INTRODUCTION

Treatment of head and neck cancer requires accurate risk stratification in order to determine the type and extent of therapy and the expected clinical outcome. Physical exam, diagnostic imaging studies, and pathologic review enable the clinician to determine the size and extent of the primary tumor, the status of cervical lymph nodes, and the likelihood of distant metastatic disease, thus generating an accurate tumor, lymph node, metastasis (TNM) stage for each patient. In addition to TNM staging, other clinical and pathologic factors not routinely incorporated into the staging system have been shown to influence response to therapy and eventual outcome. These factors may be categorized as follows: (a) prognostic factors related to the primary tumor, (b) prognostic factors related to the cervical lymph nodes, (c) prognostic factors related to patient demographics, and (d) prognostic factors related to the patient’s general medical condition.

In addition to clinical and pathologic factors, recent interest has focused on identifying molecular factors that may influence clinical outcome. These molecular markers not only provide useful prognostic information, but also serve as targets for novel pharmacotherapies that antagonize cellular proliferation by interfering with specific cellular processes. Molecular factors that influence tumor behavior fall into several broad categories, including proto-oncogenes, tumor suppressor genes, growth factors, immune-related factors, loss of heterozygosity at various genetic loci, total cellular DNA content, and parameters related to the kinetics of in vivo tumor growth. Finally, the correlation of human papilloma virus (HPV) has been crucial to furthering our understanding of this new and important subset of cancers. This will be discussed in detail in Chapter 12.

When considering prognostic factors in head and neck cancer, several specific outcomes merit attention. In addition to disease-free and overall survival, the relationship between a specific prognostic factor and local recurrence, regional recurrence, and distant metastatic disease must be considered. Factors specifically related to local recurrence may necessitate wider surgical resection, external beam radiotherapy dose escalation or altered fractionation, or the use of a brachytherapy boost to the primary tumor bed. Factors specifically related to regional recurrence may prompt prophylactic neck dissection or radiotherapy in the clinically N0 neck or determine the type of neck dissection selected in patients with clinically positive cervical lymph nodes. Finally, factors predictive of distant metastases may prompt more aggressive screening for concomitant distant metastases before local-regional treatment and influence the decision to offer systemic therapy.

FACTORS RELATED TO THE PRIMARY TUMOR

Tumor Dimensions

Tumor size and extent of invasion determine clinical and pathologic T stage for head and neck squamous cell carcinomas (HNSCC). T stage is determined clinically by measuring the maximal surface diameter of a mucosal neoplasm or pathologically by measuring the maximal cross-sectional diameter of a resected tumor. Because tumors arising from mucosal surfaces do not conform to spherical geometry, the maximal tumor diameter does not perfectly correlate with either tumor depth or tumor volume.

As the primary determinant of T stage, maximum tumor diameter has traditionally been considered an important risk factor for the presence of concomitant nodal metastases, local recurrence, and poor survival. For example, in two studies conducted on a total of 603 patients with HNSCC, Magnano et al. found that T stage was a consistent, independent predictor of pathologically positive cervical lymph nodes. In addition, pathologic maximal tumor diameter has been shown to predict local recurrence in tumors arising from the lower lip, oral cavity and oropharynx, and larynx. Finally, most studies, though not all, have shown a univariate association between either clinical or pathologic tumor diameter and survival.

One limitation of using tumor size as a prognostic determinant was highlighted by Moore et al., who stratified 155 patients with oral SCC based on surface diameter of the primary tumor. Eighty-four percent of patients with tumors ≤2 cm survived disease-free for 3 years, compared with 52% of patients with tumors larger than 2 cm. However, no significant differences in survival existed between tumors with surface diameters in the following three groups: 2.1 to 3 cm, 3.1 to 4 cm, and >4 cm. Thus, although a gross trend exists between surface diameter and survival, this trend does not follow a simple dose–response relationship. In a follow-up study conducted on 151 patients with oral and oropharyngeal SCC, Moore et al. found that tumor thickness was a more consistent predictor of concomitant nodal metastasis and 3 year disease free survival than tumor surface diameter.
The correlation between tumor thickness and risk of concomitant cervical lymph node metastases has been confirmed in multiple studies. The critical thickness, above which the risk of nodal metastasis increases substantially, varies depending upon the specific anatomic site involved, perhaps due to variations in density of lymphatic channels. Nevertheless, for most anatomic subsites, the critical thickness ranges from 3 to 5 mm depending upon the specific study. For example, a recent study of 105 patients with clinically node negative oral cavity tumors reported that the risk of occult nodal metastasis was 10% if the tumor thickness was ≤5 mm, compared to 46% if the tumor thickness was >5 mm. Furthermore, in a series of 76 patients with carcinoma of the tongue who all received neck dissection, Woolgar demonstrated that the mean reconstructed thickness of tumors with pathologically positive cervical lymph nodes was 19 mm, compared to 10 mm in patients without metastases. Tumors with a large mucosal surface area but minimal invasion were not at increased risk of nodal metastases. In such cases, clinical and pathologic T stage may overestimate the likelihood of concomitant nodal disease.

In addition to consideration of maximal tumor diameter and thickness, utilization of computed tomography (CT) enables the calculation of total tumor volume. In a series of 63 patients with supraglottic primaries treated with primary irradiation, Mancuso et al. found that tumor volume calculated from pretreatment CT scans was an independent predictor of local control, with local control rates of 89% in tumors <6 cm³ versus 52% for tumors ≥6 cm³. Furthermore, in a study of 103 patients with supraglottic SCC, tumor volume as calculated from pretreatment CT was a stronger predictor of local-regional failure than clinical T stage in multivariate analysis. Hence, calculation of tumor volume using diagnostic imaging provides important prognostic information that can supplement traditional clinical staging for tumors of the larynx. In contrast, T stage, rather than tumor volume estimated from diagnostic imaging studies, appears to be a better predictor of local control for tumors of the oropharynx and hypopharynx treated with definitive radiation.

**Margin Status**

The presence of residual carcinoma at the margins of surgical resection is an important risk factor for local recurrence in HNSCC. Although the presence of positive margins may indicate an error in surgical judgment at the time of resection, it may also imply a more biologically aggressive tumor that extends microscopically through the muscle bundles, submucosal lymphatics, and perineural spaces. The precise definition of “positive margins” varies depending on the study and may include invasive tumor involving the initial surgical margin, invasive tumor involving the final surgical margin, invasive tumor approaching within 5 mm of the final surgical margin, carcinoma in situ involving the final surgical margin, or dysplasia involving the final surgical margin. Guidelines used in the United Kingdom define “negative” margins as invasive tumor more than 5 mm away from the surgical margin, “close” margins as invasive tumor within 1 to 5 mm from the surgical margin, and “positive” margins as tumor <1 mm from the surgical margin.

In a review of resections for head and neck cancer performed at Memorial-Sloan Kettering Cancer Center, Looser et al. classified 1,775 cases according to final margin status. After excluding patients with gross residual disease, they identified 62 patients with microscopically positive margins, defined as either cancer within 0.5 cm of the margin, marked atypia or premalignant changes in the margin, carcinoma in situ in the margin, or invasive carcinoma in the margin. Interestingly, patients in all four of these groups experienced increased local recurrent rates, ranging from 64% in patients with invasive cancer at the margin to 85% for patients with carcinoma in situ at the margin, compared to a local recurrence rate of 32% in patients with negative margins.

Many other studies have confirmed the prognostic importance of margin status in HNSCC. The presence of positive margins has been shown to predict local or local-regional recurrence for the following sites: lower lip, oral cavity, buccal mucosa, oral tongue, and oropharyngeal tongue, base of tongue, oral cavity and oropharynx, larynx, and all sites combined. Furthermore, the presence of positive margins predicts poor overall survival in univariate analysis for the following sites: oral cavity, oral tongue, oral cavity and oropharynx, larynx, and all sites combined. Whether or not margin status is an independent predictor of survival remains somewhat controversial, with two studies confirming an independent association, but six others failing to find an association.

Given the negative prognostic implications of positive margins, Byers et al. demonstrated that use of intraoperative frozen sections can identify those patients with initially positive margins and thus allow resection of additional tissue to remove residual carcinoma and reduce the risk of local recurrence. In this cohort of 216 patients with SCC arising from the oral cavity, oropharynx, and hypopharynx, patients with initially positive margins on frozen section received additional resection to achieve final negative margins. Local recurrence in this group was 13%, compared with a local recurrence rate of 12% in patients with negative margins on initial frozen section. Patients with positive final margins suffered from an 80% local recurrence rate. Hence, use of intraoperative frozen section analysis of surgical margins may result in a clinically significant reduction in local recurrence.

However, in a follow-up study of 268 patients with SCC of the tongue, those patients with initially positive margins that were ultimately rendered negative experienced an increased risk of local recurrence and death compared to patients with initially negative frozen section margins. Thus, the authors recommended use of postoperative radiotherapy in all patients with initially positive frozen section margins. One additional concern regarding the use of intraoperative frozen sections to determine margin status is potential inaccuracy, with one study reporting a 14% (7/49) false negative rate for oral cancer.

For those patients with persistently positive margins, postoperative external beam radiotherapy remains an important component of therapy. However, even with combined modality therapy, most investigators, although not all, report an increased incidence of local recurrence in those patients with positive margins. One important factor influencing local control in patients with positive margins is the dose of postoperative radiotherapy, as Zelefsky et al. noted a 7-year local control rate of 92% in patients who received 260 Gy, compared with 44% in patients receiving <60 Gy. In addition, interest has grown in using postoperative brachytherapy, either alone or following postoperative external beam radiotherapy, to improve local control in patients with positive margins. Beitler et al. reported 29 patients with microscopically close or positive margins after curative surgery treated with postoperative external beam radiotherapy to a median dose of 60 Gy followed by permanent implant designed to deliver a cumulative lifetime dose of 120 to 160 Gy to the high-risk target volume. This treatment strategy resulted in a 92% 2-year actuarial local control rate and clearly merits further study. Finally, growing evidence from two large randomized trials indicates that the addition of cisplatin (100 mg/m² on days 1, 22, and 43) to postoperative radiation improves local-regional control and perhaps overall survival for patients with positive surgical margins.
Malignancy Grading

Pathologists have long recognized the potential prognostic significance of cellular morphology in squamous cell carcinoma. In 1920, Broders proposed a four-tiered grading system for carcinoma of the lip that was based on the proportion of the neoplasm resembling normal squamous epithelium. Grading provides an easily assessed scheme for assessing tumor differentiation and roughly correlates with prognosis, as poorly differentiated tumors are more likely to recur and reduce survival. Broders’ grading system has been criticized, however, for its subjectivity and for its failure to consistently predict survival in multivariate modeling. The lack of independent significance of Broders’ grading system may be due to the association between higher grade (more poorly differentiated) and advanced stage.

In 1973, Jakobsson proposed a semiquantitative grading system that considered not only histologic parameters of the tumor cell population but also the host–tumor interface. Parameters describing the tumor cell population included structure and growth of the neoplasm, degree of keratinization, nuclear pleomorphism, and the frequency of mitoses. The tumor–host interface was assessed for mode of invasion, degree or stage of invasion, vascular invasion, and lymphoplasmacytic cellular response. When this classification system was applied to a series of 42 laryngeal SCCs treated with radiotherapy, tumors with high malignancy grade were more likely to recur and result in death with disease. However, in a subsequent multivariate analysis conducted on 77 patients with oropharyngeal cancer, Crissman et al. found that invasion pattern was the only histologic parameter that independently predicted survival. None of the other histologic parameters, either independently or as a composite score, were significant predictors of outcome.

In 1987, Anneroth et al. reviewed efforts to devise a malignancy grading system and proposed that grading consist of six morphologic features: degree of keratinization, nuclear polymorphism, number of mitoses, pattern of invasion, stage of invasion, and lymphoplasmacytic infiltration. In 1989, Bryne et al. applied Anneroth’s grading system to only the most anaplastic fields in the most invasive areas of the tumor (Table 3.1). In two cohorts of 68 and 61 patients with oral SCC, Bryne’s invasive cell grading was a highly significant, independent, and reproducible predictor of survival. Those patients with a total malignancy score between 5 and 10 experienced a 57% 5-year survival, compared to a 19% 5-year survival in patients with malignancy scores >10. In contrast, Broders’ grading system failed to correlate with survival in both cohorts. The authors concluded that histologically invasive regions might be responsible for the clinical behavior of HNSCC. Further study of invasive cell grading (ICG) conducted by other groups has shown that a high ICG score strongly predicts the presence of occult cervical metastases and extracapsular extension (ECE). Due to the strong correlation between ICG and concomitant nodal metastases, this factor should be considered in decisions regarding elective treatment of the clinically negative neck.

In addition to assessment of malignancy grade, many studies have attempted to determine which individual histologic parameters contribute most strongly to prognosis. Several studies suggest that pattern of invasion may predict outcome independent of other histologic parameters. Tumors that infiltrate in small groups or cords of cells, or infiltrate with marked cellular dissociation, may behave more aggressively. For example, Spiro et al. found that oral tongue SCCs with a high-grade pattern of invasion were more likely to present with concomitant nodal metastases, develop distant metastases, and result in death. In addition, for oral carcinoma that invades the mandible, high-grade invasion pattern increases the rate of mandibular margin positivity and local recurrence and results in a fourfold increased risk of death with disease in multivariate analysis. The correlation of aggressive invasion pattern with poor local-regional control and survival has been noted in several other studies.

In addition to pattern of invasion, lymphoplasmacytic infiltration of the tumor bed has been considered as a potential independent marker of prognosis. In a study of 396 patients with HNSCC, multivariate analysis revealed that the presence of a lymphocytic tumor infiltrate decreased the risk of concomitant

**TABLE 3.1 Invasive Cell Grading System**

<table>
<thead>
<tr>
<th>Morphologic Feature</th>
<th>Score</th>
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<tbody>
<tr>
<td>Degree of keratinization</td>
<td>1</td>
</tr>
<tr>
<td>Highly keratinized (&gt;50% of the cells)</td>
<td></td>
</tr>
<tr>
<td>Moderately keratinized (20%–50% of the cells)</td>
<td></td>
</tr>
<tr>
<td>Minimal keratinization (5%–20% of the cells)</td>
<td></td>
</tr>
<tr>
<td>No keratinization (0%–5% of the cells)</td>
<td></td>
</tr>
<tr>
<td>Nuclear polymorphism</td>
<td>1</td>
</tr>
<tr>
<td>Little nuclear polymorphism (&gt;75% mature cells)</td>
<td></td>
</tr>
<tr>
<td>Moderately abundant nuclear polymorphism (50%–75% mature cells)</td>
<td></td>
</tr>
<tr>
<td>Abundant nuclear polymorphism (25%–50% mature cells)</td>
<td></td>
</tr>
<tr>
<td>Extreme nuclear polymorphism (0%–25% mature cells)</td>
<td></td>
</tr>
<tr>
<td>Number of mitoses (high-power field)</td>
<td>0–1</td>
</tr>
<tr>
<td>Pattern of invasion</td>
<td>Pushing, well-delineated infiltrating borders</td>
</tr>
<tr>
<td>Lymphoplasmocytic infiltration</td>
<td>Marked</td>
</tr>
</tbody>
</table>

cervical lymph node metastases, whereas a plasmacytic infiltrate increased the risk. In addition, several studies have reported a correlation between lymphocyte infiltration and improved local-regional control.  

Basaloid SCC (BSCC) is a rare variant of SCC that has historically been thought to be chemoresistant and radiosensitive, with similar-to-better loco-regional control than “typical” SCC, but with higher risk of distant metastases. Soriano reported a case control series of BSCC versus SCC treated with various modalities and reported that loco-regional recurrence caused death in only 15% of the BSCC patients versus 51% of the SCC patients. Distant metastasis, however, occurred in 45% versus 7%, respectively.  

Thariat reported a case control series of radiated patients with BSCC versus poorly differentiated (PSCC) or well-differentiated SCC (WSCC). The loco-regional control for BSCC, PSCC, and WSCC was similar at 81%, 86%, and 82%, respectively; distant metastases free survival was worse for BSCC at 71%, 86%, and 86%, respectively, but not significantly so.  

Why the seeming inconsistency that BSCC is considered an aggressive variant of SCC, yet HPV-associated SCC is more commonly BSCC but has a better prognosis? It may be that BSCC is a more heterogeneous disease than previously recognized. Certainly the demographics of the BSCC patient are evolving: whereas the typical BSCC patient has been thought to be in his 60s or 70s, with history of frequent, intense tobacco use and alcohol use, recent cases have also affected young women without a significant tobacco or alcohol history, suggesting a causal link between BSCC and HPV-associated SCC. Therefore, BSCC may have a dual nature similar to SCC, depending on factors such as HPV status and tobacco and alcohol use.

**Perineural Invasion**

Infiltration of perineural spaces occurs in up to 52% of HNSCCs and was first noted to influence surgical and adjuvant treatment strategies by Ballantyne et al. in 1963. Perineural invasion (PNI) may result in dysphagia secondary to involvement of the vagal trunk or pain and paresthesias along the territories of the glossopharyngeal or trigeminal nerves. CT findings suggestive of PNI include obliteration of the fat within the pterygopalatine fossa, enlargement of neural foramina, and increased enhancement in the region of Mckee’s cave. In addition, a study of 48 patients with oral tongue, base of tongue, or floor of mouth SCC utilized CT to identify the presence of vascular and/or PNI with a sensitivity of 88% and a specificity of 83% using criteria including “aggressive” tumor margins, invasion of the sublingual space, and direct adjacency to the lingual vasculature.  

PNI may be mediated by the presence of nerve cell adhesion molecule (N-CAM) on the surface of squamous carcinoma cells, which engages in homophilic binding with N-CAM expressed in neural and perineural tissues. In two studies of 76 and 66 patients with HNSCC, expression of N-CAM on the surface of neoplastic cells was significantly associated with PNI detected on review of pathologic sections.  

Numerous clinical studies have identified PNI as an important predictor of poor prognosis. In tumors arising from the lower lip, oral tongue, oral cavity, larynx, and all sites combined, the presence of PNI is associated with a higher risk of metastasis to regional lymph nodes. Furthermore, following definitive treatment of HNSCC, the presence of PNI in the primary tumor is associated with poor local control and regional control. Local-regional control, cause-specific survival, and overall survival have been associated with an increased risk of distant metastasis in some, though not all, studies.

The association between PNI and local recurrence may result from either centrifugal or centripetal propagation of malignant cells along perineural spaces and away from the primary tumor. Most primary tumors will only disseminate up to 2 cm along the perineural space, although PNI 12 cm from the primary tumor has been reported. As a result, PNI may allow malignant cells to evade surgical excision or radiotherapy and result in local recurrence. In addition, the association between PNI and regional recurrence implies that these tumors may be more biologically aggressive. The association between perineural invasion and tumor aneuploidy, a known marker of poor prognosis, lends support to this hypothesis.  

Despite the clear importance of PNI, the percentage of mucosal HNSCCs positive for PNI varies widely in the literature, from 5% to 52%. This discrepancy may result from a tendency to identify PNI only when large, named nerves are involved. However, although PNI of small, unnamed nerves may not result in clinical symptoms, the relationship between PNI and prognosis appears to be independent of nerve diameter. Hence, all HNSCC pathologic specimens should be closely examined for PNI, even in nerves <1 mm in diameter.

**Vascular Invasion**

Vascular invasion, defined as the presence of neoplastic epithelium within an endothelial-lined channel, occurs in over 50% of HNSCCs. With tumor depth and pattern of invasion, the presence of vascular invasion may identify tumors with an aggressive biologic nature due to their ability to invade normal anatomic structures. Indeed, vascular invasion has been shown to correlate with the presence of concomitant cervical metastases in both univariate and multivariate models. In addition, the presence of vascular invasion in the primary tumor specimen has been noted to increase the risk for subsequent local regional recurrence in oral cavity, oropharynx, and larynx SCC treated surgically. However, in a large series of 226 patients treated with surgery and postoperative radiotherapy, vascular invasion did not correlate with local-regional control, suggesting that postoperative radiotherapy may mitigate the poor prognosis associated with vascular invasion. Vascular invasion has also been associated with increased risk of distant metastatic disease.

**Imaging**

18F-fluorodeoxyglucose (FDG) PET scan is a functional test that combines a positron-emitting nuclide with a glucose molecule, thereby revealing areas of increased glucose metabolism. PET/CT scans are commonly performed prior to therapy to determine extent of disease, and afterward to assess response and need for further treatment. The utility of a posttreatment PET has been well studied. Isles performed a meta-analysis of radiated patients who received posttreatment FDG PET scans. The weighted mean pooled sensitivity, specificity, PPV, and NPV of posttreatment PET for the primary site was 94%, 82%, 75%, and 95%, respectively. At the neck, the corresponding numbers were 74%, 88%, 49%, and 96%, respectively. They reported that sensitivity was greater for scans performed 10 weeks or longer after completion of therapy. Gupta also performed a meta-analysis: his corresponding numbers for the primary site were 79.9%, 87.5%, 58.6%, and 95.1%, respectively, and for the neck 72.7%, 87.6%, 52.1%, and 94.5%, respectively. Scans performed ≥12 weeks after therapy had moderately higher diagnostic accuracy. Moeller prospectively compared posttreatment PET/CT with contrast-enhanced CT in radiated patients. Patients were imaged with PET/CT and CT prior to and 8 weeks after radiation.
FACTORS RELATED TO THE CERVICAL LYMPH NODES

Number of Positive Lymph Nodes

The number, size, and location of positive cervical lymph nodes define the N stage for HNSCC and provide important information regarding prognosis and selection of treatment. The number of cervical lymph nodes histologically positive for squamous cell carcinoma provides one of the simplest, and perhaps most important, prognostic markers in head and neck cancer. Mamelle et al. retrospectively reviewed 914 patients who received cervical lymph node dissection as a component of initial therapy for oral cavity, oropharyngeal, hypopharyngeal, and laryngeal SCC.

Patients with palpable lymph nodes >3 cm underwent radical lymph node dissection, and all other patients underwent selective neck dissection on sentinel nodes with immediate pathologic evaluation. Those patients with positive sentinel nodes then received ipsilateral modified radical neck dissection and contralateral selective neck dissection. Thus, all patients, regardless of their clinical N stage, received pathologic evaluation of their cervical lymph nodes. Those patients with positive cervical lymph nodes received 50 Gy to the neck with a 15 Gy boost in areas of extracapsular spread. In this study, lymph node number exhibited a strong dose–response correlation with distant metastasis and survival (Fig. 3-1). In multivariate analysis stratified by tumor site and patient age, number of positive nodes was a significant, independent predictor of survival. Furthermore, although extracapsular spread and node location (upper vs. middle vs. lower neck) were significant predictors of survival in univariate analysis, after controlling for number of positive lymph nodes,

Though postradiation maximum SUVs were significantly higher in nonresponders compared with responders, the positive and negative predictive values for PET/CT were similar to CT alone. In order to improve the PPV of the postradiation scans, they stratified patients into high-risk (HPV negative, nonoropharynx primary, tobacco history) and low-risk categories. In the high-risk patients, PET/CT outperformed CT alone, especially in terms of accurately detecting residual nodal disease, where the PPV for PET/CT was twice that of CT alone (75% vs. 37.5%).

Whether pretreatment PET/CT reveals loco-regional versus metastatic disease is clearly prognostic, but the predictive value of pretreatment SUV in localized disease is controversial. Moeller found no significant difference in the pretreatment SUVmax at the primary site or lymph nodes between responders and non-responders. Higgins came to a similar conclusion, but did report that increased pretreatment SUVmean at the primary site correlated with disease-free survival. Imsande found that SUVmax at the primary site correlated with overall survival, whereas Demirci found that disease-free survival correlated with nodal (but not primary site) SUVmax. Tang evaluated metabolic tumor volume (MTV) on pretreatment PET-CT and concluded that MTV was an independent predictor of outcomes. Specifically, MTV >17 cm³ was associated with a greater risk of both disease progression and death. Lim also reported that primary tumor MTV was predictive of local failure, distant metastases, and overall survival.

In conclusion, a negative posttreatment PET/CT that is appropriately timed portends a favorable prognosis and may be especially helpful in higher-risk patients. A positive result is less reliable, probably due to the possibility of a false positive due to residual inflammation or infection.

![Figure 3-1](image-url)
they lost their prognostic significance. Thus, with this particular treatment algorithm, the number of positive nodes emerged as an important, independent predictor of prognosis.

The importance of positive cervical nodes has been noted in many other studies and appears to correlate with risk of regional recurrence and distant metastasis. Studies have demonstrated a correlation between increasing number of positive nodes and regional recurrence in patients with advanced SCC who receive postoperative radiotherapy,122 patients who do not receive radiotherapy,64 patients with oral cavity30 and hypopharyngeal or lateral epipharyngeal primary tumors,125 and patients who receive neck dissection for mucosal HNSCC from any site.124,125 Furthermore, number of positive nodes clearly predicts risk of distant metastatic disease for all sites of HNSCC.11,108,121,126,130 Number of positive nodes predicts both regional recurrence64 and distant recurrence,129 even after controlling for other prognostic variables in multivariable analysis.

Finally, the number of positive cervical lymph nodes consistently correlates with survival in univariate analysis for all major sites of HNSCC.7,50,62,122,123,126,131–135 The relative importance of ECE versus number of positive nodes remains somewhat controversial. In addition to the Mamelle et al. study,123 Moe et al. found the number of positive nodes to predict poor survival independent of ECE in 159 patients with advanced laryngeal cancer.11 However, cohorts of 136 patients with clinically node positive oral cavity cancer63 and 281 patients with supraglottic cancer46 found that ECE, not number of positive nodes, was an independent predictor of poor survival. Differences in treatment modalities and stratification of pathologic variables may explain some of the differences noted in these studies. Regardless, patients with either multiple positive lymph nodes or ECE are at higher risk for recurrence and should be considered for aggressive therapy.

**Extracapsular Extension**

ECE occurs in roughly 60% of patients with positive cervical nodes and is of paramount importance in predicting patient outcomes.136,137 A recent study of 337 patients reported a strong association between the presence of ECE and clinical N stage, with ECE present in 10.5% (38/171) of clinical N0 patients, 35% (26/75) of clinical N1 patients, 55% (35/64) of clinical N2 patients, and 74% (20/27) of clinical N3 patients.135 The extent of ECE can be stratified into the following three levels based on the morphology of the involved cervical lymph nodes: (a) macroscopic extracapsular spread with involvement of adjacent anatomic structures such as the internal jugular vein or skeletal muscle, (b) macroscopic extracapsular spread confined to the perinodal fibro-adipose tissue, and (c) microscopic extracapsular spread.22 A recent study of 96 dissected cervical lymph nodes with ECE measured the maximum distance from the capsule border to the farthest extent of the tumor.138 The mean extension was 2.2 mm, and in 96% of lymph nodes the maximum extension was ≤5 mm. Rarely, the extension measured up to 9 mm. These findings have important implications for target delineation in the setting of intensity modulated radiation therapy to treat lymph nodes at high risk for ECE.

As a general marker of tumor invasiveness and biologic aggressiveness, some authors have proposed that the presence of ECE in cervical lymph nodes may predict recurrence at the primary site. This hypothesis remains unresolved, as two studies conducted on a total of 207 patients with laryngeal cancer noted a significant association between ECE and local recurrence,139,140 but two other studies conducted on a total of 392 HNSCC patients failed to confirm this association.123,125

Regardless of its relationship with local recurrence, ECE is a significant determinant of prognosis due to its association with increased risk of recurrence in the neck and distant metastatic disease. For example, in a review of 284 patients with HNSCC who received neck dissection as a component of initial therapy, the presence of gross or microscopic ECE tripled the risk of neck recurrence in multivariable modeling.64 In addition, patients with gross ECE were 1.5 times more likely to develop regional recurrence when compared to patients with microscopic ECE, although this trend did not reach statistical significance. Many other series have confirmed this relationship for the following sites: oral tongue,22 oral cavity and oropharynx,29 hypopharynx and lateral epipharynx,123 pyriform sinus,3 supraglottic larynx,139,140 larynx,140,142,143 and all sites combined.70,122,124,125,144 The association between ECE and regional recurrence is independent of other prognostic variables in HNSCC.64,125,142

The presence of ECE also increases the risk of distant metastatic disease. In a review of 281 patients who received a neck dissection as a component of initial therapy for HNSCC, the presence of ECE tripled the risk of distant metastasis as the first site of failure (19.1% in ECE positive patients vs. 6.7% in ECE negative patients).127 These findings have been reproduced in several large studies.108,126,128,135,145 Currently, it is unclear whether the relationship between ECE and distant metastatic disease is independent of the number, size, and location of positive cervical lymph nodes.

In addition, ECE is a significant predictor of poor disease-free, cause-specific, and overall survival. In a retrospective study of 281 patients with supraglottic SCC treated with surgery with or without postoperative radiotherapy, pathologic N0 patients experienced a 5-year overall survival of 65%, compared to 49% in node-positive patients without ECE and 20% in node-positive patients with ECE.46 In a multivariate model, the presence of ECE conferred the highest risk of death, resulting in a sixfold increased risk of death when compared with node-negative patients. In contrast, patients with positive nodes but no ECE experienced a threefold increased risk of death. This independent association between ECE and poor cause-specific or overall survival has been confirmed for the following sites: oral cavity,63 oral cavity and oropharynx,3 supraglottic larynx,62 larynx and hypopharynx,143 and all sites combined.49,125,142

The importance of ECE as compared to other pathologic factors was underscored in a prospective randomized clinical trial conducted by Peters et al. that evaluated radiation dose in the postoperative setting.146 In this trial, patients with ECE experienced significantly higher local-regional recurrence rates than patients without ECE. In addition, in the subset of patients without ECE, only those patients with four or more adverse risk factors experienced a local-regional recurrence rate comparable to those patients with ECE. Adverse factors examined included oral cavity primary, close or positive margins, PNI, two or more positive nodes, largest node >3 cm, Zubrod score ≥2, and delay in starting radiotherapy. As a result, the study authors concluded that ECE is the dominant pathologic risk factor in HNSCC. Furthermore, this study showed that, in patients with ECE, local-regional control increased from 52% to 74% as dose increased from 57.6 to 63 Gy. No such dose–response was evident for patients without ECE, suggesting that tumors with ECE require a higher dose of postoperative radiation to achieve adequate local-regional control.

Due to the poor prognosis associated with ECE, several investigators have explored the potential benefit of postoperative chemoradiation in this patient population. The first randomized data to support a benefit of chemoradiation was reported by Bachaud et al. in 1996.147 This trial included 83 patients with histologically documented ECE treated with surgery and postoperative radiation with or without concurrent cisplatin (50 mg given weekly with radiation for seven to nine cycles). The addition of cisplatin lowered the risk of local-regional recurrence, from 41% in the radiation alone group down to 23% in the chemoradiation
group. Subsequently, large multicenter trials conducted by the Radiation Therapy and Oncology Group (RTOG) and the European Organization for the Research and Treatment of Cancer (EORTC) randomized patients with high-risk, resected head and neck cancer to receive postoperative radiation or postoperative radiation with concurrent cisplatin (100 mg/m² given on days 1, 22, and 43).58,59 A post hoc pooled analysis revealed that the addition of cisplatin was specifically beneficial for patients with ECE, lowering the relative risk of local-regional recurrence by 50% and also improving overall survival.50 The updated data on the RTOG 9501 Intergroup Phase III trial has failed to show an overall benefit to the addition of chemotherapy, with a median follow-up of 9.4 years.148 However, the subgroups with positive resection margins or extracapsular spread did have improved loco regional control with the addition of chemotherapy.

**Node Location**

The anatomic location of positive cervical lymph nodes has been classically described by dividing the neck into five anatomic levels.141 Level I refers to nodes within the submandibular and submental triangles. Levels II, III, and IV refer to the chain of nodes along the upper, middle, and lower third of the jugular vein, respectively. Level V refers to nodes along the spinal accessory nerve and within the posterior cervical triangle. Using these categories, Mamelle et al. defined sentinel nodes as those nodal groups that provide the primary lymphatic drainage for a particular site within the head and neck.121 Thus, sentinel nodes for oral cavity tumors were defined as levels I, II, and III; and sentinel nodes for oropharyngeal, hypopharyngeal, and laryngeal tumors were defined as levels II and III. Applying this classification to cervical node pathologic specimens from 914 HNSCC patients, Mamelle et al. found that the presence of nodal metastases outside the sentinel node region independently decreased 5-year survival by more than 50% and nearly doubled the rate of distant metastasis. Furthermore, node location inside versus outside of the sentinel area predicted survival independent of number of positive nodes and extracapsular spread. The increased risk of regional recurrence, distant recurrence, and death associated with positive low cervical lymph nodes has been noted in several other studies of patients with HNSCC.11,36,123,129,131,134,150,151 Setton132 reported that low-lying cervical metastases (levels IV, VB) were independent predictors of distant metastasis. Finally, de Bree et al. found that 33% of HNSCC patients with low jugular lymph node metastases showed evidence of concomitant distant metastatic disease on preoperative CT of the thorax.1 Clearly, patients with positive lymph nodes outside of the sentinel node regions merit aggressive preoperative screening for distant disease and are at increased risk of treatment failure and death following aggressive local-regional therapy.

**Node Size**

The diameter of the largest metastatic cervical lymph node contributes to the assessment of N stage in HNSCC and may correspond with total tumor burden. In a review of 250 radical neck dissection specimens, Carter et al. found that pathologic nodal size >2 cm correlated with increased risk for regional recurrence.125 However, several other studies have failed to determine a relationship between node size assessed clinically or pathologically and either regional recurrence or local-regional recurrence.36,38,124 In contrast, Mamelle et al. found that nodal size increased the risk of distant metastases, with patients having nodal diameter <3 cm experiencing a distant metastasis rate of 22%, compared with 35% for patients with nodal diameter between 3 cm and 6 cm and 49% for patients with nodal diameter >6 cm.122 Furthermore, in a multivariate analysis of clinical parameters only, node size was a significant predictor of poor overall survival. In contrast, other studies have not found an independent relationship between pathologically assessed nodal size and survival when other relevant pathologic variables are considered.3,58,125,143 Thus, the diameter of the largest positive cervical lymph node may serve as a helpful clinical predictor of outcome, but may not exert an independent prognostic when other pathologic factors are considered.

**Pathologic Residual Disease on Neck Dissection After Chemotherapy**

In Chapter 14, there is a comprehensive discussion of all aspects of neck management. In the current era of definitive, concomitant chemoradiotherapy, neck dissection is often used exclusively when there is suspicion of residual disease on PET-CT performed about 3 months after treatment. This is discussed at length in Chapter 14. For this subset of patients, the presence of pathologic residual disease in the neck after chemoradiotherapy has prognostic value.153 Hu also reported the prognostic value of pathologic residual neck disease in patients who have planned neck dissections after chemotherapy. In both subsets, there is an adverse impact on risk of distant metastasis and disease-free survival.

**DEMOGRAPHIC PARAMETERS**

**Age**

Age is a commonly considered covariate and is known to influence outcome in certain types of cancer. For example, patient age and attendant comorbidities may influence the vigor of the immune response directed against the tumor and the patient’s ability to tolerate maximal therapy. In addition, head and neck cancers arising in patients at either extreme of the age continuum may result from different etiologic agents and thus manifest different clinical outcomes. For example, Schantz et al. found that cultured lymphocytes from nonsmoking young adults with HNSCC were more susceptible to bleomycin-induced chromosome damage than lymphocytes from older smokers with HNSCC and healthy controls, suggesting that genetic susceptibility to environmental carcinogens may influence the risk of HNSCC in young adults.154 Recently, investigators have noted a striking increase in the incidence of tongue and tonsillar cancer in adults under 40 years of age, thus prompting additional interest in the risk factors, natural history, and optimal treatment of HNSCC in the young.153 The relationship of HPV to these malignancies has become well established and is covered later in this chapter as well as in Chapter 12.

Risk factor profiles indicate that young adults with HNSCC are less likely to report prior exposure to tobacco or alcohol and more likely to be female.156,157 With respect to clinical outcome, Siegelman-Danieli et al. examined a retrospective cohort of 87 oral tongue SCC patients, 30 of whom were 45 years of age or younger at the time of diagnosis.157 In this study, age did not influence relapse rates, cancer-free survival, or overall survival in both univariate and multivariate analysis. Similarly, Verschuur et al. conducted a retrospective case-control study on 185 previously untreated HNSCC patients under the age of 40 who were matched to controls based on tumor site, sex, and date of presentation.158 Age did not influence cause-specific survival in univariate or multivariate analysis. However, older patients were twice as likely to develop second primary SCCs of the upper aerodigestive tract (14% vs. 7%), possibly due to their increased use of tobacco products. The effect of young age remains controversial, however, as a recent report on 1,030 HNSCC patients found that the recurrence risk was 50% less for patients under the age of...
40 as compared to those over the age of 40, even after controlling for TNM stage, primary tumor site, and comorbidity.\textsuperscript{158} Therefore, the current literature suggests that young patients experience a prognosis that is either similar to or slightly better than that experienced by older patients. Because HPV status was not known in most prior studies, its effect on this cohort is difficult to assess.

Head and neck cancers in elderly populations also present a different epidemiologic profile. In a study of 161 elderly patients with HNSCC, Leon et al. found that patients over the age of 70 were less likely to use tobacco and alcohol and more likely to be female.\textsuperscript{159} The decreased consumption of tobacco and alcohol in elderly HNSCC patients correlates with a lower incidence of p53 mutations in this population.\textsuperscript{160} With respect to survival, Leon et al. demonstrated that although elderly laryngeal cancer patients experienced worse overall survival, cause-specific survival was not influenced by patient age. Furthermore, in a prospective study of 203 head and neck cancer patients, advanced age correlated with worsening 2-year overall survival in univariate analysis, but this association was not significant when adjusted for relevant covariates, suggesting that the biologic aggressiveness of HNSCC does not fundamentally differ between White and Black patients.\textsuperscript{161,162} However, one study has shown that Black race does not appear to be associated with increased disease-specific mortality, suggesting that the biologic aggressiveness of HNSCC does not fundamentally differ between White and Black patients.\textsuperscript{161,162} However, one study has shown that Black race is associated with a higher risk of distant metastatic disease even after adjusting for relevant covariates,\textsuperscript{173} and this finding merits further study.

Recently, Settle et al. proposed an alternative reason for Black HNSCC patients’ worse prognosis. They looked retrospectively at data from the phase 3, multicenter TAX 324 trial of induction chemotherapy followed by concurrent chemoradiation in HNSCC patients. The racial disparity in overall survival was due entirely to the subgroup of patients with oropharyngeal cancer, in which the OS for Whites versus Blacks was 69.4 months and 25.2 months, respectively (\(p = 0.0006\)). OS was not reached in the HPV-positive patients versus 26.6 months in the HPV-negative patients (hazard ratio [HR] 5.1; \(p < 0.0001\)). Whites were significantly more often HPV-16 positive than Blacks, 34\% versus 4\% (\(p = 0.0004\)). Furthermore, survival was similar for Whites versus Blacks in nonopharyngeal sites and in HPV-negative patients.\textsuperscript{174} This study represents one of the very first biologic rather than socioeconomic rationales for difference in racial outcomes in HNSCC.

### Alcohol and Tobacco Exposure

Tobacco and alcohol use have long been recognized as important risk factors for the development of HNSCC. Exposure to these carcinogens results in specific molecular insults that promote neoplasia. For example, cigarette smoking has been associated with overexpression of the proto-oncogene bcl-2, a protein known to inhibit apoptosis.\textsuperscript{175} In addition, concurrent use of alcohol and tobacco has been associated with a high rate of nonspecific mutations in the tumor suppressor gene p53.\textsuperscript{176} Perhaps as a result of these deleterious mutations, prior or continued use of alcohol and tobacco in patients with head and neck cancer is a risk factor for poor outcome. Furthermore, use of these substances has been associated with immunosuppression, malnutrition, and impaired tissue oxygenation resulting in hypoxic radiosensitivity.

The most rigorous exploration of the relationship between alcohol consumption and outcome in head and neck cancer patients resulted from a prospective study of 649 patients who received chemotherapy followed by concurrent chemoradiation in HNSCC patients’ worse prognosis. They looked retrospectively at data from the phase 3, multicenter TAX 324 trial of induction chemotherapy followed by concurrent chemoradiation in HNSCC patients. The racial disparity in overall survival was due entirely to the subgroup of patients with oropharyngeal cancer, in which the OS for Whites versus Blacks was 69.4 months and 25.2 months, respectively (\(p = 0.0006\)). OS was not reached in the HPV-positive patients versus 26.6 months in the HPV-negative patients (hazard ratio [HR] 5.1; \(p < 0.0001\)). Whites were significantly more often HPV-16 positive than Blacks, 34\% versus 4\% (\(p = 0.0004\)). Furthermore, survival was similar for Whites versus Blacks in nonopharyngeal sites and in HPV-negative patients. This study represents one of the very first biologic rather than socioeconomic rationales for difference in racial outcomes in HNSCC.
Of note, this study provided indirect evidence that interventions to eliminate alcohol intake may help to improve outcomes. After controlling for relevant covariates, alcoholics abstinent at the time of diagnosis were half as likely to die as alcoholics who were currently drinking. Eighty percent of abstinent alcoholics had received formal treatment for their alcoholism, either through inpatient programs or Alcoholics Anonymous.

The interaction between cigarette smoking and response to radiotherapy was demonstrated in a prospective study of stage III to IV HNSCC patients treated with primary irradiation with or without concomitant 5-fluorouracil. Any smoking during the 6½-week course of radiotherapy decreased the complete response rate from 74% to 45%, the 2-year survival rate from 66% to 39%, and the median survival from 30 to 16 months. In addition, mortality was influenced by the length of time between smoking cessation and initiation of treatment, with a risk reduction of 40% for those patients who quit <12 weeks prior to diagnosis and of 70% for patients who quit more than 1 year prior to diagnosis. In multivariate analysis, smoking was the only significant predictor of survival, with those who abstained 2.5 times more likely to survive. The authors speculated that the deleterious impact of smoking may be related to lower levels of natural-killer-cell activity, reduced cell-mediated immunity, and increased blood carboxyhemoglobin concentrations, resulting in tissue hypoxia and increased radioresistance. The adverse effect of continued smoking during radiotherapy has also been noted in two studies conducted on patients with T1 and T2 glottic carcinomas treated with primary radiation. In addition to mortality, continued smoking doubles the risk of long-term complications in patients with T1 glottic cancer treated with primary radiation.

To evaluate the effect of alcohol and tobacco consumption on the risk of second primary malignancies, Day et al. conducted a nested case-control study composed of 80 case patients with second cancers and 189 sex- and survival-matched oral cancer patients free of second cancers. The risk of a second primary tumor of the aerodigestive tract (oral cavity, pharynx, larynx, esophagus, and lung) increased with both number of cigarettes smoked per day and years of cigarette smoking, peaking at odds ratio of 4.7 for those patients who smoked 40 or more cigarettes per day for 20 or more years. Similarly, consumers of 15 or more beers per week experienced a 3.8-fold increased risk compared to nondrinkers or light drinkers (0–4 drinks per week). Smoked 40 or more cigarettes per day for 20 or more years. Similar results were reported in a study of 1,086 patients with primary head and neck cancer, the presence of comorbidity was a significant, independent predictor of 2-year survival, even after controlling for age, sex, race, and stage. As compared to patients without comorbidity, the mortality HR was 1.9 for patients with moderate comorbidity and 2.5 for patients with severe comorbidity. Similar results were reported in a study of 9,386 elderly Medicare beneficiaries with HNSCC. Given the importance of comorbidity as a predictor of survival, efforts have been made to develop new systems for staging head and neck cancer that combine TNM staging with symptom severity and comorbidity indices.

The general scheme for such a staging system of oral cavity cancer is presented in Figure 3.2. Of note, the most advanced stage, stage D, is determined solely by the presence of severe comorbidity or moderate or severe symptom severity. Hence, advanced TNM stage alone is not sufficient to merit placement in the worse prognostic category. Utilizing multivariate modeling, the investigators convincingly demonstrated that this model was more strongly predictive of disease-free and overall survival than TNM staging alone in a retrospective cohort of 277 patients with oral cavity SCC. Similar results were obtained in other retrospective studies conducted on oral cavity cancers. Utilizing multivariate modeling, the investigators convincingly demonstrated that this model was more strongly predictive of disease-free and overall survival than TNM staging alone in a retrospective cohort of 277 patients with oral cavity SCC. Similar results were obtained in other retrospective studies conducted on oral cavity, oropharyngeal, and laryngeal cancers. Clearly, the combination of comorbidity, symptom severity, and TNM stage holds promise for more accurate prognostication in head and neck cancer and merits prospective validation.

### Nutritional Status

Malnutrition is common in patients with head and neck cancer and attributable to a number of causes including poor dietary habits, excessive alcohol consumption, local tumor effects, tumor-induced cachexia, and the effects of various therapies. In 1984, Goodwin and Torres applied a prognostic nutritional index to a retrospective cohort of 50 consecutive patients with advanced head and neck cancer to determine whether nutritional status influenced postoperative complications and survival. The prognostic nutritional index considers serum albumin, serum transferrin, serum albumin, serum transferrin, triceps skin-fold thickness, and cutaneous delayed hypersensitivity to mumps, streptokinase-streptodornase, or candida. This index was generated from retrospective review of 161 patients undergoing gastrointestinal surgery and then prospectively validated in 100 patients as an indicator of risk for postoperative complications. Goodwin and Torres found that 89% (8/9) of patients with prognostic nutritional index <39% suffered major postoperative complications, compared to 14% (4/29) of patients with prognostic nutritional index ≥39%. In addition, 64% (9/14) of the patients with prognostic nutritional index ≥39% died of their disease within 1 year, compared to 28% (10/36) of patients with prognostic nutritional index <39%.

The relationship between nutritional status, postoperative complications, and survival has been further explored in several prospective studies. In an analysis of six different nutritional parameters, 10% weight loss in the 6 months preceding surgery was the only significant, independent predictor of major

### Comorbidity

Comorbidity refers to the presence of other diseases, illnesses, or conditions not directly related to the index cancer. Although not included in TNM staging, comorbidity directly influences the care of cancer patients, selection of treatment modalities, and evaluation of treatment effectiveness. Multiple instruments have been utilized to characterize comorbidity in head and neck cancer patients, and no single instrument has been consistently shown to be superior. Using a four-tiered classification (none, mild, moderate, and severe), Piccirillo found that 24% of head and neck cancer patients suffer from moderate or severe comorbidity. Examples of moderate comorbidity include poorly controlled hypertension, old stroke with residua, and history of an alcoholic seizure. Examples of severe comorbidity include congestive heart failure or myocardial infarction within the last 6 months, recent stroke, and severely decompensated alcoholism. In a prospective study of 1,086 patients with primary head and neck cancer, the presence of comorbidity was a significant, independent predictor of 2-year survival, even after controlling for age, sex, race, and stage.
postoperative complications in a cohort of 64 patients with T2-4 carcinomas of the oral cavity, oropharynx, hypopharynx, and larynx. In a subsequent study, 5% weight loss in the 6 months preceding initial treatment for advanced HNSCC was identified as an independent predictor of poor disease-specific survival in men but not women.

Given the strong evidence for an association between malnutrition and an increased risk of postoperative complications, interest has grown in evaluating the benefits of nutritional support. A meta-analysis of 28 trials revealed that preoperative parenteral nutrition lowers the risk of major surgical complications and surgical mortality for patients with gastrointestinal cancers, yet a small randomized trial of preoperative tube feeding in 49 severely malnourished head and neck cancer failed to show a similar benefit. An observational study of 1,073 patients treated with definitive radiation therapy on RTOG 90-03 examined the correlation between baseline nutritional support (BNS), radiation toxicity, and survival. BNS was defined as either oral supplements, enteral feedings, or parenteral nutrition used by the patient prior to beginning radiation. BNS was associated with a slightly lower risk of grade 3 or 4 radiation-induced mucositis: 34% with BNS versus 40% without BNS. However, even after adjusting for potential confounders, BNS was associated with a highly significant 1.5-fold increased risk of local-regional failure. This finding generated the hypothesis that nutritional support of head and neck cancer patients, though beneficial for their general medical condition and for minimizing toxicity of therapy, may actually exert a deleterious effect on cancer outcomes by providing the cancer with nutrients needed to resist the effects of radiation therapy. This hypothesis clearly merits further prospective study.

**Anemia**

Anemia commonly occurs in patients with head and neck cancer and may be due to a number of causes, including comorbid illness, intraoperative blood loss, toxicity from chemotherapy and/or radiation, and malignancy-associated anemia of chronic disease. Anemia is commonly thought to enhance radioresistance via enhancing tumor hypoxia. Numerous retrospective studies have suggested a strong association between anemia and inferior local-regional control and survival among patients treated for HNSCC (Table 3.2). This association has been reported for patients with both early and advanced disease treated with a variety of treatment modalities, including radiation alone, chemoradiation, surgery and postoperative radiation, and surgery alone. The finding that anemia correlates with inferior local-regional control among patients treated with
### Table 3.2: Prognostic Significance of Anemia in Head and Neck Cancer

<table>
<thead>
<tr>
<th>Author</th>
<th>Description of Patients</th>
<th>Treatment Given</th>
<th>MVT</th>
<th>Local/Regional Recurrence</th>
<th>Survival</th>
<th>Measure of Anemia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overgaard et al.</td>
<td>950 Larynx or pharynx SCC</td>
<td>RT</td>
<td>No</td>
<td>Yes (p &lt; 0.01)</td>
<td>OS (p &lt; 0.01)</td>
<td>Hemoglobin &lt;14.5 g/dL for men and &lt;13.0 g/dL for women pretreatment</td>
</tr>
<tr>
<td>van Acht et al.</td>
<td>357 T1-4 N0-2 glottic or supraglottic SCC</td>
<td>RT</td>
<td>Yes</td>
<td>NR</td>
<td>DFS (p &lt; 0.05)</td>
<td>Hemoglobin &lt;11.0 mmol/L for men and &lt;10.0 mmol/L for women at day 35 of RT</td>
</tr>
<tr>
<td>Dubray et al.</td>
<td>217 T1-4 N0-3 oral cavity and oropharynx SCC</td>
<td>RT and surgical salvage</td>
<td>Yes</td>
<td>Trend (p = 0.06)</td>
<td>OS (p = 0.04)</td>
<td>Decrease in hemoglobin ≥1.0 from before treatment to day 35 of RT</td>
</tr>
<tr>
<td>Tarnawski et al.</td>
<td>847 T1-4 N0-2 laryngeal supraglottic SCC</td>
<td>RT</td>
<td>Yes</td>
<td>Yes (p &lt; 0.0001)</td>
<td>NR</td>
<td>Hemoglobin as continuous variable and change in hemoglobin during treatment period</td>
</tr>
<tr>
<td>Lee et al.</td>
<td>451 Stage III or IV HNSCC</td>
<td>RT</td>
<td>Yes</td>
<td>Yes (p = 0.003)</td>
<td>OS (p = 0.0007)</td>
<td>Hemoglobin &lt;14.5 g/dL for men and &lt;13.0 g/dL for women pretreatment</td>
</tr>
<tr>
<td>Overgaard et al.</td>
<td>422 (414 eligible) T1-4 N0-3 pharynx and supraglottic larynx SCC</td>
<td>RT and surgical salvage</td>
<td>Yes</td>
<td>Yes (p = 0.001)</td>
<td>Trend DFS and recurrence (p = 0.09)</td>
<td>Hemoglobin &lt;9.0 mmol/L for men and &lt;8.0 mmol/L for women pretreatment</td>
</tr>
<tr>
<td>Warde et al.</td>
<td>736 T1-2 glottic SCC</td>
<td>RT</td>
<td>Yes</td>
<td>Yes (p &lt; 0.05)</td>
<td>NR</td>
<td>Hemoglobin &lt;10.0 g/dL to &lt;14.0 g/dL</td>
</tr>
<tr>
<td>Lutterbach et al.</td>
<td>258 T1-4 glottic SCC</td>
<td>Surgery</td>
<td>Yes</td>
<td>Yes (p &lt; 0.05)</td>
<td>NR</td>
<td>Hemoglobin &lt;13.0 g/dL for men and &lt;12.0 g/dL for women pretreatment</td>
</tr>
<tr>
<td>Glaser et al.</td>
<td>191 T2-4 N0-3 M0 oral cavity or oropharynx SCC</td>
<td>RT, CTX, surgery</td>
<td>No</td>
<td>Yes (p &lt; 0.05)</td>
<td>OS (p &lt; 0.05)</td>
<td>Hemoglobin &lt;14.5 g/dL with or without exogenous erythropoietin treatment</td>
</tr>
<tr>
<td>Nguyen-Tan et al.</td>
<td>223 T3-4 larynx SCC</td>
<td>Any</td>
<td>Yes</td>
<td>Yes (p = 0.01) univariate only</td>
<td>OS (p &lt; 0.001)</td>
<td>Hemoglobin nadir during RT as continuous variable</td>
</tr>
<tr>
<td>Jin et al.</td>
<td>238 T1 N0 glottic SCC</td>
<td>RT</td>
<td>Yes</td>
<td>Yes (p = 0.03)</td>
<td>No</td>
<td>Hemoglobin drop &gt;1 g/dL during treatment</td>
</tr>
<tr>
<td>Daly et al.</td>
<td>238 Stage III–IV HNSCC</td>
<td>RT</td>
<td>Yes</td>
<td>Yes (p = 0.009)</td>
<td>CSS (p = 0.009)</td>
<td>Hemoglobin &lt;13 g/dL in men and &lt;12 g/dL in females pretreatment</td>
</tr>
<tr>
<td>Grau et al.</td>
<td>478 Stage III–IV HNSCC</td>
<td>RT +/- mitomycin C</td>
<td>No</td>
<td>Yes (p = 0.02)</td>
<td>No</td>
<td>Hemoglobin drop &gt;1.3 g/dL during treatment</td>
</tr>
<tr>
<td>Reichel et al.</td>
<td>120 Stage I–IV HNSCC</td>
<td>surgery + postoperative chemoradiation</td>
<td>Yes</td>
<td>Yes (p = 0.02)</td>
<td>OS (p = 0.008)</td>
<td>Hemoglobin &lt;13 g/dl in men and &lt;12 g/dl in females postoperatively</td>
</tr>
<tr>
<td>Schafer et al.</td>
<td>214 Stage I–IV HNSCC</td>
<td>RT +/- surgery +/- chemotherapy</td>
<td>Yes</td>
<td>NR</td>
<td>OS (p = 0.01) DFS (p = 0.05)</td>
<td>Hemoglobin stratified into quartiles pretreatment</td>
</tr>
<tr>
<td>Cho et al.</td>
<td>246 T1-2 N0 larynx SCC</td>
<td>RT</td>
<td>Yes</td>
<td>Yes (p = 0.03)</td>
<td>OS (p = 0.001)</td>
<td>Hemoglobin stratified into quartiles pretreatment</td>
</tr>
</tbody>
</table>

(continued)
surgery alone suggests that anemia may influence outcome in head and neck cancer patients independent of its influence on hypoxic radioresistance.\textsuperscript{205}

The optimal timepoint to assess anemia for the purposes of prognostication is unclear and varies widely throughout the literature (Table 3.2). Options include pretreatment hemoglobin, midradiation hemoglobin, postoperative hemoglobin, and drop in hemoglobin concentration during radiation. Due to collinearity in these measures, they all likely confer some degree of prognostic significance. The optimal hemoglobin cutpoint for defining anemia is also unclear, ranging from 10 to 14 g/dL, depending on the study (Table 3.2). Denis et al. used an ROC curve to conclude that 12.5 g/dL is the best cutpoint for predicting mortality.\textsuperscript{215} In addition, at least two studies have shown a strong dose-response relationship when hemoglobin is divided into quartiles,\textsuperscript{212,213} suggesting that a single cutpoint for defining anemia may be inadequate.

Given the strong association between anemia and adverse outcomes, two randomized trials were launched to assess the benefit of treating anemic patients with recombinant erythropoietin (EPO) during radiotherapy. The first study, a double-blind, placebo-controlled randomized trial reported by Henke et al. in 2003, found that administration of epoetin-\(\beta\) increased the median hemoglobin concentration from 11.7 g/dL pretreatment to 14.8 g/dL posttreatment.\textsuperscript{218} However, patients who received epoetin-\(\beta\) experienced inferior local-regional control (HR, 1.62; 95% confidence interval [CI], 1.22–2.14) and inferior overall survival (HR, 1.39; 95% CI, 1.05–1.84). This study has been criticized due to unequal distribution of certain covariates among the study arms. RTOG 99-03 similarly found that taking epoetin increased hemoglobin concentration, by 1.66 g/dL in the experimental arm compared with 0.24 g/dL decrease in the control arm (\(p = 0.0001\)). However, there was no statistically significant difference in the primary endpoint of local-regional failure (LRF) or overall survival.\textsuperscript{219}

The possibility that exogenous EPO could exert a deleterious effect on control of HNSCC has prompted research into possible mechanisms. Winter et al. demonstrated that 99% of HNSCCs express the EPO receptor.\textsuperscript{220} and Lai et al. reported that treatment of HNSCC cell lines with EPO increased invasion and proliferation.\textsuperscript{221} Further, a secondary analysis of the trial reported by Henke et al. examined the interaction of EPO administration with EPO receptor expression among 154 patients treated at a single institution.\textsuperscript{222} In this study, 67% of tumors had moderate or strong expression of the EPO receptor and were considered EPO receptor positive. Among EPO receptor positive tumors, administration of EPO was associated with an increased risk of local-regional failure (relative risk, 2.07; 95% CI, 1.27–3.36). In contrast, among EPO receptor negative tumors, administration of EPO did not affect local-regional control (relative risk, 0.94; 95% CI, 0.47–1.90). The \(p\)-value for the interaction of EPO administration with EPO receptor expression was 0.08, suggesting that EPO administration exerts a deleterious effect only among tumors that are EPO receptor positive.

In summary, although there is a strong association between anemia and adverse outcomes, there is little evidence to suggest that modification of anemia through administration of growth factors produces a clinical benefit. Clinicians should be cognizant that administration of EPO may stimulate tumor growth and should therefore utilize extreme caution when considering this medication.

### Molecular Prognostic Factors

The vast array of molecular factors studied in head and neck cancer can be divided into several broad categories. Proto-oncogenes code for proteins that promote cellular proliferation. A proto-oncogene is transformed into an oncogene when its protein product becomes unresponsive to the normal regulatory processes that control cell division. Activation of proto-oncogenes occurs by point mutations, chromosomal translocations, or gene amplification. At the cellular level, an oncogene exerts a dominant phenotype over its proto-oncogene counterpart because only one copy of an oncogene is necessary to promote neoplasia. In contrast, tumor suppressor genes (anti-oncogenes) inhibit cellular proliferation. At the cellular level, both copies of a tumor suppressor gene must be disabled to promote neoplasia. Another class of markers includes proteins and growth factors that mediate the interaction between neoplastic cells and their local microenvironment. In addition to these specific molecular markers expressed in neoplastic cells, other factors such as tumor ploidy and the rate of tumor cell proliferation may yield important prognostic information. This chapter will focus on molecular markers that may become important targets for new pharmacotherapies.
p53. p53 is a transcription factor with tumor suppressor function that negatively regulates the cell cycle and serves to protect the integrity of the genome. Its gene resides on chromosome 17p13 and is composed of 11 exons spanning 20 kb in length. Activation of p53 occurs in response to a variety of cellular stressors, such as DNA damage, hypoxia, and cell cycle abberations. Such stressors lead to G1/S checkpoint arrest through transactivation of at least two genes: p21WAF1/CIP1, an inhibitor of the G1 cyclin-dependent kinases essential for the G1/S transition, and GADD45, a gene implicated in growth arrest and DNA excision repair. p53 induction also results in apoptosis, presumably when the extent of DNA damage exceeds the capacity of cellular repair mechanisms. Thus, p53 protects the cell from propagating mutations to subsequent generations and is considered the "guardian of the genome." Loss of p53 function may contribute to tumor aggressiveness by promoting resistance to radiation and chemotherapy, accelerated growth in hypoxic conditions, and tumor neovascularization. When compared to cells with wild-type p53, p53 mutant cell lines exhibit more resistance to ionizing radiation and certain chemotherapeutic agents. This evidence led to the hypothesis that tumors lacking a functional p53 gene will exhibit a radioresistant and chemotherapy-resistant phenotype due to a deficiency in DNA damage-induced apoptosis. This hypothesis has been strengthened by experiments conducted in p53 mutant cell lines derived from HNSCCs. Transfection with an adenoviral vector or a liposomal system containing a functional p53 gene resulted in a dose-dependent increase in radiation sensitivity, restoration of the G1 checkpoint, and induction of apoptosis. p53 may also mediate hypoxia-induced apoptosis. Graeber et al. demonstrated that mutations in p53 reduce hypoxic cell death and confer a competitive advantage to cells growing in a hypoxic environment. Thus, mutations in p53 may increase the number of hypoxic cells, conferring hypoxia-mediated radioresistance. Finally, loss of p53 function results in the down-regulation of thrombospondin-1, an inhibitor of angiogenesis. Indeed, a strong correlation between p53 overexpression, a surrogate marker for p53 gene mutation, and both elevated tumor microvessel density and vascular endothelial growth factor (VEGF) expression has been reported in HNSCC. As a whole, the data from basic science studies support the hypothesis that p53 mutations contribute to biologically aggressive tumor behavior by conferring resistance to radiotherapy and chemotherapy and by promoting angiogenesis.

Inactivation of p53 is a common event in HNSCC and may result from spontaneous or tobacco-induced mutations or from sequestration by cellular proteins such as mdm2 or the E6 oncoprotein of HPV. Detection of p53 mutations has been attempted through both protein-based and DNA-based techniques. Many studies have utilized immunohistochemistry (IHC), capitalizing on the differential expression of wild type and mutant p53. Wild-type p53 has a half-life of 20 minutes, whereas many p53 mutations confer stability to the protein and thus result in higher intracellular levels detectable via IHC. Unfortunately, this technique often fails to detect nonsense or frameshift mutations that result in p53 protein truncation. Induction of wild-type p53 in response to DNA damage can result in sufficient quantities for IHC detection. Saunders et al. sequenced all exons of the p53 gene from 39 laryngeal SCCs and found that p53 IHC resulted in a 64% concordance, 42% false positive rate, and 23% false negative rate. Larger studies that examined only the conserved regions of the p53 gene reported 59% to 71% concordance with IHC. Thus, although relatively simple and inexpensive, IHC is not an accurate method for detecting p53 mutations.

DNA-based techniques for detection of p53 mutations have focused on exons 5 to 8, a highly conserved region that codes for a DNA-binding domain. However, recent studies have found that 20% to 25% of p53 gene mutations lie outside the conserved region, although these mutations may not influence in vitro p53 function. Finally, loss of heterozygosity at the p53 gene locus has been examined using polymorphic markers and fluorescent in situ hybridization (FISH).

Recent studies correlating p53 mutation with outcome in HNSCC have suggested that p53 mutations, as assessed via direct sequencing, are associated clinically with resistance to radiotherapy and chemotherapy (Table 3.3). In the largest study to employ direct sequencing, Koch et al. evaluated the prognostic significance of p53 mutations in exons 5 to 9 in 110 HNSCCs treated with either primary or postoperative radiotherapy. These tumors spanned all major sites for HNSCC and included all clinical stages. In multivariate analysis, patients with p53 mutations were 2.4 times more likely to develop local-regional recurrence, equal to the risk of local-regional recurrence conferred by the presence of cervical lymph nodes. In contrast, p53 expression as assessed via IHC failed to correlate with any outcome variable. Similarly, two other studies showed that p53 mutation, but not protein overexpression, predicted poor local-regional control and survival in cohorts composed of 58 and 68 patients with HNSCC, most of whom received radiotherapy. Furthermore, a study conducted on 22 recurrent HNSCCs previously treated with radiation found that 21 of the 22 recurrences had evidence for p53 inactivation by either gene mutation, mdm2 overexpression, or HPV infection.

In addition to the relationship between p53 mutation and response to radiotherapy, several groups have considered the relationship between directly sequenced p53 mutations and response to chemotherapy. In a cohort of 105 patients with HNSCC treated with platinum- and fluorouracil-based induction chemotherapy, Temam et al. directly sequenced all coding regions of the p53 gene from biopsy specimens taken prior to the initiation of chemotherapy. Multivariate modeling revealed that tumors with a mutant p53 gene were 70% less likely to experience major response to chemotherapy and, independently, tumors with high levels of p53 protein expression were 60% less likely to experience a major response. The correlation between p53 overexpression and resistance to chemotherapy persisted in the subset of patients with wild-type p53, suggesting that p53 IHC conveys independent prognostic information in patients treated with chemotherapy. An additional study conducted on 106 HNSCC patients treated with cisplatin and 5-fluorouracil neoadjuvant chemotherapy screened for p53 mutations using denaturing gradient gel electrophoresis (DGGE) on PCR products from exons 4 to 9 of the p53 gene followed by direct sequencing of those exons that demonstrated a variant DGGE pattern. In agreement with Temam et al. this study found that p53 mutation was the only significant predictor of response to chemotherapy in multivariate analysis, with p53 mutant tumors 63% less likely to respond to chemotherapy. At least three other studies have reported a correlation between p53 overexpression and poor survival in HNSCC patients who receive chemotherapy.

Thus, strong evidence supports a role for p53 alterations in predicting poor initial response and long-term survival following treatment with chemotherapy.

At least one group has considered whether the location of p53 mutations influences the clinical phenotype of HNSCC. Erber et al. sequenced exons 5 to 8 and assessed for loss of heterozygosity at the p53 allele in 86 oral cavity, oropharyngeal, hypopharyngeal, and laryngeal SCCs, stage I to IV. All patients received surgical resection, and 79% (68/86) received postoperative radiotherapy. Twenty-eight percent (24/86) of tumors...
## TABLE 3.3  Prognostic Significance of \( p53 \) in Head and Neck Cancer

<table>
<thead>
<tr>
<th>Author</th>
<th>Description of Patients</th>
<th>Treatment Given</th>
<th>MVT</th>
<th>Detection of ( p53 ) Abnormality</th>
<th>% Positive</th>
<th>Local/Regional Recurrence</th>
<th>Survival</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overgaard et al.(^{250})</td>
<td>68 HNSCC (stage not specified)</td>
<td>RT + Nimorazole</td>
<td>Yes</td>
<td>IHC, DGGE + sequencing exons 5–9</td>
<td>67% 47%</td>
<td>No Yes</td>
<td>No</td>
<td>( p53 ) mutants at risk for LR recurrence</td>
</tr>
<tr>
<td>Saunders et al.(^{244})</td>
<td>35 Laryngeal SCC (mainly T1-2)</td>
<td>Primary RT</td>
<td>No</td>
<td>IHC, Sequenced exons 2–11</td>
<td>67% 48%</td>
<td>No No</td>
<td>NR</td>
<td>( p53 ) status not correlated with local failure</td>
</tr>
<tr>
<td>Gallo et al.(^{251})</td>
<td>85 HNSCC (mostly T1-2 NO)</td>
<td>Primary RT</td>
<td>Yes</td>
<td>SSCP + sequencing exons 5–8</td>
<td>45%</td>
<td>No No</td>
<td>(HR = 4.1)</td>
<td>( p53 ) mutants trended to LR failure in univariate analysis only (( p = 0.10 ))</td>
</tr>
<tr>
<td><strong>( p53 ) sequencing in heterogeneous series</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Koch et al.(^{260})</td>
<td>110 Stage I–IV HNSCC</td>
<td>Surgery + RT 42 primary RT</td>
<td>Yes</td>
<td>Sequenced Exons 5–9</td>
<td>44%</td>
<td>Yes NR</td>
<td>NR</td>
<td>( p53 ) mutants more likely to develop LR recurrence</td>
</tr>
<tr>
<td>Erber et al.(^{252})</td>
<td>86 Stage I–IV HNSCC</td>
<td>Surgery (68 received post-op RT)</td>
<td>No</td>
<td>sequenced exons 5–8</td>
<td>15%</td>
<td>NR NR</td>
<td>DFS (( p = 0.05 )) OS (( p = 0.01 )) Only DNA contact mutations conferred poor prognosis</td>
<td></td>
</tr>
<tr>
<td>Mineta et al.(^{253})</td>
<td>58 Stage I–IV oral and oropharyngeal SCC</td>
<td>Mostly primary or preoperative RT</td>
<td>Yes</td>
<td>IHC, SSCP + sequencing exons 5–8</td>
<td>74% 18%</td>
<td>NR NR</td>
<td>NR CSS (RR = 9.9)</td>
<td></td>
</tr>
<tr>
<td><strong>( p53 ) assessment in chemotherapy series</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Etienne et al.(^{255})</td>
<td>82 Stage II–IV HNSCC</td>
<td>Induction cisplatin/5-FU, 21 concurrent chemoRT</td>
<td>Yes</td>
<td>Immuno-luminometric assay</td>
<td>NA</td>
<td>Trend for chemoRT group only</td>
<td>No</td>
<td>( p53 ) status correlated with poor CSS in univariate but not MVT analysis</td>
</tr>
<tr>
<td>Temam et al.(^{246})</td>
<td>105 Stage III–IV HNSCC</td>
<td>Induction cisplatin and 5-FU</td>
<td>Yes</td>
<td>IHC, Sequenced exons 2–11</td>
<td>61% 37%</td>
<td>Yes Yes</td>
<td>NR NR</td>
<td>Both ( p53 ) IHC and mutation were independent predictors of response</td>
</tr>
<tr>
<td>Cabelguenne et al.(^{254})</td>
<td>106 Stage I–IV HNSCC</td>
<td>Induction cisplatin and 5-FU</td>
<td>Yes</td>
<td>( p53 ) LOH, DGGE + sequencing exons 4–9</td>
<td>54% 68%</td>
<td>Trend Yes</td>
<td>NR</td>
<td>Presence of ( p53 ) mutation or anti-( p53 ) serum antibodies conferred highest risk of not responding (RR = 4.9)</td>
</tr>
</tbody>
</table>

CSS, cause-specific survival; CTX, chemotherapy; DFS, disease-free survival; DGGE, denaturing gradient gel electrophoresis; HNSCC, head and neck squamous cell carcinoma; HR, hazard ratio; IHC, immunohistochemistry; LOH, loss of heterozygosity; LR, local-regional; MVT, multivariate analysis; NR, not reported; OS, overall survival; RT, radiotherapy; RR, relative risk; SCC, squamous cell carcinoma; SSCP, single-strand conformation polymorphism.
exhibited a mutation within structural components of the p53 gene, and 15% (13/86) of tumors exhibited mutations within regions critical for DNA binding of the p53 protein. DNA contact mutations were associated with nodal positivity, advanced stage, and poor recurrence-free and overall survival in univariate analysis. Furthermore, in the subset of stage IV patients, the presence of contact mutations correlated with poor overall survival. No such correlation between structural mutations and stage at presentation or outcome was observed. These results imply that p53 mutations resulting in the specific loss of DNA binding and thus transcriptional regulation result in radioresistance and particularly poor survival. Clearly, the clinical significance of specific p53 mutations merits further study.

Given the association between p53 mutation and adverse outcomes, multiple groups have investigated the potential benefit of p53 gene therapy, and phase III clinical trials are currently ongoing in the United States. In China, a recombinant human adenovirus in which the E1 region has been replaced by the p53 expression cassette has been trademarked Gendicine and approved for clinical use. To date, approximately 3,500 patients have received this gene therapy in China. However, data on the effectiveness of this intervention in the English literature is sparse.

**Angiogenesis-Related Markers**

Angiogenesis, the sprouting of new blood vessels from a preexisting endothelium, enables the growth of tumors beyond microscopic size. Many growth factors and cytokines, including the VEGF family, basic and acidic fibroblast growth factor (bFGF, aFGF), interleukin-8 (IL-8) and platelet-derived endothelial cell growth factor (PD-ECGF) have been shown to promote angiogenesis. The VEGF family consists of five members including VEGF, PlGF, VEGF-B, VEGF-C, VEGF-D, and the Orf virus VEGFs (VEGF-E). Of these factors, VEGF plays a pivotal role in vasculogenesis and angiogenesis, and VEGF-C is a potent inducer of lymphangiogenesis. Receptors for the VEGF family include VEGFR-1, VEGFR-2, and VEGFR-3, also known as flt, KDR/flk-1, and flt-4, respectively. VEGFR-1 and VEGFR-2 are expressed on vascular endothelium, whereas VEGFR-3 is expressed in the lymphatic endothelium. VEGF, a 34-50 kDa dimer composed of two identical disulfide-linked subunits that arise from differential splicing of a single gene, is frequently expressed in HNSCC and has received the most study as a potential mediator of angiogenesis. Overexpression of VEGF may result from hypoxia-induced upregulation of transcription and eIF4E-mediated upregulation of translation. Because hypoxic tumors display increased radioresistance, VEGF protein levels may serve as a surrogate marker for hypoxia-radioresistance. Furthermore, angiogenic activity may influence a tumor’s metastatic potential by exposing it to a greater endothelial surface area, thus increasing the likelihood of hematogenous or lymphatic dissemination. In addition, once a tumor has formed a distant micrometastasis, it must recruit a vascular supply in order to proliferate to a clinically relevant size.

Angiogenic activity can be assessed in archival tumor tissue directly by staining with antibodies targeted against proteins that reside on endothelial cells, such as Factor VIII, CD-31, CD-34, and CD-105, in order to calculate the density of microvessels in the tumor bed. In addition, expression of angiogenesis-promoting factors has been assessed via IHC and ELISA. Expression of both VEGF and PD-ECGF has been reported to correlate with microvessel density (MVD) in HNSCC. Elevated tumor MVD as assessed via IHC has been shown to correlate with risk for concomitant cervical lymph node metastasis in oral cavity and nasopharyngeal carcinomas.

Furthermore, elevated MVD has been shown to correlate with risk of regional relapse in clinically N0 oral cavity carcinomas treated with surgery. These studies strongly suggest that the finding of high MVD in the primary tumor suggests a higher risk of concomitant nodal metastasis. In contrast, studies are mixed regarding the potential correlation of elevated MVD with either local-regional control or survival. However, a recent study of 127 HNSCC patients suggested that endothelial expression of CD-105 (endoglin), which is a marker of neovascularization, was strongly correlated with poor disease-free and overall survival. In contrast, expression of CD34, which is commonly expressed on both quiescent and proliferating endothelial cells, was not associated with outcome, suggesting that the proliferating endothelial component is the primary determinant of tumor behavior.

**Cyclin D1**

Cyclin D1, also known as PRAD1, is a proto-oncogene located on chromosome 11q13 that serves as the rate-limiting controller of G1-phase progression through the cell cycle. In response to extracellular mitogens, cyclin D1 levels increase and complex with the cyclin-dependent kinases cdk4 or cdk6 in order to mediate progression through G1 by phosphorylation of the retinoblastoma protein. Cyclin D1 is the most commonly amplified oncogene in HNSCC, with approximately 35% of tumors revealing increased gene copy number in FISH analysis. Overexpression of cyclin D1 shortens the G1 interval and reduces the cell’s dependence on mitogens for proliferation. For example, overexpression of cyclin D1 in a human epithelial breast cancer cell line reduced the fraction of cells entering quiescence under conditions where growth factors were limited, resulting in accelerated growth. Such observations suggest that overexpression of cyclin D1 may increase the aggressiveness of certain cancers by desensitizing cellular proliferation to inhibitory signals.

Although the literature remains somewhat mixed, a growing body of evidence suggests a relationship between cyclin D1 amplification and poor prognosis (Table 3.4). In patients with clinically negative cervical lymph nodes, expression or amplification of cyclin D1 in the primary tumor increases the risk of cervical nodal metastasis by four- to eightfold. In patients with postoperative radiotherapy, cyclin D1 overexpression was an independent predictor of shortened disease-free interval in 115 stage I to IV HNSCCs treated with surgery (73 received postoperative radiotherapy).
EGFR and TGF-α

The receptor tyrosine kinase epidermal growth factor receptor (EGFR) and its ligand transforming growth factor-alpha (TGF-α) are frequently overexpressed in HNSCC. Activation of EGFR induces autophosphorylation, resulting in activation of several signaling pathways including Ras-MAP kinase, phospholipase C, phosphatidylinositol 3-kinase, and STATs (signal transducers and activators of transcription). In HNSCC, autocrine activation of EGFR by TGF-α functions to promote cellular proliferation and inhibit apoptosis. For example, in vitro experiments...
CHAPTER 3 | PROGNOSTIC FACTORS IN PATIENTS WITH HEAD AND NECK CANCER

conducted on HNSCC-derived cell lines demonstrated that inhibition of TGF-α with antisense oligonucleotides and inhibition of EGFR with antisense oligonucleotides, monoclonal antibodies, or specific inhibitors of EGFR kinase activity resulted in reduced cellular proliferation.\textsuperscript{307,308} Furthermore, in vitro transfection of a vector expressing EGFR antisense oligonucleotides resulted in growth inhibition and induction of apoptosis in nude mice tumor xenografts.\textsuperscript{309}

EGFR expression may also modulate tumor radioresistance. In murine tumors, Akimoto et al. found that the magnitude of EGFR expression positively correlated with increased radioreistance and inversely correlated with radiation-induced apoptosis.\textsuperscript{310} Blockade of EGFR with the monoclonal antibody C225 enhances radiosensitivity of SCCs both \textit{in vitro} and \textit{in vivo}. In cell lines derived from HNSCC patients, concomitant C225 administration and radiotherapy enhances of radiosensitivity, in part because inhibition of EGFR results in down-regulation of antiapoptotic proteins such as Stat3 and thus promotes radiation-induced apoptosis.\textsuperscript{311,312} Furthermore, \textit{in vivo} experiments suggest that C225 inhibits repair of radiation-induced DNA damage and promotes tumor necrosis.\textsuperscript{313,314}

In agreement with laboratory data, clinical studies have established a correlation of EGFR overexpression with poor prognosis and radioresistance (Table 3.5).\textsuperscript{315–323} Initial evidence for the prognostic utility of EGFR levels was derived from Dassonville et al. who found that EGFR expression independently predicted poor relapse-free survival in 109 stage I to IV HNSCC patients treated primarily with either chemotherapy or surgery.\textsuperscript{315} Maurizi et al. reported an independent relationship between EGFR protein expression and poor disease-free and overall survival in 140 stage I to IV laryngeal SCCs treated primarily with surgery.\textsuperscript{316} Similarly, Grandis et al. utilized quantitative IHC to show that increasing levels of both TGF-α and EGFR predicted poor disease-free and cause-specific survival in a cohort of 91 stage I to IV HNSCCs\textsuperscript{316} treated with surgery (56 received postoperative radiotherapy). Finally, Chung et al. used FISH in 75 patients with stage I to IV HNSCC to determine that patients with more than two copies per cell of the EGFR gene experienced a higher risk of progression and death.\textsuperscript{317} Thus, the cumulative evidence suggests that EGFR protein overexpression or gene amplification is associated with poor prognosis.

Overexpression of EGFR has also been shown to correlate with radioresistance. For example, Ang et al. reported that, in a cohort of 155 patients with stage II to IV HNSCC treated with conventional radiotherapy on RTOG study 90-03, overexpression of EGFR was associated with poor local-regional control, disease-free survival, and overall survival in multivariate analysis.\textsuperscript{319} In addition, growing evidence suggests that accelerating the course of radiotherapy may mitigate the adverse effect of EGFR overexpression on local-regional control. For example, Bentzen et al. reported a cohort of 304 patients with stage II to IV HNSCC randomized to receive either conventional daily radiation to a total dose of 66 Gy in 33 fractions over 45 days or continuous hyperfractionated accelerated radiation therapy (CHART) to a total dose of 54 Gy in 36 fractions over 12 days.\textsuperscript{321} Patients with high EGFR expression derived a significant local-regional control benefit from CHART, whereas patients with low EGFR expression experienced similar local-regional control regardless of whether they received conventional radiation or CHART. Two other recent studies also suggest that a short overall treatment time may compensate for the adverse effects of EGFR.\textsuperscript{320,323} One potential explanation for this observation is that EGFR may mediate accelerated repopulation late in the course of conventional radiotherapy, thus allowing EGFR-positive tumors to become increasingly resistant to radiation during the last few weeks of conventional treatment.

Emerging evidence suggests that targeting the EGFR receptor with either small molecule tyrosine kinase inhibitors such as erlotinib or monoclonal antibodies such as cetuximab results in clinical gains. In the locally advanced setting, a recent randomized trial of 424 patients with stage III to IV HNSCC revealed that median overall survival for patients treated with cetuximab and radiotherapy was 49.0 months versus 29.3 months in the radiotherapy-alone arm (HR, 0.73; 95% CI, 0.56–0.95; \( p = 0.018 \)).\textsuperscript{324} In the first-line recurrent/metastatic setting, the EXTREME trial found that adding cetuximab to platinum-fluorouracil significantly prolonged median OS from 7.4 months to 10.1 months (HR for death, 0.80; 95% CI, 0.64–0.99; \( p = 0.04 \)) and median progression-free survival from 3.3 to 5.6 months (HR, 0.54; \( p < 0.001 \)).\textsuperscript{325} In addition, for patients with recurrent or metastatic disease that is refractory to first-line chemotherapy, cetuximab monotherapy has been shown to produce response rates ranging from 5% to 15%,\textsuperscript{326} and the addition of platinum agents to cetuximab further improves response rates from approximately 18% to 26%.\textsuperscript{327} These trials have provided the first conclusive evidence that molecularly targeted therapies can produce meaningful gains in the treatment of head and neck cancer.

Human Papillomavirus

HPV is a small, circular DNA virus that infects basal cells in the squamous epithelium. HPV is transmitted through sexual contact and the recent rise in HPV-related HNSCC is thought to be due to increased oral sexual practices. D’Souza found that a high lifetime number of vaginal-sex partners (26 or more) was associated with oropharyngeal SCC (OR 3.1; 95% CI, 1.5–6.5), as was a high lifetime number of oral-sex partners (6 or more) (OR 3.4; 95% CI, 1.3–8.8).\textsuperscript{328} HPV causes cancer by means of viral oncoproteins E6 and E7, which inactivate two human tumor suppressor genes, p53 and RB. Though there are over 100 different subtypes of HPV, the vast majority of HPV-related HNSCC is related to HPV16, which is found in up to 90% of these cases. HPV-associated HNSCC patients are typically younger, more often white, nonsmokers, nonalcohol abusers, have higher socioeconomic status, with primary tumors more commonly in the oropharynx.\textsuperscript{329,330} Ang looked at patients treated on RTOG 0129, which randomized patients with advanced HNSCC treated with cisplatinum to either standard versus accelerated fractionation radiation therapy. While the radiation fractionation scheme made no significant difference in OS, subgroup analysis of the oropharyngeal SCCs revealed that the HPV-positive patients had better outcomes compared to HPV-negative patients (3-year OS, 82.4% vs. 57.1%; \( p < 0.001 \)). Using recursive-partitioning analysis, they subdivided these patients into low, intermediate, or high risk of death on the basis of four factors: HPV status, greater or less than 10 pack-years of tobacco use, T stage, and N stage.\textsuperscript{331} The improved prognosis of HPV-related HNSCC does not appear confined to patients treated with primary radiation, but extends to surgical patients as well.\textsuperscript{332–334} How to take advantage of the improved prognosis of HPV-related HNSCC will be a major area of research in the coming years, specifically whether it is possible to de-escalate radiation dose or systemic therapy and maintain these patients’ favorable prognosis. RTOG 1016 is a phase III trial randomizing patients with HPV-positive oropharyngeal SCC to concurrent cisplatinum radiation versus cetuximab radiation, the rationale being that if concurrent cetuximab can offer similarly excellent outcomes in this population, the toxicity of concurrent cetuximab is much less than concurrent cisplatinum.\textsuperscript{335} HPV and its implications in head and neck cancer is also discussed in detail in Chapter 12.
<table>
<thead>
<tr>
<th>Author</th>
<th>Description of Patients</th>
<th>Treatment Given</th>
<th>MVT</th>
<th>Marker</th>
<th>Method (% Positive)</th>
<th>Local/Regional Recurrence</th>
<th>Survival</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dassonville et al.</td>
<td>109 Stage I–IV HNSCC</td>
<td>73 CTX, 29 surgery, 6 RT, 1 none</td>
<td>Yes</td>
<td>EGFR</td>
<td>RRA</td>
<td>NR</td>
<td>DFS (p = 0.03)</td>
<td>EGFR status correlated with overall survival in univariate but not mvt model</td>
</tr>
<tr>
<td>Maurizi et al.</td>
<td>140 Stage I–IV laryngeal SCC</td>
<td>Surgery (post-op RT not mentioned)</td>
<td>Yes</td>
<td>EGFR</td>
<td>RRA: 20%</td>
<td>NR</td>
<td>OS (p = 0.0001)</td>
<td>DFS (p = 0.01)</td>
</tr>
<tr>
<td>Almadori et al.</td>
<td>140 Stage I–IV laryngeal SCC</td>
<td>Surgery (post-op RT not mentioned)</td>
<td>Yes</td>
<td>EGFR</td>
<td>RRA: 20%</td>
<td>Yes (p = 0.001)</td>
<td>NR</td>
<td>EGFR status divided into tertiles using computerized image analysis</td>
</tr>
<tr>
<td>Grandis et al.</td>
<td>91 Stage I–IV HNSCC</td>
<td>Surgery (56 received post-op RT)</td>
<td>Yes</td>
<td>TGF-α</td>
<td>IHC</td>
<td>NR</td>
<td>CSS (p = 0.0001)</td>
<td></td>
</tr>
<tr>
<td>Ang et al.</td>
<td>155 Stage II–IV HNSCC</td>
<td>Conventional RT</td>
<td>Yes</td>
<td>EGFR</td>
<td>IHC</td>
<td>NR</td>
<td>Yes (p = 0.002)</td>
<td>DFS (p = 0.003)</td>
</tr>
<tr>
<td>Eriksen et al.</td>
<td>336 T1-4 HNSCC</td>
<td>Conventional RT + misronidazole or nimorazole</td>
<td>No</td>
<td>EGFR</td>
<td>IHC: 65%</td>
<td>Yes (p &lt; 0.05)</td>
<td>NR</td>
<td>EGFR was associated with poor local-regional control only for patients who received RT over 9.5 weeks, and not for patients who received RT over 5.5–6.5 weeks</td>
</tr>
<tr>
<td>Bentzen et al.</td>
<td>304 Stage II–IV HNSCC</td>
<td>120 Conventional RT, 184 CHART</td>
<td>No</td>
<td>EGFR</td>
<td>IHC</td>
<td>NR</td>
<td>No</td>
<td>For tumor with high EGFR expression, CHART, but not conventional RT, improved local-regional control</td>
</tr>
<tr>
<td>Chung et al.</td>
<td>75 Stage I–IV HNSCC</td>
<td>51 Surgery, 24 not surgery</td>
<td>Yes</td>
<td>EGFR</td>
<td>FISH: 58%</td>
<td>NR</td>
<td>PFS &lt; (p = 0.02)</td>
<td>OS (p = 0.01)</td>
</tr>
<tr>
<td>Smid et al.</td>
<td>165 Stage I–IV oral SCC</td>
<td>Surgery and post-operative radiation</td>
<td>Yes</td>
<td>EGFR</td>
<td>IHC: 80%</td>
<td>No</td>
<td>see notes</td>
<td>For patients with EGFR positive tumors only, long overall treatment time &gt;42 days was associated with poor local-regional control</td>
</tr>
</tbody>
</table>

CHART, continuous hyperfractionated accelerated radiation therapy; FISH, fluorescent in situ hybridization; HNSCC, head and neck squamous cell carcinoma; IHC, immunohistochemistry; MVT, multivariate model; NR, not reported; PFS, progression-free survival; RT, radiotherapy; RRA, radioligand receptor assay; SCC, squamous cell carcinoma; TGF-α, transforming growth factor-alpha.
SUMMARY

Understanding the vast array of factors that contribute to the prognosis of patients with HNSCC enables an accurate assessment of patient risk and promotes the development of optimal treatment strategies. In addition, novel targeted therapies are emerging, which enable clinicians to mitigate the risk associated with adverse molecular factors.

REFERENCES


