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Review paper

Prognostic markers in cancers of the head and neck region

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Increasing research initiatives in head and neck cancer over the past 5 years have resulted in the identification of several 'new' biologic parameters which may be of prognostic importance. This review discusses the limitations of traditional parameters and outlines several promising developments in the study of proliferation markers, molecular biology, immunobiology and flow cytometry as they relate to head and neck cancer.

Key words: Biology, head and neck cancer, prognostic factors, squamous cell cancer.

Introduction

Although squamous cell cancers (SCCs) of the head and neck region represent only 5–10% of newly diagnosed malignancies in the Western world, in many Third World countries, they represent one of the most common forms of solid tumor in adult life.[1] Desai[2] estimates that head and neck cancers constitute 28% of the total cancer load in India and represent nearly 5000 newly diagnosed cancer patients presenting annually to the Tata Memorial Institute.

Traditional therapy with surgery and/or radiotherapy may be curative in selective cases; however, the prognosis for head and neck SCCs is poor, with a 5 year survival rate of approximately 25–40%.[3] Encouraging data from the SEER Program demonstrate a reduction in mortality of 16.2% for patients with cancers of the oral cavity and pharynx when during the same period (1973–1987) the incidence of these cancers declined by 1.3%.[4] Quite apart from the early and late treatment-related functional cosmetic morbidity, it is also important to appreciate the considerable socio-economic impact of head and neck cancer. Incurable disease following treatment failure almost invariably remains localized to the head and neck region and terminal suffering is frequently protracted and distressing.

To provide each patient with optimal treatment, it is important to predict the future biologic behavior of head and neck cancers as precisely as possible. Prognostic information is therefore essential for the evaluation, judgement and optimal treatment of patients.

Given the world-wide impact of head and neck cancer, it is somewhat surprising that basic research into this disease has been a relatively neglected cause. Recent technologic advances have proven to be powerful tools in understanding basic biologic aspects of cancer growth and behavior. It is therefore timely to review the prognostic factors associated with cancers of the head and neck region, emphasizing how some of the more recent advances in molecular biology and related disciplines may be adding to our understanding of this disease.

Tumor stage, histologic grade and other pathologic criteria

The size of the primary malignant lesion and the status of cervical lymph nodes (T,N status) are fundamental characteristics used to describe the extent or stage of a tumor and these clinical factors alone greatly influence therapy and the clinical course of head and neck SCCs.[5] However, there has been much debate over the clinical relevance of the TNM classification (International Union Against Cancer), given that it does not account for other important tumor–host characteristics such as gross tumor appearance, site of the primary lesion, degree of local tumor infiltration, total nodal bulk, anatomic level of neck nodal involvement, patient age nor performance status.[3,6] Furthermore, the

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TNM classification assumes that anatomic sites are accessible to accurate measurement. Hence, the appearance of several modifications to the original TNM system.

In general, appropriately treated stage T1 cancers can be cured in a high proportion of cases, whereas stage T4 cancers of the head and neck have a gloomy prognosis. However, Platz *et al.*[7] have used the traditional UICC-TNM (1978) criteria and found that stage 2 and 3 patients did not differ with respect to their prognosis. They also found that T4 lesions did better than patients with T3 primary tumors. Even within the stage 4 category, there was a marked lack of homogeneity as far as prognosis was concerned. Predicting the survival of patients with stage T2 and stage T3 disease remains a serious concern[5] and it is a common clinical observation that patients with T3 N0 M0 may have a more favorable prognosis than those which are T1 N1 or T2 N1 at presentation. The presence of cervical lymph node metastases has a major impact on survival[8] and is the main determinant of prognosis within the TNM system. The presence of a single histologically positive lymph node in a patient with an oral cavity tumor reduces the 5 year survival expectation by 45%.[9] Furthermore, survival is directly proportional to the number of affected lymph nodes and to the number of nodes with capsular rupture.[10] Extracapsular spread reduces survival to 11%.[5] Not only may the measurement of tumor size be difficult and inaccurate, but the high proportion of clinically false negative cervical lymph nodes reflects the subjectivity of current staging systems.[10] Nevertheless, the TNM staging system provides a basis for planning therapy in many centers and allows comparison between reported series. It is axiomatic, however, that stage is intimately related to other biological determinants of tumor behavior.

The grade of the tumor is based on its histologic features, specifically the degree of differentiation, and there is a widely held belief that this has a significant impact on clinical decision-making and patient prognosis. For instance, poorly differentiated cancers are clinically more aggressive than well differentiated ones, regardless of their site of origin.[11] With decrease in the degree of differentiation there is an increased likelihood of regional lymph node involvement at presentation for SCCs of the larynx, oral cavity and oropharynx, a reduced interval between primary treatment and the presence of recurrent malignancy and a reduced 5 year survival rate. For SCCs, histologic grading is based primarily on the degree of keratinization within the tumor, abnormal nuclear features, pleomorphism and the number of mitoses, particularly abnormal mitotic forms, per high power field. While precise figures vary from site to site, marked differences in 5 year survival rates have been reported for oral and oropharyngeal cancers according to histologic grade, varying from 55% for the highest differentiated tumors to 20% for the least differentiated.[12]

However, studies have demonstrated a high degree of intra- and interobserver differences in the histologic grading of a tumor.[13,14] Wide variation among observers has been shown in the bias to one end or the other of the spectrum of differentiation or their reluctance to leave the middle ground and to allocate extreme grades. In practical terms pathologists tend to grade most of the head and neck SCCs as moderately differentiated. The aforesaid shortcomings explain, at least in part, the finding in a number of recent studies that patient survival is poorly related to conventional histologic grading.

Several attempts have been made to evaluate histologic parameters in relation to grading and biologic behavior. Jakobsson *et al.*[15,16] undertook a comprehensive multiparameter histologic grading approach to head and neck SCCs. His system employed eight different morphologic parameters for the tumor cell population and its relation to adjacent stromal tissue. An infiltrative growth pattern, the presence of vascular invasion and lymphoplasmacytic infiltrates were evaluated as additional factors to the usual parameters of cell cytology, mitoses and the degree of keratin production. Using multivariate analyses, the pattern of invasion was prognostically significant for small laryngeal carcinomas, whereas for larger laryngeal tumors, nuclear pleomorphism became a more important prognostic index of tumor behavior. Holm *et al.*[17] modified Jakobsson's criteria and employed three tumor cell parameters (differentiation, nuclear pleomorphism and mitoses) and three tumor–host relationships (mode of invasion, stage of invasion and cellular response). Similar studies have been performed for carcinomas of the gingiva,[18] palate,[19] floor of mouth[20] and oropharynx.[21] Although the degree of keratinization and nuclear pleomorphism contribute to the determination of tumor grade as single parameters, they have less value than the pattern of invasion and the frequency of mitoses in predicting survival.[21] Byrne *et al.*[22] have suggested that tumor grade at the most invasive margin has highly significant prognostic value.

In a further semi-quantitative study of the relationship between 39 individual histologic features of 100 intra-oral carcinomas and subsequent behavior,[23,24] it was demonstrated that many of the commonly used histologic criteria had little or no predictive value; these included mitotic activity and the degree of keratinization. Apart from the poor prognostic finding of lymph node involvement, histopathologic features which were significantly correlated with a poor prognosis included loss of epithelial stratification, the presence of individual tumor cells in the stroma, and a poorly developed immune inflammatory response within and around the tumor.[23,24] However, these findings have been disputed by others[25] who reaffirm the traditional belief that 5 year survival is proportional to degree of tumor differentiation. Much of this confusion has arisen because the majority of head and neck SCCs are graded as moderately differentiated (*vide supra*) and tumor grade does not correlate as well with biologic behavior as other strong predictors such as tumor size and nodal status. It is now becoming clear that tumor grade, taken alone, is of limited prognostic value in comparison with clinical staging parameters.

Histologic grades together with other prognostic factors have been variously combined with or used as alternatives to the conventional TNM system to improve the predictive value of tumor staging of oral cancers.[7,26,27] The TNM system has been extended to incorporate factors such as the precise site of the tumor, various histologic criteria (*vide supra*) and crude measures of the rate of tumor growth. Group-based staging data may offer better prognostic guidelines than the standard TNM staging system; however, their value to the individual as a determinant of therapy and survival is uncertain. Griffin *et al.*[28] have previously reported a prognostic model based on tumor site, T-stage, N-stage, histology, degree of infiltration, degree of differentiation, patient age, sex and Karnofsky performance score for patients receiving radiotherapy to head and neck cancers. For an entire group of 2066 patients predicted to have a 90% or better complete primary tumor response, 94% remained in initial complete remission at the primary site at 1 year and 87% at 2 years.

Proliferation markers

Evidence from several sources suggests that the degree of cellular proliferation within tumors holds some promise of enabling an estimate of their biologic aggression.[29-31] Estimation of the labeling index (LI) by *in vitro* incorporation of tritiated thymidine by tumor fragments has been a reliable, albeit long and tedious technique which has yielded important information in many types of cancer, confirming the clinical wisdom that fast growing cancers are more rapidly fatal than slowly growing ones.[32]

The advent of monoclonal antibodies to various cellular antigens and oncogene products has provided a novel, relatively quick, reproducible and simple means of studying various characteristics of their malignant phenotype. Cellular proliferation in a number of malignancies has been variously assessed by studying the cellular expression of Ki-67 antigen,[32] transferrin receptor,[33] epidermal growth factor receptor,[34] proliferating cellular nuclear antigen[35] and c-*myc* oncoprotein.[36] In one of the few studies assessing markers of cellular proliferation in head and neck SCCs, Kearsley *et al.*[37] have reported strong staining for Ki-67 antigen, transferrin and epidermal growth factor (EGF) receptor in cells in mitotic phase at the periphery of SCC whorls and at the invading cellular margin. Sakai *et al.*[38] demonstrated a similar correlation between mitotic state and expression of the EGF receptor and c-*myc* oncogene product. It is now apparent that the cellular differentiation of SCCs bears little relationship to the underlying cellular proliferation kinetics.[39,40] Dische *et al.*[41] have demonstrated high LI and short duration of DNA synthesis in a series of verrucous (highly differentiated) SCCs using bromodeoxyuridine (BrdU) incorporation.

SCC growth rates measured by *in vivo* BrdU incorporation and flow cytometry currently hold the greatest promise in determining the prognosis for patients treated with radiotherapy. Ploidy [DNA index (DI)], LI, duration of S-phase (T_s) and potential doubling time (T_{pot}) can be assessed within 24 h after BrdU administration to the patient.[42] It has been reported that head and neck SCCs have widely variable T_{pot}s and that 70% of these tumors have T_{pot}s of 5 days or less.[43] Potential doubling time did not correlate with ploidy status nor TN status, but shorter T_{pot}s were observed in moderate or poorly differentiated tumors.[43] The BrdU technique is a useful tool for studying the proliferative behavior of human tumors and pre-treatment knowledge of individual T_{pot}s for tumors scheduled for radiotherapy may assist the radiation oncologist in choosing the most effective fractionation regimen for tumors with T_{pot}s ≤ 5 days and standard fractionation for tumors with higher values.[43]

Cellular DNA content

In contrast to the inaccuracies inherent in some of the more traditional prognostic criteria, analysis of solid tumors by flow cytometry (FCM) permits rapid, objective, quantitative evaluation of cellular DNA content.[44] Abnormal cellular DNA content (aneuploidy), a reflection of chromosomal imbalance, is a well-recognized and common feature of human cancer which appears to be an important determinant of survival at a number of tumor sites.[45–47] In addition to assessing DNA content, FCM also allows some assessment of cellular proliferative activity (S-phase fraction) and the presence of high-degree aneuploidy, all of which may add a new dimension to current clinical and pathologic classifications of malignancy.

Results of several studies add to a growing body of evidence which increasingly recognizes the prognostic significance of cellular DNA content (ploidy) in a range of solid malignancies.[47] There is now compelling evidence in cancers of the ovary,[46] breast,[45] colo-rectum[48] and osteosarcoma[49] that ploidy status is an independent prognostic factor which has the potential to influence clinical decision-making.

Kearsley *et al.*[50] reported flow cytometric DNA ploidy measurements on formalin-fixed tumor specimens from 172 patients with SCCs of the head and neck region. One hundred and two samples were chosen retrospectively and a further 70 consecutive patients were analyzed prospectively in order to assess the prognostic significance of DNA ploidy and DI. There were no statistically significant differences between retrospective and prospective groups in regard to age, sex, TNM stage, ploidy or DI. Sixty-seven percent of patients were aneuploid and the proportion of aneuploid tumors was significantly higher among poorly differentiated tumors. Survival analysis using Cox multivariate regression modeling revealed that DNA aneuploidy and increasing DI were significant independent prognostic factors for both relapse-free and overall survival.

Although an assessment of ploidy profile has now been shown to be an important prognostic factor in head and neck SCCs (Table 1), not all specimens with poor prognosis are aneuploid. This finding may simply mean that all malignant cells were diploid or the result may represent a sampling error in situations when a biopsy has been too superficial to include more infiltrative aneuploid elements.

Alternatively, small aneuploid cellular clones may have been masked by the large numbers of diploid cells in some tumors. It is anticipated that these results will have a significant impact on clinical decision-making and that assessment of ploidy status may become a routine test to assist oncologists in the treatment of individual patients with head and neck cancers.

Immunobiologic markers

Immunologic deficiencies have been documented in patients with carcinomas and a number of investigators have attempted to use this information in determining prognosis more accurately. Studies

Table 1. A comparison of ploidy studies in head and neck cancer patients

Reference (year)	Number of evaluable patients	Technique	Aneuploidy (%)	Comment(s)
Holm (1982)[51]	45	static	75	worse prognosis for aneuploid SCCs
Johnson *et al.* (1985)[52]	73	flow	86	broad overlap of ploidy and clinicopathologic features; prognostic evaluation not attempted
Kaplan *et al.* (1986)[53]	46	flow	41	ploidy related to surgical (T) stage; prognostic evaluation not attempted
Goldsmith (1987)[54]	69	flow	74	better prognosis for aneuploid SCCs; ploidy a powerful prognositic indicator
Tytor *et al.* (1987)[55]	50	static	44	non-significant trend for aneuploid SCCs to have worse prognosis; aneuploid SCCs more aggressive
Kokai *et al.* (1988)[56]	76	not stated	71	ploidy the single most important prognostic factor for relapse and death
Ensley *et al.* (1989)[57]	165	flow	70	prognostic evaluation not attempted
Kearsley *et al.* (1991)[50]	172	flow	63 71	aneuploid SCCs had significantly higher risk of relapse and death

have shown that patients with head and neck SCCs who have a positive reaction to recall antigens have a better short-term prognosis than those patients who are anergic.[58] Skin testing using recall antigens is a measure of cell-mediated immunity and is theoretically helpful. Eiber and Morton[58] have demonstrated that the response to sensitizing the skin with dinitrochlorobenzene (DNCB) correlates better with the stage and clinical course of head and neck cancer than do results of recall skin tests. Patients with impaired immunological activity to DNCB have a high incidence of recurrence following cancer surgery.[58] DNCB reactivity has correlated well with prognosis and inversely with the extent of disease.[58]

More sophisticated indices of cell-mediated immune system involve *in vitro* assays of the ability of patients' lymphocytes to respond to various stimuli, including common recall antigens, specific tumor antigens or non-specific immune stimulants.[59] In general, the more impaired the response of lymphocytes to the antigen, the worse the prognosis. A more convenient method of obtaining comparable information has been demonstrated in the study of serum glycoproteins, especially alpha 2HS-glycoprotein.[5,60] Serum levels of acute phase proteins correlate inversely with *in vitro* and *in vivo* parameters of cellular immunity, whereas serum levels of alpha 2HS-glycoprotein correlate directly with these same parameters.[60]

Another attempt to simplify the study of cell-mediated immunity has been the analysis of circulating T lymphocytes.[61] While the prognostic value of the percent circulating T lymphocytes of the total lymphocyte count is controversial,[62] there is general agreement that a low absolute number of circulating T cells is present in patients who develop carcinomas and that this decreased number correlates with increasing stage of disease.[63,64]

Schantz *et al.*[65,66] have investigated a series of potential immunologic markers of treatment response. In an initial study,[65] they found that untreated patients with head and neck cancer expressed significantly higher levels of C1q-binding macromolecules, compared to non-cancer controls. Furthermore, an elevated level of C1q-binding macromolecules within the head and neck cancer population was predictive of subsequent non-response to induction chemotherapy. In a subsequent study examining a series of 17 cellular and humoral immunologic parameters, Schantz *et al.*[66] demonstrated that failure to respond to chemotherapy was significantly related to higher levels of total circulating immunoglobulin and C1q-binding activity (C1qBA). However, in multivariate logistic regression analysis, only C1qBA levels were predictive of cytotoxic response despite a broad overlap of C1qBA levels between responders and non-responders. This result requires prospective confirmation. Further research into the precise nature of the macromolecules capable of binding complement and the biologic mechanisms underpinning the association between C1qBA and chemotherapy response is indicated.

Yamanaka *et al.*[67] assayed the sera of 85 patients with a range of head and neck cancer sites for immune complexes and for squamous cell carcinoma-related antigen. Results suggested that post-treatment levels of both serum proteins may provide some broadly useful information regarding the presence of recurrence. Finally, an intriguing recent unconfirmed report involving a small number of head and neck cancer patients by Byrne *et al.*[68] attaches prognostic significance to rhesus blood groups and hemoglobin levels in multivariate survival analysis.

Molecular biology

Relatively little is known at the molecular level about the mechanisms which control the proliferation and neoplastic behavior of head and neck SCCs. However, the results of several studies suggest that oncogene amplifications and allelic deletions may contribute to the development and progression of specific human cancers. For instance, in neuroblastoma N-*myc* amplification is found at a high frequency in patients with disease stages 3 and 4.[69] In addition, the three members of the *myc* family are amplified in small cell lung carcinomas, and c-*myc* amplification is related to an aggressive histologic subtype.[70] Slamon *et al.*[71] have demonstrated that amplification of the HER-2/neu (c-*erb*B-2) oncogene occurs relatively frequently in patients with breast cancer and that it is associated with early disease relapse and poor overall patient survival. These data suggest that analysis of oncogenes and their protein products may have important benefits for cancer patients in the development of more precise prognostic indices.

Several studies have been undertaken to determine the incidence and potential significance of proto-oncogene amplification in head and neck cancer patients. Although the incidence of oncogene amplification has been documented at a number of loci (Table 2), no clear cut prognostic results have been obvious. Berenson *et al.*[80] have

Table 2. Proto-oncogene amplification in fresh head and neck SCCs

Reference	Locus studied	Number amplified/ number tested	Percent amplified
Hunts *et al.* (1985)[72]	EGFR	1/10	10
Yamamoto *et al.* (1986)[73]	EGFR	1/6	16
Yokota *et al.* (1986)[74]	c-*myc*	2/7	28
Yokota *et al.* (1986)[75]	EGFR	1/8	12
	c-*cerb*B-2	0/8	0
Eisbruch *et al.* (1987)[76]	EGFR	0/17	0
Tsutsumi *et al.* (1988)[77]	*hst*-1/*int*-2	2/5	40
Zhou *et al.* (1988)[78]	*int*-2	2/8	25
Berenson *et al.* (1989)[79]	*bcl*-1	8/23	34
	c-*myc*/Ha-*ras*	0/17	0
Berenson (1989)[80]	*bcl*-1/*int*-2 (co-amplification)	7/20	35
Ishitoya *et al.* (1989)[81]	EGFR	4/21	19
Saranath *et al.* (1989)[82]	c-*myc*/Ki-*ras*	4/23	17
	N-*ras*	7/23	30
	N-*myc*	9/23	39
	L-*myc*/Ha-*ras*	0/23	0
Leonard *et al.* (1991)[83]	c-*myc*	6/66	9
	EGFR	7/66	10
	bcl-1/*int*-2 (co-amplification)	5/66	7
	TGF-α	0/66	0
	c-Ha-*ras*-1	0/66	0
	c-*mos*	0/54	0
	c-*erb*B-2	0/31	0
	c-*erb*A-2	0/38	0

suggested that amplification of the *bcl*-1 locus is more frequently observed in patients with poorly differentiated head and neck SCCs.

Overexpression of the c-*myc* gene has been reported in a number of tumors without amplification of the gene[84] and this also appears to be so in patients with head and neck cancer.[85] Elevated levels of c-*myc* RNA transcripts were initially reported by Spandidos *et al.*[88] in head and neck SCCs and have since been reported by others.[87,88] Field *et al.*[89] have reported that quantitation of c-*myc* oncoproteins by ELISA was a useful prognostic marker in a small number of head and neck cancer patients. There was an inverse relationship between the level of c-*myc* oncoprotein and duration of survival. A similar correlation was found between elevated c-*myc* expression and clinical outcome using the *myc*1-9E10 monoclonal antibody in a subsequent immunohistochemical analysis.[90]

The clinical significance of p21*ras* expression and prognosis in head and neck SCCs is unclear. Field *et al.*[91] have demonstrated that overexpression of the p21*ras* correlated with a favorable prognosis in head and neck SCCs, but this finding is at variance with that reported by two Japanese groups,[92,93] raising the possibility that different environmental factors inter-relate in the initiation of head and neck SCCs in these two different geographical regions.

Leonard *et al.*[83] have recently reported a study of oncogene amplification in head and neck cancers; amplification of at least one gene was found in 18% of samples. The incidence of proto-oncogene amplification in head and neck SCC patients is comparable to that reported for other solid tumors. However, there was no statistically significant difference in survival between patients with or without gene amplification. The presence of multiple gene amplifications in several patients with advanced primary tumors suggests that the accumulation of genetic changes may correlate more closely with tumor size than with inherent biologic aggression.

Nucleolar organizer region-associated proteins

Argyrophilic staining of intranucleolar, non-histone proteins which are specifically associated with transcriptionally-active sites of ribosomal DNA have been investigated as a prognostic index in a number of malignancies.[94–96] These nucleolar

organizer region-associated proteins (AgNORs) are of central importance in the regulation of protein synthesis of a given cell, and their size and number have been correlated with aneuploidy, tumor grade and proliferative kinetics.[96] The AgNOR technique is a simple, inexpensive and accurate technique which can be applied both to formalin-fixed, paraffin-embedded tissue and to cytologic preparations. Sano *et al.*[97] have reported that high AgNOR counts in head and neck cancer patients are highly suggestive of a poor prognosis. Mourad *et al.*[96] found that tumors with a high AgNOR count were significantly correlated with aneuploidy, tumor grade and high S-phase fraction.

Other antigenic markers

Since the development of hybridoma technology, there have been numerous reports of antigenic expression by malignant squamous cells. Monoclonal antibodies have now been reported to react with a range of antigenic determinants, including several cytokeratins,[98,99] desmosomal glycoproteins,[100] cell adhesion molecules[101] and other hitherto unidentified cytoplasmic or membrane antigens.[102–104]

Cell surface carbohydrates are produced during the post-translational modification (glycosylation) of proteins/lipids to glycoproteins/glycolipids involving the sequential action of various glycotransferases. Unlike normal epithelia, aberrant glycosylation is a common feature of malignancy which results in the appearance of both tumor-associated and altered antigens of cell surface carbohydrates.[105] These changes are apparently due to an incomplete or neosynthesis of carbohydrate chains caused by either absence of some normal glycotransferases or activation of new tumor-related glycotransferases. Cell surface carbohydrates play an important role in the regulation of cell proliferation and epithelial growth in the oral mucosa.[106] Alterations in cell surface carbohydrates have also been suggested to influence the mechanisms of both tumor growth and spread.[107]

Wolf *et al.*[108] have recently identified an antigen found on the basal surface of epithelial cells and expressed on all squamous cell carcinomas of the head and neck region. These authors have demonstrated that cell lines from metastatic or recurrent SCCs exhibit stronger expression of the A9 cell membrane antigen than cell lines from the primary tumor of the same patients. Loss of expression in tumor tissue of normal A, B and H (ABH) blood group antigens has also been linked to clinical behavior in some epithelial cancers. A combination of A9 pattern and ABH blood group antigen expression in SCCs was the variable most strongly associated with duration of disease-free survival, even after adjustment for the traditional prognostic factors of tumor site, stage and TNM classification. Loss of blood group antigens was the most significant single variable associated with early recurrence, but among patients whose tumors retained ABH blood group antigen expression, the A9 pattern distinguished good and poor prognostic groups.

Schipper *et al.*[101] have recently suggested that loss of the cell adhesion molecule E-cadherin plays an important role in the progression of head and neck SCCs. E-cadherin was expressed in well and moderately differentiated tumors, but not in poorly differentiated ones. Furthermore, seven of eight lymph nodes were found to have down-regulated E-cadherin, independent of the primary tumor grade. It is thus possible that E-cadherin down-regulation may provide a very powerful method to assess the invasive and metastatic potential of head and neck cancers.

Robinson[98] has recently reported a study demonstrating that the expression of certain classes of keratin varies with cell maturation and differentiation. SCCs cannot be considered homogeneous entities with uniform growth characteristics and, like other tumors, they may express complex patterns of cytokeratins and produce keratins that are absent from or only a minor component of the tissue in the area of origin. As squamous cancer cells penetrate more deeply into adipose tissue and muscle, they relinquish contact with normal dermal and epidermal elements and factors which may help modulate cell growth. When analyzed with a battery of six monoclonal antibodies to keratin, serial sections of large tumors demonstrate progressive changes in the keratin proteins expressed as the tumor invades. At the deep margins of invasion, well-differentiated SCCs cease production of high molecular weight keratins. This selective loss of keratin polypeptide markers in SCCs is associated with progressively more aggressive biologic behavior. While tumor cells retain the specificity of the intermediate filament keratin, the individual cells express products of differentiation as measured by keratin expression independently of their cytologic atypia. Attempts have been made to correlate aggressive biologic behavior by SCCs with the cellular expression of keratin subsets.

Finally, Kearsley *et al.*[109] have reported the reactivity of a novel murine IgM monoclonal antibody, 3H-1, in formalin-fixed tissue from a series of patients with SCCs of the head and neck region. The pattern of cellular staining with 3H-1 made it possible to identify different cellular phenotypic subpopulations more accurately than is possible by conventional means; multivariate analyses demonstrated that perinuclear focal or nil staining was a significant independent prognostic factor for survival and was the only significant prognostic factor for relapse.

While it is clear that several of these antibodies provide information about SCCs not currently demonstrated on hematoxylin & eosin sections, some are applicable only to frozen sections and there is occasional cross-reactivity with other non-SCC tissues. Prognostic studies using archival formalin-fixed material are few.

Serum sialic acid

Bhatavdeker *et al.*[110] have studied the prognostic value of protein-bound (PSA), lipid-bound (LSA) and free sialic acid (FSA) levels in the serum of patients with head and neck cancer. Although there was a broad overlap of raised PSA in patients with benign disorders and head and neck cancer patients, a strong correlation was demonstrated between PSA and disease activity. PSA levels tended to increase or remain at high levels in patients with a poor prognosis. Altevogt *et al.*[111] have previously reported a correlation between metastatic potential and the position of sialic acid molecules on the surface of head and neck cancer cell lines.

Conclusion

Over the past few years, a growing list of factors believed to influence the prognosis for patients with head and neck cancer has been reported. Although a number of these so-called 'prognostic factors' require independent validation in larger prospective studies, it is likely that new parameters such as tumor cell proliferation activity, DNA ploidy status and molecular biologic markers will prove valuable in predicting both relapse and survival. However, it is at present unclear whether any marker is sufficiently reliable to influence individual therapy, and a more critical assessment is required of the proportion of patients who may actually benefit from more accurate prognostic data. One can only appeal to clinical investigators that future studies be suitably designed so that the precise combination and the hierarchy of these prognostic indices can be determined. Only time will tell whether many of these newer prognostic factors generated by sophisticated and potentially costly techniques will be as routinely applicable to head and neck cancers as are those currently available.

References

1. Boring CC, Squires TS, Tong T. Cancer statistics, 1993. *CA Cancer J Clin* 1993; **43**: 7–26.
2. Desai PB. Keynote address: predictive criteria for treatment responses in oral cancer– histomorphologic and biologic approaches. *Second ICOC*, New Delhi. December 1991: 3.
3. Platz H, Fries R, Hudec M, *et al.* The prognostic relevance of various factors at the time of the first admission of the patient. Retrospective DOSAK study of carcinoma of the oral cavity. *J Maxillofac Surg* 1983; **11**: 3–12.
4. Henderson BE, Ross RK, Pike MC. Towards the primary prevention of cancer. *Science* 1991; **254**: 1131–8.
5. Davis RK. Prognostic variable in head and neck cancer. *Otolaryngol Clin North Am* 1985; **18**: 411–19.
6. Bennett SH, Futrell JW, Roth JA, *et al.* Prognostic significance of histologic host response in cancer of the larynx or hypopharynx. *Cancer* 1971; **28**: 1255–65.
7. Platz H, Fries R, Hudec M. *Prognosis of oral cavity carcinomas.* Munich: Carl Hanser 1986.
8. Kalnins IK, Leonard AG, Sako KJ, *et al.* Correlation between prognosis and degree of lymph node involvement in carcinoma of the oral cavity. *Am J Surg* 1977; **134**: 450–4.
9. Ditroia JF. Nodal metastases and prognosis in carcinoma of the oral cavity. *Otolaryngol Clin North Am* 1972; **5**: 333–42.
10. Cachin Y, Sancho-Garnier H, Micheau C, *et al.* Nodal metastasis from carcinomas of the oropharynx. *Otolaryngol Clin North Am* 1979; **12**: 145–54.
11. Henson DE. The histological grading of neoplasms. *Arch Pathol Lab Med* 1988; **112**: 1091–6.
12. Arthur K, Farr HW. Prognostic significance of histologic grade in epidermoid carcinoma of the mouth and pharynx. *Am J Surg* 1972; **124**: 489–92.
13. Stenkvist B, Westman-Naeser S, Vegelius J, *et al.* Analysis of reproducibility of subjective grading systems for breast carcinoma. *J Clin Pathol* 1979; **32**: 979–85.
14. Thomas GDH, Dixon MF, Smeeton NC, *et al.* Observer variations in the histologic grading of rectal carcinoma. *J Clin Pathol* 1983; **36**: 385–91.
15. Jakobsson P, Killander D, Moberger G, *et al.* Histologisk Klassifikation och malignitets gradering vid larynxcancer. *Proc Swedish Soc Med Radiol* 1972; **62**: 836–48.
16. Jakobsson PA. Histologic grading of malignancy and prognosis in glottic carcinoma of the larynx. Workshop No. 14. In: Alberti PW, Bryce DP, eds. *Centennial conference on laryngeal cancer.* New York: Appleton-Century-Crofts 1976: 847–52.
17. Holm L-E, Lundquist P-G, Silfverward C, *et al.* Histologic grading of malignancy in squamous cell carcinoma of the oral tongue. *Acta Otolaryngol* 1982; **94**: 185–92.

18. Willen R, Nathauson A, Moberger G, *et al.* Squamous cell carcinoma of the gingiva. *Acta Otolaryngol* 1975; **79**: 146–54.
19. Eneroth C-M, Moberger G. Histologic malignancy grading of squamous cell carcinoma of the palate. *Acta Otolaryngol* 1973; **75**: 293–5.
20. Crissman JD, Gluckman J, Whiteley J, *et al.* Squamous cell carcinoma of the floor of mouth. *Head Neck Surg* 1980; **3**: 2–7.
21. Crissman JD, Liu WY, Gluckman JL, *et al.* Prognostic value of histopathologic parameters in squamous cell carcinoma of the oropharynx. *Cancer* 1984; **54**: 2995–3001.
22. Byrne M, Koppang HS, Lilleng R, *et al.* New malignancy grading is a better prognostic indicator than Broders' grading in oral squamous cell carcinomas. *J Oral Path Med* 1989; **18**: 432–7.
23. Johnson NW. The role of histopathology in diagnosis and prognosis of oral squamous cell carcinoma. *Proc Roy Soc Med* 1976; **69**: 740–6.
24. Johnson NW. Histological and histochemical studies of oral cancer. *Int Dent J* 1977; **27**: 25–34.
25. Langdon JD, Harvey PW, Rapidis AD, *et al.* Oral cancer: the behaviour and response to treatment of 194 cases. *J Maxillofac Surg* 1977; **5**: 221–7.
26. Rapidis AD, Langdon JD, Patel MF, *et al.* STNMP—a new system for clinico-pathologic classification and identification of oral carcinoma. *Cancer* 1977; **39**: 204–9.
27. Aneroth G, Hansen LS, Silverman S. Malignancy grading in oral squamous cell carcinoma. Part 1: squamous cell carcinoma of the tongue and floor of mouth; histologic grading in the clinical evaluation. *J Oral Pathol* 1986; **15**: 162–8.
28. Griffin TW, Pajak TF, Wilson Gillespie B, *et al.* Predicting the response of head and neck cancers to radiation therapy with multivariate modelling system: an analysis of the RTOG head and neck registry. *Int J Radiat Oncol Biol Phys* 1984; **10**: 481–7.
29. Young GAR, Hedley DW, Rugg CA, *et al.* The prognostic significance of proliferative activity in poor histology non-Hodgkin's lymphoma: a flow cytometry study using archival material. *Eur J Cancer Clin Oncol* 1987; **23**: 1497–504.
30. Sledge GW, Eble JN, Roth BJ, *et al.* Relation of proliferative activity to survival in patients with advanced germ cell cancer. *Cancer Res* 1988; **48**: 3864–8.
31. Tubiana M, Courdi A. Cell proliferation kinetics in human solid tumours: relation to probability of metastatic dissemination and long-term survival. *Radiother Oncol* 1989; **15**: 1–18.
32. Brown DC, Cole D, Gatter KC, *et al.* Carcinoma of the cervix uteri: an assessment of tumour proliferation using the monoclonal antibody Ki-67. *Br J Cancer* 1988; **57**: 178–81.
33. Shindelman JE, Ortmeyer AE, Sussman HH. Demonstration of the transferrin receptor in human breast cancer tissue. Potential marker for identifying dividing cells. *Br J Cancer* 1981; **27**: 329–34.
34. Sainsbury JRC, Farndom JR, Needham GK, *et al.* Epidermal growth factor receptor status as a predictor of early recurrence of and death from breast cancer. *Lancet* 1987; **i**: 1398–402.
35. Hall PA, Levinson DA, Woods AL, *et al.* Proliferating cell nuclear antigen (PCNA) immunolocalization in paraffin sections—an index of cell proliferation with evidence of deregulated expression in some neoplasms. *J Pathol* 1990; **162**: 285–94.
36. Sikora K, Chan S, Evan G, *et al.* c-*myc* oncogene expression in colorectal cancer. *Cancer* 1987; **59**: 1289–95.
37. Kearsley JH, Furlong KL, Cooke RA, *et al.* An immunohistochemical assessment of cellular proliferation markers in head and neck squamous cell cancers. *Br J Cancer* 1990; **61**: 821–7.
38. Sakai H, Kawano K, Okamura K, *et al.* Immunohistochemical localisation of c-*myc* and EGF receptor gene products in oral squamous cell carcinoma. *J Oral Pathol Med* 1990; **19**: 1–4.
39. Chauvel P, Courdi A, Gioanni J, *et al.* The labelling index: a prognostic factor in head and neck carcinoma. *Radiother Oncol* 1989; **14**: 231–7.
40. Bennett MH, Wilson GD, Dische S, *et al.* Tumour proliferation assessed by combined histological and flow cytometric analysis: implications for therapy in squamous cell carcinoma in the head and neck. *Br J Cancer* 1992; **65**: 870–8.
41. Dische S. Saunders MI, Bennett MH, *et al.* Cell proliferation and differentiation in squamous cancer. *Radiother Oncol* 1989; **15**: 19–23.
42. Wilson GD, McNally NJ, Dische S, *et al.* Measurement of cell kinetics in human tumours *in vivo* using bromodeoxyuridine incorporation and flow cytometry. *Br J Cancer* 1988; **58**: 423–31.
43. Corvo R, Giaretti W, Margarino G, *et al.* The *in vivo* use of bromodeoxyuridine for the study of cell kinetics in squamous tumors of the neck and head. *Radiologia Medica* 1991; **82**: 147–52.
44. Laerum OD, Farsund T. Clinical application of flow cytometry: a review. *Cytometry* 1981; **2**: 1–13.
45. Cornelisse CJ, Van De Velde CJH, Caspers RJC, *et al.* DNA ploidy and survival in breast cancer patients. *Cytometry* 1987; **8**: 225–34.
46. Friedlander ML, Hedley DW, Taylor IW, *et al.* Influence of cellular DNA content on survival in advanced ovarian cancer. *Cancer Res* 1984; **44**: 397–400.
47. Hedley DW. Flow cytometry using paraffin-embedded tissue: five years on. *Cytometry* 1989; **10**: 229–41.
48. Blijham G, Schutte B, Reynders M, *et al.* Flow cytometric determination of ploidy level and life cycle analysis on 297 paraffin-embedded colorectal carcinoma specimens. *Proc Am Soc Clin Oncol* 1985; **4**: 22.
49. Look AT, Douglass EC, Meyer WH. Clinical importance of near-diploid tumour stem lines in patients with osteosarcoma of an extremity. *N Engl J Med* 1988; **318**: 1567–72.
50. Kearsley JH, Bryson G, Battistutta D, *et al.* Prognostic importance of cellular DNA content in head and neck squamous cell cancers. A comparison of retrospective and prospective series. *Int J Cancer* 1991; **47**: 31–7.
51. Holm LE. Cellular DNA amounts of squamous cell carcinomas of the head and neck region in relation to prognosis. *Laryngoscope* 1982; **92**: 1064–9.
52. Johnson TS, Williamson KD, Cramer MM, *et al.* Flow cytometric analysis of head and neck carcinoma DNA index and s-fraction from paraffin-embedded sections: comparison with malignancy grading. *Cytometry* 1985; **6**: 461–70.
53. Kaplan AS, Caldarelli DD, Chacho MS, *et al.* Retrospective DNA analysis of head and neck squamous

cell carcinoma. *Arch Otolaryngol Head Neck Surg* 1986; **112**: 1159–62.
54. Goldsmith MM, Cresson DH, Arnold LA, *et al.* DNA flow cytometry as a prognostic indicator in head and neck cancer. *Otolaryngol Head Neck Surg* 1987; **96**: 307–18.
55. Tytor M, Franzen G, Olfsson G, *et al.* DNA content, malignancy grading and prognosis in T1 and T2 oral cavity carcinomas. *Br J Cancer* 1987; **56**: 647–52.
56. Kokal WA, Gardiner RL, Sheibani K, *et al.* Tumour DNA content as a prognostic indicator in squamous cell carcinoma of the head and neck region. *Proc Am Soc Clin Oncol* 1988; **7**: 149.
57. Ensley JF, Maciorowski Z, Hassan M, *et al.* Cellular DNA content parameters in untreated and recurrent squamous cell cancers of the head and neck. *Cytometry* 1989; **10**: 334–8.
58. Eilber FR, Morton DL. Impaired immunologic reactivity and recurrence following cancer surgery. *Cancer* 1970; **25**: 362–7.
59. Katz AE. Immunobiologic staging of patients with carcinoma of the head and neck. *Laryngoscope* 1983; **93**: 445–63.
60. Baskies AM, Chretien PB, Weiss JF, *et al.* Serum glycoproteins in cancer patients. *Cancer* 1980; **45**: 3050–60.
61. Deegan MJ, Coulthard SW. Spontaneous rosette formation and rosette inhibition assays in patients with squamous cell carcinoma of the head and neck. *Cancer* 1977; **39**: 2137–41.
62. Mason JM, Kitchens GG, Eastham RJ, *et al.* T-lymphocytes and survival of head and neck squamous cell carcinoma. *Arch Otolaryngol* 1977; **103**: 223–7.
63. Olkowski ZL, Wilkins SA. T-lymphocyte levels in the peripheral blood of patients with cancer of the head and neck. *Am J Surg* 1975; **130**: 440–3.
64. Veltri RW, Sprinkle PM, Maxim PE, *et al.* Immune monitoring protocol for patients with carcinoma of the head and neck. *Ann Otol Rhino Laryngol* 1978; **87**: 692–700.
65. Schantz SP, Savage HE, Brown BW, *et al.* Association of levels of circulating C/q binding macromolecules with induction chemotherapy response in head and neck cancer patients. *Cancer Res* 1988; **48**: 5868–73.
66. Schantz SP, Savage HE, Rocz T, *et al.* Immunologic determinants of head and neck cancer response to induction chemotherapy. *J Clin Oncol* 1989; **7**: 857–64.
67. Yamanaka N, Himi T, Harabuchi Y, *et al.* Soluble immune complexes and squamous cell carcinoma-related antigens in patients with head and neck cancer. *Cancer* 1988; **62**: 1932–8.
68. Byrne M, Eide GE, Lilleng R, *et al.* A multivariate study of the prognosis of oral squamous cell carcinomas. *Cancer* 1991; **68**: 1994–8.
69. Schwab M, Ellison J, Busch M, *et al.* Enhanced expression of the human gene N-*myc* consequent to amplification of DNA may contribute to malignant progression neuroblastoma. *Proc Natl Acad Sci USA* 1984; **81**: 4940–4.
70. Johnson BE, Ihde DC, Makuch RW. *myc* family oncogene amplification in tumour cell lines established from small cell lung cancer patients and its relationship to clinical status and course. *J Clin Invest* 1987; **79**: 1629–34.
71. Slamon DJ, Clark GM, Wong SG, *et al.* Human breast cancer: correlation of relapse and survival with amplification of the HER-2/*neu* oncogene. *Science* 1987; **235**: 177–82.
72. Hunts J, Ueda M, Ozawa S, *et al.* Hyperproduction and gene amplification of the epidermal growth factor receptor in squamous cell carcinomas. *Jpn J Cancer Res* 1985; **76**: 663–6.
73. Yamamoto T, Kamata N, Kawano H, *et al.* High incidence of amplification of the epidermal growth factor receptor gene in human squamous carcinoma cell lines. *Cancer Res* 1986; **46**: 414–6.
74. Yokota J, Tsunetsugu-Yokota Y, Battifora H, *et al.* Alterations of *myc*, *myb*, *ras*^{Ha} proto-oncogenes in cancers are frequent and show clinical correlation. *Science* 1986; **231**: 261–5.
75. Yokota J, Toyoshima K, Sugimara T, *et al.* Amplification of c-erbB-2 oncogene in human adenocarcinomas *in vivo*. *Lancet* 1986; **1**: 765–7.
76. Eisbruch A, Blick M, Lee JS, *et al.* Analysis of the epidermal growth factor receptor gene in fresh human head and neck tumours. *Cancer Res* 1987; **47**: 3603–5.
77. Tsutsumi M, Sakamoto H, Yoshida T, *et al.* Coamplification of the *hst*-1 and *int*-1 genes in human cancers. *Jpn J Cancer* 1988; **79**: 428–32.
78. Zhou DJ, Casey G, Cline MJ. Amplification of human *int*-2 in breast cancers on squamous carcinomas. *Oncogene* 1988; **2**: 279–82.
79. Berenson JR, Yang J, Alessi DM, *et al.* Amplification of the putative oncogene BCL-1 in head and neck squamous carcinomas. *Proc Am Ass Can Res* 1989; **30**: 1754 (abstr).
80. Berenson JR, Yang J, Michel RA. Frequent amplification of the *bcl*-1 locus in head and neck squamous cell carcinomas. *Oncogene* 1989; **4**: 1111–6.
81. Ishitoya J, Toriyama M, Oguchi N, *et al.* Gene amplification and overexpression of EGF receptor in squamous cell carcinomas of the head and neck. *Br J Cancer* 1989; **59**: 559–62.
82. Saranath D, Panchal G, Nair R, *et al.* Oncogene amplification in squamous cell carcinoma of the oral cavity. *Jpn J Cancer Res* 1989; **80**: 430–7.
83. Leonard JH, Kearsley JH, Chenevix-Trench G, *et al.* Analysis of gene amplification in head and neck squamous cell carcinomas. *Int J Cancer* 1991; **48**: 511–5.
84. Alitalo K, Koshkinein C, Makela TP, *et al.* *myc* oncogenes: activation and amplification. *Biochim Biophys Acta* 1987; **907**: 1–32.
85. Field JK. Oncogenes and tumour-suppressor genes in squamous cell carcinoma of the head and neck. *Oral Oncol* 1992; **1**: 67–76.
86. Spandidos DA, Lamothe A, Field JK. Multiple transcriptional activation of cellular oncogenes in human head and neck solid tumours. *Anticancer Res* 1985; **5**: 221–4.
87. Riviere A, Wilckens C, Loning T. Expression of c-*erb*B-2 and c-*myc* in squamous epithelia and squamous cell carcinomas of the head and neck and the lower female genital tract. *J Oral Pathol Med* 1990; **19**: 408–13.
88. Kawano SH, Okamura K, Hashimoto N. Immunohistochemical localisation of c-*myc* oncogene product and EGF receptor in oral squamous cell carcinoma. *J Oral Pathol Med* 1990; **19**: 1–4.
89. Field JK, Spandidos DA, Stell PM, *et al.* Elevated expression of the c-*myc* oncoprotein correlates with poor prognosis in head and neck squamous cell carcinoma. *Oncogene* 1989; **4**: 1463–8.
90. Butt SA, Field JK, Cosney JR, *et al.* Immunohistochemi-

cal study of c-*myc* expression in head and neck cancer: a prognostic indicator. *In Vivo* 1990; **4**: 8.
91. Field JK, Yiagnisis M, Spandidos DA, *et al.* Low levels of *ras* p21 oncogene expression correlates with clinical outcome in head and neck squamous cell carcinoma. *Eur J Surg Oncol*, in press.
92. Azuma M, Furumoto N, Kawamata H, *et al.* The relation of *ras* oncogene product p21 expression to clinicopathological status and clinical outcome in squamous cell head and neck cancer. *Cancer J* 1987; **1**: 375–80.
93. Tsuji T, Sasaki K, Hiraoka F, *et al.* The immunohistochemical detection of *ras* p21 and its correlation with differentiation in oral cancers. *J Tumour Marker Oncol* 1989; **4**: 415–9.
94. Ruschoff J, Plate K, Bittinger A, *et al.* Nucleolar organiser regions (NORs). Basic concepts and practical applications in tumor pathology. *Path Res Pract* 1989; **185**: 878–85.
95. Crocker J, Skilbeck N. Nucleolar organiser region associated proteins in cutaneous melanotic lesions: a quantitative study. *J Clin Pathol* 1987; **40**: 885–9.
96. Mourad WA, Erkman-Balis B, Livingston S, *et al.* Argyrophilic nucleolar organiser regions in breast carcinoma. *Cancer* 1992; **69**: 1739–44.
97. Sano K, Takahashi H, Fujita S, *et al.* Prognostic implication of silver-binding nucleolar organiser regions (AgNORs) in oral squamous cell carcinoma. *J Oral Pathol Med* 1991; **20**: 53–6.
98. Robinson JK. Expression of keratin proteins in deeply invasive basal and squamous cell carcinoma: an immunohistochemical study. *J Dermatol Surg Oncol* 1987; **13**: 283–94.
99. Huszar M, Gigi-Leitner O, Moll R, *et al.* Monoclonal antibodies to various acidic (type I) cytokeratins of stratified epithelia. *Differentiation* 1986; **31**: 141–53.
100. Moll R, Cowia P, Kapprell H-P, *et al.* Desmosomal proteins: new markers for identification and classification of tumours. *Lab Invest* 1986; **54**: 4–25.
101. Schipper JH, Frixen UH, Behrens J, *et al.* E-Cadherin expression in squamous cell carcinomas of head and neck: inverse correlation with tumor dedifferentiation and lymph node metastasis. *Cancer Res* 1991; **51**: 6328–37.
102. Ranken R, White CF, Gottfried TG, *et al.* Reactivity of monoclonal antibody 17.13 with human squamous cell carcinoma and its application to tumour diagnosis. *Cancer Res* 1987; **47**: 5684–90.
103. Samuel J, Noujaim AA, Willans DJ, *et al.* A novel marker for basal (stem) cells of mammalian stratified squamous epithelia and squamous cell carcinomas. *Cancer Res* 1989; **49**: 2465–70.
104. Tatake RJ, Amin KM, Maniar HS, *et al.* Monoclonal antibody against human squamous cell carcinoma associated antigen. *Int J Cancer* 1989; **44**: 840–5.
105. Vigneswaran N, Petus K-P, Hornstein OP, *et al.* Alteration of cell surface carbohydrates associated with ordered and disordered proliferation of oral epithelia: a lectin histochemical study in oral leukoplakias, papillomas and carcinomas. *Cell Tissue Kinet* 1990; **23**: 41–55.
106. Hakomori SI. Aberrant glycosyletion in cancer cell membranes as focused on glycolipids: overview and perspectives. *Cancer Res* 1985; **45**: 2405–10.
107. Fenlon S, Ellis IO, Bell J, *et al. Helix pomatia* and *Ulex europeus* lectin binding in human breast carcinoma. *J Pathol* 1987; **152**: 169–75.
108. Wolf GT, Carey TE, Schmaltz SP, *et al.* Altered antigen expression predicts outcome in squamous cell carcinoma of the head and neck. *JNCI* 1990; **82**: 1566–72.
109. Kearsley JH, Leonard JH, Takahashi H, *et al.* Prognostic significance of monoclonal antibody 3H-1 reactivity with squamous cell head and neck cancers. *Int J Cancer* 1991; **47**: 853–7.
110. Bhatavdekar JM, Vora HH, Patel DD. Serum sialic acid forms as markers for head and neck malignancies. *Neoplasma* 1988; **35**: 425–34.
111. Altevogt P, Fogel M, Cheinsong-Popov R, *et al.* Different patterns of lectin binding and cell surface sialylation detected on high- and low-metastatic tumour lines. *Cancer Res* 1983; **43**: 5138–43.

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