464–74.
22. Erovic BM, Pammer J, Hollemann D, et al. Motility-related protein-1/CD9 expression in head and neck squamous cell carcinoma. Head Neck 2003;25: 848–57.
23. Chan TA, Hermeking H, Lengauer C, Kinzler KW, Vogelstein B. 14-3-3σ is required to prevent mitotic catastrophe after DNA damage. Nature 1999;401: 616–20.
24. Cheng L, Pan CX, Zhang JT, et al. N. Loss of 14-3-3σ in prostate cancer and its precursors. Clin Cancer Res 2004;10:3064–8.
25. Gasco M, Bell AK, Heath V, et al. Epigenetic inactivation of 14-3-3σ in oral carcinoma: association with p16(INK4a) silencing and human papillomavirus negativity. Cancer Res 2002;62:2072–6.
26. Hotz MA, Bosq J, Zbaeren P, et al. Spontaneous apoptosis and the expression of p53 and Bcl-2 family proteins in locally advanced head and neck cancer. Arch Otolaryngol Head Neck Surg 1999; 125:417–22.
27. Skvara H, Thallinger C, Wachek V, et al. Mcl-1 blocks radiation-induced apoptosis and inhibits clonogenic cell death. Anticancer Res 2005;25:2697–703.
28. Geng L, Donnelly E, McMahon G, et al. Inhibition of vascular endothelial growth factor receptor signaling leads to reversal of tumor resistance to radiotherapy. Cancer Res 2001;61:2413–9.
29. Schueneman AJ, Himmelfarb E, Geng L, et al. SU11248 maintenance therapy prevents tumor regrowth after fractionated irradiation of murine tumor models. Cancer Res 2003;63:4009–16.
30. Abdollahi A, Lipson KE, Han X, et al. SU5416 and SU6668 attenuate the angiogenic effects of radiation-induced tumor cell growth factor production

and amplify the direct anti-endothelial action of radiation *in vitro*. Cancer Res 2003;63:3755–63.

31. Fu KK, Pajak TF, Trotti A, et al. A Radiation Therapy Oncology Group (RTOG) phase III randomized study to compare hyperfractionation and two variants of accelerated fractionation to standard fractionation radiotherapy for head and neck squamous cell carcinomas: first report of RTOG 9003. Int J Radiat Oncol Biol Phys 2000;48:7–16.

32. Lemaire F, Millon R, Young J, et al. Differential expression profiling of head and neck squamous cell carcinoma (HNSCC). Br J Cancer 2003;89:1940–9.

33. El-Naggar AK, Kim HW, Clayman GL, et al. Differential expression profiling of head and neck squamous carcinoma: significance in their phenotypic and biological classification. Oncogene 2002;21:8206–19.

34. Leethanakul C, Knezevic V, Patel V, et al. Head and Neck Cancer Genome Anatomy Project. Gene discovery in oral squamous cell carcinoma through the Head and Neck Cancer Genome Anatomy Project: confirmation by microarray analysis. Oral Oncol 2003;39:248–58.

35. Al Moustafa AE, Alaoui-Jamali MA, Batist G, et al. Identification of genes associated with head and neck carcinogenesis by cDNA microarray comparison between matched primary normal epithelial and squamous carcinoma cells. Oncogene 2002;21:2634–40.

36. Gallo O, Boddi V, Calzolari A, et al. bcl-2 protein expression correlates with recurrence, and survival in early stage head, and neck cancer treated by radiotherapy. Clin Cancer Res 1996;2:261–7.

37. Homma A, Furuta Y, Oridate N, et al. Prognostic significance of clinical parameters and biological markers in patients with squamous cell carcinoma of the head and neck treated with concurrent chemoradiotherapy. Clinical Cancer Res 1999;5:801–6.

38. Brizel DM, Sibley GS, Prosnitz LR, Scher RL, Dewhirst MW. Tumor hypoxia adversely affects the prognosis of carcinoma of the head and neck. Int J Radiat Oncol Biol Phys 1997;38:285–9.

39. Arcasoy MO, Amin K, Chou SC, Haroon ZA, Varia M, Raleigh JA. Erythropoietin and erythropoietin receptor expression in head and neck cancer: relationship to tumor hypoxia. Clin Cancer Res 2005;11:20–7.

40. Chu K, Teele N, Dewey MW, Albright N, Dewey WC. Computerized video time lapse study of cell cycle delay and arrest, mitotic catastrophe, apoptosis and clonogenic survival in irradiated 14-3-3σ and CDKN1A (p21) knockout cell lines. Radiat Res 2004;162:270–86.

41. Thallinger C, Wolschek MF, Maierhofer H, et al. Mcl-1 is a novel therapeutic target for human sarcoma: synergistic inhibition of human sarcoma xenotransplants by a combination of mcl-1 antisense oligonucleotides with low-dose cyclophosphamide. Clin Cancer Res 2004;10:4185–91.

42. Buffa FM, Bentzen SM, Daley FM, et al. Molecular marker profiles predict locoregional control of head and neck squamous cell carcinoma in a randomized trial of continuous hyperfractionated accelerated radiotherapy. Clin Cancer Res 2004;10:3745–54.