



Lu Jian Jun. *Memories*, 1999. Oil on canvas, 36" × 48". Courtesy of Weinstein Gallery, San Francisco, California.

Genetic abnormalities are commonly detected in head and neck cancers and may impact the future management of this cancer.

Molecular Genetics of Head and Neck Cancer

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Background: *Head and neck cancers have multiple genetic abnormalities that influence tumor behavior and may be useful in developing new treatments.*

Methods: *Genetic alterations implicated in head and neck cancer oncogenesis and behavior are reviewed, and molecular techniques for detection and treatment are evaluated.*

Results: *The large number of genetic changes present in head and neck cancer cells precludes meaningful use of simple molecular tests and treatments. Detection of abnormalities in multiple genes provides better prognostic information than the detection and assessment of single mutations. Screening tests that rely on amplification of genetic material present in bodily fluids are hindered by the genomic complexity of head and neck cancer. Introduction of genetic material into head and neck cancer cells for gene therapy has shown some efficacy.*

Conclusions: *Head and neck cancers comprise a complex genetic disease. Although much has been learned about the molecular genetics of head and neck cancers, continued study of multiple genes is critical for further progress. Gene therapy, although promising, must also overcome this complexity.*

Introduction

Genetic abnormalities in head and neck squamous cell cancer have been studied extensively, and frequent changes have been found. A key motivation for investigating head and neck cancer genetics is to find prognostic indicators of patient survival or markers to select the type of treatment. Further understanding of head and neck cancer genetics may also permit the development of new cancer therapies. This article discusses information on changes in tumor suppressor genes and oncogenes, presents the current state of the art of molecular detection of head and neck cancer, and reviews the status and promise of gene therapy.

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Tumor Suppressor Genes

Tumor suppressor genes have been intensely investigated in head and neck cancer. These genes act to limit growth of tumors by slowing or halting cell cycle progression, and mutations in tumor suppressor genes are commonly seen in head and neck cancer. Aberrations in specific tumor suppressor genes may be predictive of patient outcome.

p53

Dysfunction in the p53 tumor suppressor gene (located at 17p13) is implicated in many cancers, including head and neck cancer, and has received the most attention. The production of p53 is increased in response to cellular insults or DNA damage, and p53 then induces cell cycle arrest at the G₁/S junction. If the damage is irreparable, p53 can initiate cell death by apoptosis.¹

The steady-state concentration of p53 in normal cells is low, and the half-life of normal (wild type) p53 is short. In contrast, if the p53 gene is mutated, the genetic product is often present in high concentrations.² Therefore, immunohistochemical (IHC) methods can be used to detect abnormal p53, although the exact protein that is stained is of questionable significance. Polymerase chain reaction (PCR)-based methods, such as direct sequencing of the p53 gene or loss of heterozygosity (LOH) analysis, also allow detection of mutant p53. LOH analysis detects the loss of a genome-specific allele and thus can detect changes that may not be apparent with IHC analysis.

In head and neck cancer, p53 mutations are present in 33% to 59% of tumors using PCR, LOH occurs in 38% of tumors, and abnormal IHC staining is seen in 37% to 76% of tumors (Table 1). Unfortunately, the tumors that have mutations do not always have abnor-

Table 1. — Selected Studies of p53 Expression and Mutation That Included Evaluation of Prognosis

| Study, Year | Patients | Method | p53 Abnormal | Measure | Results |
|--|----------|--------|--------------|--------------------|---|
| Bova et al 1999 ³ | 143 | IHC | 62% | Survival | No correlation |
| Bradford et al 1995 ⁴ | 178 | IHC | 61% | Organ preservation | More likely with staining |
| Hirvikoski et al 1997 ⁵ | 103 | IHC | 68% | Survival | Better with staining |
| Kapranos et al 2001 ⁶ | 93 | IHC | 48% | Survival | No correlation |
| Kaur et al 1998 ⁷ | 145 | IHC | 70% | Survival | Worse with staining only for oral cavity cancer |
| Kokoska et al 1996 ⁸ | 66 | IHC | 61% | Survival | No correlation |
| Mao et al 1995 ⁹ | 111 | IHC | 69% | Survival | No correlation |
| Michalides et al 1997 ¹⁰ | 115 | IHC | 42% | Survival | No correlation |
| Narayana et al 2000 ¹¹ | 102 | IHC | 37% | Survival | No correlation |
| Osaki et al 2000 ¹² | 225 | IHC | 57% | Survival | Worse with staining |
| Portugal et al 1997 ¹³ | 100 | IHC | 66% | Survival | No correlation |
| Pruneri et al 1998 ¹⁴ | 149 | IHC | 50% | Survival | No correlation |
| Raybaud-Diogene et al 1997 ¹⁵ | 101 | IHC | 49% | Recurrence | Worse with staining |
| Wilson et al 1995 ¹⁶ | 99 | IHC | 76% | Survival | No correlation |
| Gleich et al 1996 ¹⁷ | 50 | LOH | 38% | Survival | No correlation |
| Bradford et al 1997 ¹⁸ | 44 | PCR | 39% | Survival | Worse with mutation |
| Chomchai et al 1999 ¹⁹ | 45 | PCR | 33% | Survival | Better with mutation |
| Hegde et al 1998 ²⁰ | 39 | PCR | 33% | Survival | Worse with mutation |
| Hiranuma et al 1998 ²¹ | 45 | PCR | 40% | Survival | No correlation |
| Hogmo et al 1999 ²² | 34 | PCR | 59% | Recurrence | No correlation |
| Koch et al 1996 ²³ | 110 | PCR | 44% | Survival | No correlation |
| Ostwald et al 2000 ²⁴ | 94 | PCR | 43% | Survival | No correlation |
| Saunders et al 1999 ²⁵ | 42 | PCR | 48% | Recurrence | No correlation |
| Shima et al 2000 ²⁶ | 46 | PCR | 43% | Survival | No correlation |

LOH = loss of heterozygosity
 IHC = immunohistochemistry
 PCR = polymerase chain reaction

mal IHC. This poor correlation between PCR analysis, LOH, and IHC is a confounding factor in evaluating head and neck cancer p53 data.

Given the large number of studies without a clear correlation between p53 status and survival, it is evident that mutation of p53 is not a powerful predictive marker. In general, the studies that did show a relationship between p53 and outcome found, as expected, that overexpression of p53 as detected by IHC or the presence of LOH or mutations are associated with recurrence or death. However, an analysis of the Veterans Affairs Laryngeal Cancer Study, in which patients were randomized to induction chemotherapy followed by radiation therapy vs surgery and postoperative radiation, found unexpectedly that p53 overexpression as detected by IHC was associated with an increased rate of organ preservation.⁴ A further analysis of these same patients by direct sequencing for mutations reported worse survival with p53 mutations present.¹⁸ The clinical utility of p53 mutations as a predictor of survival or as an aid in selecting the method of therapy is therefore unclear.

Retinoblastoma

Retinoblastoma (Rb, located at 13q14) is a key tumor suppressor gene involved in controlling the cell cycle.²⁷ Hypophosphorylated Rb binds and inactivates a transcription factor responsible for cell cycle progression (EF1). Mutation of Rb or loss of Rb activity can therefore cause unchecked cell growth.

IHC studies demonstrate Rb abnormalities (diminished expression) in 6% to 74% of head and neck cancers (Table 2). LOH analysis demonstrates loss of an Rb allele in 14% to 59% of tumors. As with p53, there is no clear correlation between Rb mutation and poor outcome; however, two studies suggested that underexpression correlates with poor survival.^{28,34} One study found that LOH at p53 and Rb occurring simultaneously is associated with poorer survival.¹⁷

p16/p21/p27

The p16, p21, and p27 tumor suppressor genes act to modulate cell proliferation. The p16 gene (located at 9p21) produces p16 protein, which in turn inhibits phosphorylation of Rb, thus inhibiting the Rb-induced release of transcription factor EF1 and cell cycle progression.⁴⁰ The p21 and p27 genes (located at 6p21 and 14q32, respectively) produce proteins that are activated by p53 and induce cell cycle arrest.^{41,42} Abnormalities can be found using PCR, LOH analysis, or Western blotting, which can evaluate protein expression.

Abnormalities in p16 are common in head and neck cancers. PCR methods have shown mutations in 19% to 58% of tumors, while LOH analysis revealed allelic losses in 57% (Table 3). IHC methods have shown low p16 expression in 55% to 89% of tumors. Low expression of p16 therefore occurs in the vast majority of head and neck cancers. A study examining p16 using PCR and Western blotting for the same set of tumors found mutated p16 in only 19% of tumors;

Table 2. — Selected Studies of Retinoblastoma (Rb) Expression and Mutation

| Study, Year | Patients | Method | Rb Abnormal | Measure | Results |
|--|----------|--------------|-------------|----------|----------------------------------|
| Dokiya et al 1998 ²⁸ | 72 | IHC | 29% | Survival | Worse with abnormality |
| El-Naggar et al 1999 ²⁹ | 35 | IHC | 6% | | |
| Ioachim et al 1999 ³⁰ | 41 | IHC | 30% | | |
| Koontongkaew et al 2000 ³¹ | 53 | IHC | 74% | | |
| Nakahara et al 2000 ³² | 78 | IHC | 56% | | |
| Pande et al 1998 ³³ | 35 | IHC | 66% | | |
| Pavelic et al 1996 ³⁴ | 182 | IHC | 27% | Survival | T1 tumors worse with abnormality |
| Regezi et al 1999 ³⁵ | 55 | IHC | 40% | | |
| Xu et al 1998 ³⁶ | 34 | IHC | 9% | | |
| Gleich et al 1996 ¹⁷ | 57 | LOH | 37% | Survival | No correlation |
| Scholnick et al 1994 ³⁷ | 37 | LOH | 59% | | |
| Yokoyama et al 1996 ³⁸ | 28 | LOH | 14% | | |
| Sartor et al 1999 ³⁹ | 25 | Western blot | 44% | | |
| LOH = loss of heterozygosity IHC = immunohistochemistry | | | | | |

however, decreased p16 expression was found in 69% of tumors.³⁹ Thus, aberrations in the regulation of p16 protein production are common in head and neck cancer. Transcriptional inactivation by hypermethylation of the p16 gene promoter may contribute to this down-regulation.^{48,49} Abnormal p16 is associated with worse survival, increased recurrences, tumor progression, and

nodal metastasis in many of the studies assessing patient outcome.

Expression of p21 was shown in 29% to 92% of head and neck tumors using IHC methods (Table 4). There is no clear relationship between p21 staining and clinical parameters. Expression of p27 was demonstrated in 18%

Table 3. — Studies of p16 Expression and Mutation

| Study, Year | Patients | Method | p16 Abnormal | Measure | Results |
|---|----------|--------------|--------------|------------------|------------------------|
| Bova et al 1999 ³ | 143 | IHC | 55% | Survival | Worse with abnormality |
| El-Naggar et al 1999 ²⁴ | 35 | IHC | 89% | | |
| Pande et al 1998 ³³ | 35 | IHC | 63% | | |
| Jares et al 1997 ⁴³ | 42 | LOH | 57% | Nodal metastasis | More with abnormality |
| Danahey et al 1999 ⁴⁴ | 26 | PCR | 58% | Recurrence | More with abnormality |
| Jares et al 1999 ⁴³ | 46 | PCR | 22% | | |
| Matsuda et al 1996 ⁴⁵ | 20 | PCR | 20% | | |
| Olshan et al 1997 ⁴⁶ | 33 | PCR | 36% | | |
| Sartor et al 1999 ³⁹ | 26 | PCR | 19% | | |
| Shintani et al 2001 ⁴⁷ | 32 | PCR | 56% | | |
| Sartor et al 1999 ³⁹ | 26 | Western blot | 69% | | |
| LOH = loss of heterozygosity IHC = immunohistochemistry PCR = polymerase chain reaction | | | | | |

Table 4. — Studies of p21 Expression

| Study, Year | Patients | Method | p21 Expression | Measure | Results |
|-------------------------------------|----------|--------|----------------|------------------|----------------------|
| Agarwal et al 1998 ⁵⁰ | 51 | IHC | 69% | | |
| Erber et al 1997 ⁵¹ | 42 | IHC | 67% | Survival | Worse with staining |
| Hirvikoski et al 1999 ⁵² | 144 | IHC | 68% | Survival | No correlation |
| Jeannon et al 2000 ⁵³ | 60 | IHC | 58% | Survival | Worse with staining |
| Kapranos et al 2001 ⁶ | 93 | IHC | 55% | Survival | No correlation |
| Kuo et al 1995 ⁵⁴ | 51 | IHC | 92% | Nodal metastasis | More with staining |
| Mineta et al 1999 ⁵⁵ | 72 | IHC | 29% | Survival | No correlation |
| Ng et al 1999 ⁵⁶ | 88 | IHC | 82% | | |
| Osaki et al 2000 ¹² | 225 | IHC | 29% | Survival | No correlation |
| Pruneri et al 1999 ⁵⁷ | 132 | IHC | 69% | Metastasis | Less with staining |
| Ralhan et al 2000 ⁵⁸ | 30 | IHC | 53% | | |
| Regezi et al 1999 ³⁵ | 64 | IHC | 77% | | |
| Saunders et al 1999 ²⁵ | 36 | IHC | 86% | | |
| Tatemoto et al 1998 ⁵⁹ | 150 | IHC | 29% | Nodal metastasis | More with staining |
| van Oijen et al 1998 ⁶⁰ | 43 | IHC | 67% | | |
| Yook et al 1998 ⁶¹ | 20 | IHC | 75% | Survival | No correlation |
| Yuen et al 2001 ⁶² | 87 | IHC | 56% | Survival | Better with staining |
| IHC = immunohistochemistry | | | | | |

to 62% of tumors by IHC. The presence of p27 has been correlated with improved survival (Table 5).

Oncogenes

Oncogenes produce proteins that promote cell and tumor growth. The cellular changes necessary for malignant transformation involve the activation of many oncogenes.

Cyclin D1

The cyclins are proteins that are involved in cell cycle regulation. The cyclin D1 gene product (*CCND1*, located at 11q13) phosphorylates Rb, leading to cell

cycle progression. The activity of cyclin D1 may be inhibited by many tumor suppressor genes including p16, p21, and p27.⁶⁵ In assessing cyclin D1, multiple techniques have been used including IHC, fluorescence in situ hybridization (FISH) allowing detection of gene copy number, and Southern blotting techniques allowing quantification of gene copy number.

In head and neck cancers, cyclin D1 has been shown to be amplified in 36% of tumors using FISH and in 18% to 58% of tumors using Southern blotting, and it is overexpressed in 12% to 68% using IHC (Table 6). Studies that showed a relationship between cyclin D1 and outcome found, as expected, that amplification or overexpression was associated with recurrence, nodal metastasis, or death.

Table 5. — Studies of p27 Expression

| Study, Year | Patients | Method | p27 Expression | Measure | Results |
|----------------------------------|----------|--------|----------------|----------|----------------------|
| Kapranos et al 2001 ⁶ | 93 | IHC | 53% | Survival | No correlation |
| Kudo et al 2000 ⁶³ | 17 | IHC | 18% | | |
| Mineta et al 1999 ⁵⁵ | 81 | IHC | 32% | Survival | Better with staining |
| Pruneri et al 1999 ⁵⁷ | 132 | IHC | 62% | Survival | Better with staining |
| Tamura et al 2001 ⁶⁴ | 102 | IHC | 47% | Survival | Better with staining |
| IHC = immunohistochemistry | | | | | |

Table 6. — Studies of Cyclin D1 Expression and Amplification

| Study, Year | Patients | Method | Cyclin D1 Abnormal | Measure | Results |
|---|----------|---------------|--------------------|------------------|---------------------------|
| Okami et al 1999 ⁶⁶ | 11 | FISH | 36% | | |
| Akervall et al 1997 ⁶⁷ | 75 | IHC | 12% | Survival | Worse with staining |
| Bova et al 1999 ³ | 147 | IHC | 68% | Survival | Worse with staining |
| Capaccio et al 1997 ⁶⁸ | 96 | IHC | 44% | Nodal metastasis | More with staining |
| Koontongkaew et al 2000 ³¹ | 53 | IHC | 40% | | |
| Kyomoto et al 1997 ⁶⁹ | 45 | IHC | 53% | Survival | Worse with staining |
| Masuda et al 1996 ⁷⁰ | 42 | IHC | 55% | Survival | Worse with staining |
| Michalides et al 1997 ¹⁰ | 115 | IHC | 49% | Survival | Worse with heavy staining |
| Mineta et al 2000 ⁵⁵ | 94 | IHC | 19% | Survival | Worse with staining |
| Nakahara et al 2000 ³² | 78 | IHC | 36% | | |
| Pignataro et al 1998 ⁷¹ | 149 | IHC | 32% | Survival | Worse with staining |
| Bellacosa et al 1996 ⁷² | 51 | Southern blot | 18% | Survival | Worse with amplification |
| Callender et al 1994 ⁷³ | 32 | Southern blot | 34% | | |
| Fortin et al 1997 ⁷⁴ | 50 | Southern blot | 20% | Survival | No correlation |
| Gleich et al 1999 ⁷⁵ | 24 | Southern blot | 42% | Survival | No correlation |
| Meredith et al 1995 ⁷⁶ | 56 | Southern blot | 39% | Survival | Worse with amplification |
| Muller et al 1997 ⁷⁷ | 201 | Southern blot | 58% | Survival | No correlation |
| IHC = immunohistochemistry FISH = fluorescence in situ hybridization | | | | | |

EGF/EGFR

Human epidermal growth factor receptor (EGFR, located at 7p12) is a transmembrane protein with intrinsic tyrosine kinase activity expressed primarily on cells of epithelial origin. EGFR regulates cell growth in response to activation by EGF and transforming growth factor- α (TGF- α) binding.⁷⁸ EGFR is overexpressed in head and neck tumors, leading to increased tyrosine kinase activity and cell proliferation.⁷⁹ In addition, tumors can overexpress EGF, causing autocrine stimulation of the EGFR.

EGFR expression is found in a high percentage of head and neck cancers (43% to 62%).^{80,81} EGFR expression has been correlated with worse survival; however, the studies are few, and there are negative studies. Blockage of EGFR receptors in cell lines inhibits tumor growth and has led to active clinical trials.⁸²

STAT3

The STAT tyrosine kinase system has recently been the subject of much investigation. Activated EGFR activates STAT proteins through a complex mechanism. The activated STAT then induces cell proliferation.⁸³

STAT3 expression and DNA binding are significantly increased in the mucosa of patients with head and neck cancer.⁸⁴ In addition, blocking EGFR expression leads to a decrease in STAT3 activation.⁸⁵ No studies have been performed to demonstrate an association between STAT activity and head and neck cancer survival, but this kinase appears to be involved in tumor progression.

Multiple Molecular Aberrations in Head and Neck Cancers

Multiple genetic abnormalities are present in head and neck cancers. The exact number and type of abnormality differs from tumor to tumor, resulting in different phenotypes. Evaluating the genotype of head and neck tumors at multiple loci may provide more prognostic information.

The multiple genetic abnormalities present in head and neck cancer cells can be evaluated at the chromosomal level using spectral karyotyping (SKY) and comparative genomic hybridization (CGH). The SKY technique involves labeling each chromosome with a unique colored marker. Rearrangements can be identified when a combination of colors is seen on a single derived chromosome. Using this technique, frequent breakpoints were found in multiple chromosomes of

head and neck cancer cells.⁸⁶ Identification of rearrangement sites allows for investigation of candidate oncogenes and tumor suppressor genes at these locations. Similar information can be obtained using CGH, in which tumor DNA is labeled with a specific colored marker and hybridized with DNA isolated from normal cells and stained with a different marker. Differences in gene copy number can be determined by the relative amount of staining. CGH has revealed frequent losses and gains on multiple chromosomes.⁸⁷

LOH analysis provides a rapid method of screening for multiple genetic abnormalities. In a study of 68 head and neck cancers, significant LOH was seen at chromosome bands 3p21, 3p25-26, 8pter-21.1, 13q14, and 17p12. LOH at more than two loci was correlated with a poor prognosis.⁸⁸ A study of 43 head and neck tumors also revealed that tumors with abnormalities in more than one gene (p53, Rb, q16, cyclin D1, p16, p21, and p27 were tested) had a poorer prognosis.⁷⁵

Newer techniques such as gene array technology that can evaluate a number of genes simultaneously have also shown additional potential sites of genetic abnormalities.⁸⁹ As more information on these techniques accumulates, patterns of gene abnormalities that correlate with prognosis may be found.

Molecular Immunology

Immunocompromised patients are more susceptible to many malignancies, and it is hypothesized that the immune system is involved in active surveillance for tumor cells. Patients with head and neck cancer in particular exhibit impairments in immune cell function and cytokine production. This suppression is present at the primary site, in the neck nodes, and systemically.⁹⁰ Tumor cells also secrete substances that further suppress the immune system. The treatments for head and neck cancers also cause immunosuppression.

As a part of the cellular immune system, major histocompatibility (MHC) class I proteins present peptide antigens to CD8⁺ cytotoxic T lymphocytes. Thus, loss of class I MHC activity may allow tumor cells to elude detection. IHC and LOH studies have shown abnormalities in MHC expression in many head and neck tumors.⁹¹

Molecular Detection of Head and Neck Cancers

Screening tests for head and neck cancers are being developed. These cancers are bathed in saliva, and cells exfoliate into this fluid. Analysis of saliva for

abnormal cancer genes may allow tumor screening. An analysis of saliva from 44 head and neck cancers using a panel of PCR probes found microsatellite alterations present in both the saliva and the tumor in 36 cases.⁹² Although saliva samples have the potential for screening for disease or recurrence, these tests are not currently in clinical use and have not yet been verified for clinical application.

Attempts have been made at finding p53 immunoglobulin G (IgG) antibodies in the serum and saliva of head and neck cancer patients with mixed results. In a study of 271 patients with oral squamous carcinoma, p53 antibodies were present in 25% of serum samples.⁹³ A low percentage of patients with head and neck cancer exhibit p53 antibody in their saliva.⁹⁴ These results are not surprising, given that p53 is abnormal in approximately 50% of head and neck cancers.

Abnormal promoter methylation is common in head and neck cancer genes. Using PCR, the presence of promoter hypermethylation can be detected in the serum and saliva of patients with head and neck cancer.^{48,49}

Molecular Determination of Surgical Margins

An analysis of the histologically negative margins from 25 head and neck cancer patients demonstrated p53 mutations in 13 of these patients. None of the patients with histologically and genetically negative margins recurred, while 5 of the 13 patients with p53 mutation in the margin recurred locally.⁹⁵ In another study, surgical margins from patients who underwent larynx cancer resections were tested for eIF4E status.⁹⁶ Of the 54 patients studied, 32 had eIF4E-positive margins. Of the 25 patients who recurred, 21 had eIF4E-positive margins. These studies show that histologically negative margins are not necessarily genetically negative and that genetically positive margins are more likely to recur. However, the relevance of this information in clinical management has not yet been fully elucidated.

Gene Therapy

The goal of gene therapy for cancer is to introduce genetic material into malignant cells to cause tumor regression. Once introduced, these genes may directly replace the function of a mutated gene, convert prodrugs into antineoplastic compounds, or induce other mechanisms that lead to cancer cell death.

Vectors are the means by which genes are delivered to the cell. Viral and nonviral vectors (eg, adenovirus, retrovirus, and liposomal) are used. Despite the high transfection efficiency of some vectors, delivery to all tumor cells is not technically feasible.

p53

Replacing a mutated p53 gene with a wild-type (normal) p53 gene is a potential approach to head and neck cancer treatment. This approach is limited by the lack of mutated p53 in many tumors and also by the current limitations of vector technology in delivering the gene. In a study of 17 patients with advanced recurrent or refractory unresectable head and neck cancer, treatment with delivery of the p53 gene using an adenoviral vector found only 2 patients with tumor regression of more than 50%.⁹⁷ An additional 17 patients with resectable disease were treated, and 2 remained disease-free for longer than 2 years.

ONYX-015

ONYX-015 is an adenovirus from which the E1B region has been deleted. E1B inactivates p53, thereby allowing virus replication. Consequently, ONYX-015 should be able to replicate only in cells lacking functional p53 and thus potentially target cancer cells. Some conflicting data have emerged regarding the specificity of ONYX-015, although its proponents claim that the most stringent tests, ie, those comparing cell lines differing only in p53 status, validate the efficacy of ONYX-015.⁹⁸

ONYX-015 was intratumorally injected in 22 patients with recurrent refractory head and neck cancer that had abnormal p53 immunohistochemistry.⁹⁹ A partial response was seen in 3 patients, and 2 had a minor response. A study involving 40 patients showed a partial response in 14%.¹⁰⁰ In another report, ONYX-015 was given in combination with cisplatin and 5-fluorouracil to treat 37 patients with recurrent head and neck cancer.¹⁰¹ A partial response was seen in 15 patients. However, this was not a randomized trial, and it is not possible to conclude the true efficacy of ONYX-015 and chemotherapy combinations.

Alloantigen Therapy

Head and neck cancer commonly has reduced MHC expression. MHC antigens can incite a vigorous immune response. A potential application in treating head and neck cancer is the use of gene therapy to deliver a class I MHC. If the MHC is human but foreign to the patient, it can induce an antitumor response either by presenting tumor antigens or by itself being

an antigen. Allovectin-7 is a gene therapy product that uses a liposomal vector and encodes the class I MHC HLA-B7.

A study of recurrent, advanced, unresectable head and neck cancer included 18 patients, all of whom were HLA-B7-negative. Patients received intratumoral injection of a gene transfer product (Allovectin-7), which resulted in complete or partial response in 4 patients.¹⁰² A multi-institutional study, also of advanced unresectable head and neck cancer, included 60 patients who were HLA-B7-negative. After the first cycle of treatment, 23 patients had stable disease or a partial response and proceeded to the second cycle. After the second cycle and 16 weeks after the initiation of gene therapy, 6 patients had stable disease, 4 had a partial response, and 1 had a complete response.¹⁰³

p16/p21/Rb

The tumor suppressor genes p16, p21, and Rb are frequently mutated in head and neck cancer and therefore are potential gene therapy targets. Studies in animal models support the potential for these genes in head and neck cancer therapy,^{104,105} but the application of vector technology is currently limited, as seen with p53 gene therapy.

Biologic Therapy

EGF/EGFR

The role of EGFR in head and neck cancer progression has led to the development of drugs to block this receptor. In a study of 16 patients with stage III and IV head and neck cancer, EGFR blocking antibody was combined with radiation therapy, and a complete response was seen in 13 patients.¹⁰⁶ This study was not randomized, so the true efficacy of the antibody cannot be assessed. EGFR blocking antibody in combination with cisplatin was also used in 12 patients with incurable recurrent or metastatic head and neck cancer. A complete response was achieved in 2 patients and a partial response in 4.¹⁰⁷ Again, due to the combination with cisplatin the true efficacy of the antibody cannot be assessed. Currently there are ongoing trials of EGFR blocking antibody.

Conclusions

Abnormalities of many genes critically involved in the regulation of the cell cycle are found in head and neck cancer. Detection of these genetic changes may assist in both the diagnosis and treatment of head and

neck cancer in the future. Further research is needed to clarify how tumor genotype correlates with prognosis. In addition, simpler and less costly methods of detection need to be developed.

Although genetic and biologic therapies are still in the early phases of development, they hold promise for the treatment of head and neck cancer by addressing the fundamental defect of the malignant cell.

References

1. Lane DP. Cancer. p53, guardian of the genome. *Nature*. 1992;358:15-16.
2. Jenkins JR, Rudge K, Chumakov P, et al. The cellular oncogene p53 can be activated by mutagenesis. *Nature*. 1985;317:816-818.
3. Bova RJ, Quinn DI, Nankervis JS, et al. Cyclin D1 and p16INK4A expression predict reduced survival in carcinoma of the anterior tongue. *Clin Cancer Res*. 1999;5:2810-2819.
4. Bradford CR, Zhu S, Wolf GT, et al. Overexpression of p53 predicts organ preservation using induction chemotherapy and radiation in patients with advanced laryngeal cancer. Department of Veterans Affairs Laryngeal Cancer Study Group. *Otolaryngol Head Neck Surg*. 1995;113:408-412.
5. Hirvikoski P, Kumpulainen E, Virtaniemi J, et al. p53 expression and cell proliferation as prognostic factors in laryngeal squamous cell carcinoma. *J Clin Oncol*. 1997;15:3111-3120.
6. Kapranos N, Stathopoulos GP, Manolopoulos L, et al. p53, p21 and p27 protein expression in head and neck cancer and their prognostic value. *Anticancer Res*. 2001;21:521-528.
7. Kaur J, Srivastava A, Ralhan R. Prognostic significance of p53 protein overexpression in betel-and tobacco-related oral oncogenesis. *Int J Cancer*. 1998;79:370-375.
8. Kokoska MS, Piccirillo JF, el-Mofty SK, et al. Prognostic significance of clinical factors and p53 expression in patients with glottic carcinoma treated with radiation therapy. *Cancer*. 1996;78:1693-1700.
9. Mao C, Lu Y, Lai Q, et al. Expression of p53 gene in oral squamous cell carcinoma and its relation with clinical and pathological parameters and prognosis of patients. *Chin Med Sci J*. 1995;10:199-203.
10. Michalides RJ, van Veelen NM, Kristel PM, et al. Overexpression of cyclin D1 indicates a poor prognosis in squamous cell carcinoma of the head and neck. *Arch Otolaryngol Head Neck Surg*. 1997;123:497-502.
11. Narayana A, Vaughan AT, Kathuria S, et al. P53 overexpression is associated with bulky tumor and poor local control in T1 glottic cancer. *Int J Radiat Oncol Biol Phys*. 2000;46:21-26.
12. Osaki T, Kimura T, Tatemoto Y, et al. Diffuse mode of tumor cell invasion and expression of mutant p53 protein but not of p21 protein are correlated with treatment failure in oral carcinomas and their metastatic foci. *Oncology*. 2000;59:36-43.
13. Portugal LG, Goldenberg JD, Wenig BL, et al. Human papillomavirus expression and p53 gene mutations in squamous cell carcinoma. *Arch Otolaryngol Head Neck Surg*. 1997;123:1230-1234.
14. Pruneri G, Pignataro L, Carbone N, et al. Clinical relevance of p53 and bcl-2 protein over-expression in laryngeal squamous-cell carcinoma. *Int J Cancer*. 1998;79:263-268.
15. Raybaud-Diogene H, Fortin A, Morency R, et al. Markers of radioresistance in squamous cell carcinomas of the head and neck: a clinicopathologic and immunohistochemical study. *J Clin Oncol*. 1997;15:1030-1038.
16. Wilson GD, Richman PI, Dische S, et al. p53 status of head and neck cancer: relation to biological characteristics and outcome of radiotherapy. *Br J Cancer*. 1995;71:1248-1252.
17. Gleich LL, Li YO, Biddinger PW, et al. The loss of heterozygosity in retinoblastoma and p53 suppressor genes as a prognostic indicator for head and neck cancer. *Laryngoscope*. 1996;106:1378-1381.
18. Bradford CR, Zhu S, Poore J, et al. p53 mutation as a prognostic marker in advanced laryngeal carcinoma. Department of Veterans Affairs Laryngeal Cancer Cooperative Study Group. *Arch Otolaryngol Head Neck Surg*. 1997;123:605-609.

19. Chomchai J, Du W, Sarkar F, et al. Prognostic significance of p53 gene mutations in laryngeal cancer. *Laryngoscope*. 1999;109:455-459.
20. Hegde PU, Brenski AC, Caldarelli DD, et al. Tumor angiogenesis and p53 mutations: prognosis in head and neck cancer. *Arch Otolaryngol Head Neck Surg*. 1998;124:80-85.
21. Hiranuma H, Jikko A, Maeda T, et al. An analysis of the prognostic significance of p53 status for squamous cell carcinoma of the oral cavity treated by radiotherapy. *Oral Oncol*. 1998;34:513-518.
22. Hogmo A, Borresen-Dale AL, Blegen H, et al. TP53 mutations do not correlate with locoregional recurrence in stage I tongue carcinomas. *Anticancer Res*. 1999;19:3433-3438.
23. Koch WM, Brennan JA, Zahurak M, et al. p53 mutation and locoregional treatment failure in head and neck squamous cell carcinoma. *J Natl Cancer Inst*. 1996;88:1580-1586.
24. Ostwald C, Gogacz P, Hillmann T, et al. p53 mutational spectra are different between squamous-cell carcinomas of the lip and the oral cavity. *Int J Cancer*. 2000;88:82-86.
25. Saunders ME, MacKenzie R, Shipman R, et al. Patterns of p53 gene mutations in head and neck cancer: full-length gene sequencing and results of primary radiotherapy. *Clin Cancer Res*. 1999;5:2455-2463.
26. Shima K, Kobayashi I, Saito I, et al. Incidence of human papillomavirus 16 and 18 infection and p53 mutation in patients with oral squamous cell carcinoma in Japan. *Br J Oral Maxillofac Surg*. 2000;38:445-450.
27. Buchkovich K, Duffy LA, Harlow E. The retinoblastoma protein is phosphorylated during specific phases of the cell cycle. *Cell*. 1989;58:1097-1105.
28. Dokiya F, Ueno K, Ma S, et al. Retinoblastoma protein expression and prognosis in laryngeal cancer. *Acta Otolaryngol*. 1998;118:759-762.
29. El-Naggar AK, Lai S, Clayman GL, et al. Expression of p16, Rb, and cyclin D1 gene products in oral and laryngeal squamous carcinoma: biological and clinical implications. *Hum Pathol*. 1999;30:1013-1018.
30. Ioachim E, Assimakopoulos D, Agnantis NJ, et al. Altered patterns of retinoblastoma gene product expression in benign, premalignant and malignant epithelium of the larynx: an immunohistochemical study including correlation with p53, bcl-2 and proliferating indices. *Anticancer Res*. 1999;19:541-545.
31. Koontongkaew S, Chareonkitkajorn L, Chanvitan A, et al. Alterations of p53, pRb, cyclin D(1) and cdk4 in human oral and pharyngeal squamous cell carcinomas. *Oral Oncol*. 2000;36:334-339.
32. Nakahara Y, Shintani S, Mihara M, et al. Alterations of Rb, p16(INK4A) and cyclin D1 in the tumorigenesis of oral squamous cell carcinomas. *Cancer Lett*. 2000;160:3-8.
33. Pande P, Mathur M, Shukla N, et al. pRb and p16 protein alterations in human oral tumorigenesis. *Oral Oncol*. 1998;34:396-403.
34. Pavelic ZP, Lasmar M, Pavelic L, et al. Absence of retinoblastoma gene product in human primary oral cavity carcinomas. *Eur J Cancer B Oral Oncol*. 1996;32B:347-351.
35. Regezi JA, Dekker NP, McMillan A, et al. p53, p21, Rb, and MDM2 proteins in tongue carcinoma from patients < 35 versus > 75 years. *Oral Oncol*. 1999;35:379-383.
36. Xu J, Gimenez-Conti IB, Cunningham JE, et al. Alterations of p53, cyclin D1, Rb, and H-ras in human oral carcinomas related to tobacco use. *Cancer*. 1998;83:204-212.
37. Scholnick SB, Sun PC, Shaw ME, et al. Frequent loss of heterozygosity for Rb, TP53, and chromosome arm 3p, but not NME1 in squamous cell carcinomas of the supraglottic larynx. *Cancer*. 1994;73:2472-2480.
38. Yokoyama J, Shiga K, Sasano H, et al. Abnormalities and the implication of retinoblastoma locus and its protein product in head and neck cancers. *Anticancer Res*. 1996;16:641-644.
39. Sartor M, Steingrimsdottir H, Elamin F, et al. Role of p16/MTS1, cyclin D1 and RB in primary oral cancer and oral cancer cell lines. *Br J Cancer*. 1999;80:79-86.
40. Lukas J, Parry D, Aagaard L, et al. Retinoblastoma-protein-dependent cell-cycle inhibition by the tumour suppressor p16. *Nature*. 1995;375:503-506.
41. Rasmussen UB, Wolf C, Mattei MG, et al. Identification of a new interferon-alpha-inducible gene (p27) on human chromosome 14q32 and its expression in breast carcinoma. *Cancer Res*. 1993;53:4096-4101.
42. el-Deiry WS, Tokino T, Velculescu VE, et al. WAF1, a potential mediator of p53 tumor suppression. *Cell*. 1993;75:817-825.
43. Jares P, Fernandez PL, Nadal A, et al. p16MTS1/CDK4I mutations and concomitant loss of heterozygosity at 9p21-23 are frequent events in squamous cell carcinoma of the larynx. *Oncogene*. 1997;15:1445-1453.
44. Danahey DG, Tobin EJ, Schuller DE, et al. p16 mutation frequency and clinical correlation in head and neck cancer. *Acta Otolaryngol*. 1999;119:285-288.
45. Matsuda H, Konishi N, Hiasa Y, et al. Alterations of p16/CDKN2, p53 and ras genes in oral squamous cell carcinomas and premalignant lesions. *J Oral Pathol Med*. 1996;25:232-238.
46. Olshan AF, Weissler MC, Pei H, et al. Alterations of the p16 gene in head and neck cancer: frequency and association with p53, PRAD-1 and HPV. *Oncogene*. 1997;14:811-818.
47. Shintani S, Nakahara Y, Mihara M, et al. Inactivation of the p14(ARF), p15(INK4B) and p16(INK4A) genes is a frequent event in human oral squamous cell carcinomas. *Oral Oncol*. 2001;37:498-504.
48. Sanchez-Cespedes M, Esteller M, Wu L, et al. Gene promoter hypermethylation in tumors and serum of head and neck cancer patients. *Cancer Res*. 2000;60:892-895.
49. Rosas SL, Koch W, Carvalho MG, et al. Promoter hypermethylation patterns of p16, O6-methylguanine-DNA-methyltransferase, and death-associated protein kinase in tumors and saliva of head and neck cancer patients. *Cancer Res*. 2001;61:939-942.
50. Agarwal S, Mathur M, Shukla NK, et al. Expression of cyclin dependent kinase inhibitor p21waf1/cip1 in premalignant and malignant oral lesions: relationship with p53 status. *Oral Oncol*. 1998;34:353-360.
51. Erber R, Klein W, Andl T, et al. Aberrant p21(CIP1/WAF1) protein accumulation in head-and-neck cancer. *Int J Cancer*. 1997;74:383-389.
52. Hirvikoski P, Kellokoski JK, Kumpulainen EJ, et al. Downregulation of p21/WAF1 is related to advanced and dedifferentiated laryngeal squamous cell carcinoma. *J Clin Pathol*. 1999;52:440-444.
53. Jeannon JP, Soames J, Lunec J, et al. Expression of cyclin-dependent kinase inhibitor p21(WAF1) and p53 tumour suppressor gene in laryngeal cancer. *Clin Otolaryngol*. 2000;25:23-27.
54. Kuo MY, Chang HH, Hahn LJ, et al. Elevated ras p21 expression in oral premalignant lesions and squamous cell carcinoma in Taiwan. *J Oral Pathol Med*. 1995;24:255-260.
55. Mineta H, Miura K, Suzuki I, et al. p27 expression correlates with prognosis in patients with hypopharyngeal cancer. *Anticancer Res*. 1999;19:4407-4412.
56. Ng IO, Lam KY, Ng M, et al. Expression of p21/waf1 in oral squamous cell carcinomas: correlation with p53 and mdm2 and cellular proliferation index. *Oral Oncol*. 1999;35:63-69.
57. Pruner G, Pignataro L, Carboni N, et al. Clinical relevance of expression of the CIP/KIP cell-cycle inhibitors p21 and p27 in laryngeal cancer. *J Clin Oncol*. 1999;17:3150-3315.
58. Ralhan R, Agarwal S, Mathur M, et al. Association between polymorphism in p21(Waf1/Cip1) cyclin-dependent kinase inhibitor gene and human oral cancer. *Clin Cancer Res*. 2000;6:2440-2447.
59. Tatemoto Y, Osaki T, Yoneda K, et al. Expression of p53 and p21 proteins in oral squamous cell carcinoma: correlation with lymph node metastasis and response to chemoradiotherapy. *Pathol Res Pract*. 1998;194:821-830.
60. van Oijen MG, Tilanus MG, Medema RH, et al. Expression of p21 (Waf1/Cip1) in head and neck cancer in relation to proliferation, differentiation, p53 status and cyclin D1 expression. *J Oral Pathol Med*. 1998;27:367-375.
61. Yook JI, Kim J. Expression of p21WAF1/CIP1 is unrelated to p53 tumour suppressor gene status in oral squamous cell carcinomas. *Oral Oncol*. 1998;34:198-203.
62. Yuen PW, Chow V, Choy J, et al. The clinicopathologic significance of p53 and p21 expression in the surgical management of lingual squamous cell carcinoma. *Am J Clin Pathol*. 2001;116:240-245.
63. Kudo Y, Takata T, Ogawa I, et al. Reduced expression of p27(Kip1) correlates with an early stage of cancer invasion in oral squamous cell carcinoma. *Cancer Lett*. 2000;151:217-222.
64. Tamura N, Dong Y, Sui L, et al. Cyclin-dependent kinase

inhibitor p27 is related to cell proliferation and prognosis in laryngeal squamous cell carcinomas. *J Laryngol Otol.* 2001;115:400-406.

65. Serrano M, Hannon GJ, Beach D. A new regulatory motif in cell-cycle control causing specific inhibition of cyclin D/CDK4. *Nature.* 1993;366:704-707.

66. Okami K, Reed AL, Cairns P, et al. Cyclin D1 amplification is independent of p16 inactivation in head and neck squamous cell carcinoma. *Oncogene.* 1999;18:3541-3545.

67. Akervall JA, Michalides RJ, Mineta H, et al. Amplification of cyclin D1 in squamous cell carcinoma of the head and neck and the prognostic value of chromosomal abnormalities and cyclin D1 overexpression. *Cancer.* 1997;79:380-389.

68. Capaccio P, Carbone N, Pignataro L, et al. Cyclin D1, p53, mdm2, and Ki67 protein expression in preneoplastic lesions of the larynx. *J Chemother.* 1997;9:113-114.

69. Kyomoto R, Kumazawa H, Toda Y, et al. Cyclin-D1-gene amplification is a more potent prognostic factor than its protein overexpression in human head-and-neck squamous-cell carcinoma. *Int J Cancer.* 1997;74:576-581.

70. Masuda M, Hirakawa N, Nakashima T, et al. Cyclin D1 overexpression in primary hypopharyngeal carcinomas. *Cancer.* 1996;78:390-395.

71. Pignataro L, Pruneri G, Carbone N, et al. Clinical relevance of cyclin D1 protein overexpression in laryngeal squamous cell carcinoma. *J Clin Oncol.* 1998;16:3069-3077.

72. Bellacosa A, Almadori G, Cavallo S, et al. Cyclin D1 gene amplification in human laryngeal squamous cell carcinomas: prognostic significance and clinical implications. *Clin Cancer Res.* 1996;2:175-180.

73. Callender T, El-Naggar AK, Lee MS, et al. PRAD-1 (CCND1)/cyclin D1 oncogene amplification in primary head and neck squamous cell carcinoma. *Cancer.* 1994;74:152-158.

74. Fortin A, Guerry M, Guerry R, et al. Chromosome 11q13 gene amplifications in oral and oropharyngeal carcinomas: no correlation with subclinical lymph node invasion and disease recurrence. *Clin Cancer Res.* 1997;3:1609-1614.

75. Gleich LL, Li YQ, Wang X, et al. Variable genetic alterations and survival in head and neck cancer. *Arch Otolaryngol Head Neck Surg.* 1999;125:949-952.

76. Meredith SD, Levine PA, Burns JA, et al. Chromosome 11q13 amplification in head and neck squamous cell carcinoma. Association with poor prognosis. *Arch Otolaryngol Head Neck Surg.* 1995;121:790-794.

77. Muller D, Millon R, Velten M, et al. Amplification of 11q13 DNA markers in head and neck squamous cell carcinomas: correlation with clinical outcome. *Eur J Cancer.* 1997;33:2203-2210.

78. Derynck R. The physiology of transforming growth factor- α . *Adv Cancer Res.* 1992;58:27-52.

79. Santini J, Formento JL, Francoual M, et al. Characterization, quantification, and potential clinical value of the epidermal growth factor receptor in head and neck squamous cell carcinomas. *Head Neck.* 1991;13:132-139.

80. Krecicki T, Jelen M, Zaleska-Krecicka M, et al. Epidermal growth factor receptor (EGFR), proliferating cell nuclear antigen (PCNA) and Ki-67 antigen in laryngeal epithelial lesions. *Oral Oncol.* 1999;35:180-186.

81. Wen QH, Miwa T, Yoshizaki T, et al. Prognostic value of EGFR and TGF- α in early laryngeal cancer treated with radiotherapy. *Laryngoscope.* 1996;106:884-888.

82. Rubin Grandis J, Chakraborty A, Melhem ME, et al. Inhibition of epidermal growth factor receptor gene expression and function decreases proliferation of head and neck squamous carcinoma but not normal mucosal epithelial cells. *Oncogene.* 1997;15:409-416.

83. Zhong Z, Wen Z, Darnell JE, Jr. Stat3: a STAT family member activated by tyrosine phosphorylation in response to epidermal growth factor and interleukin-6. *Science.* 1994;264:95-98.

84. Grandis JR, Drenning SD, Zeng Q, et al. Constitutive activation of Stat3 signaling abrogates apoptosis in squamous cell carcinogenesis in vivo. *Proc Natl Acad Sci U S A.* 2000;97:4227-4232.

85. Grandis JR, Zeng Q, Drenning SD. Epidermal growth factor receptor—mediated stat3 signaling blocks apoptosis in head and neck cancer. *Laryngoscope.* 2000;110:868-874.

86. Singh B, Gogineni S, Goberdhan A, et al. Spectral karyotyping analysis of head and neck squamous cell carcinoma. *Laryngoscope.* 2001;111:1545-1550.

87. Bockmuhl U, Wolf G, Schmidt S, et al. Genomic alterations associated with malignancy in head and neck cancer. *Head Neck.* 1998;20:145-151.

88. Li X, Lee NK, Ye YW, et al. Allelic loss at chromosomes 3p, 8p, 13q, and 17p associated with poor prognosis in head and neck cancer. *J Natl Cancer Inst.* 1994;86:1524-1529.

89. Villaret DB, Wang T, Dillon D, et al. Identification of genes overexpressed in head and neck squamous cell carcinoma using a combination of complementary DNA subtraction and microarray analysis. *Laryngoscope.* 2000;110(3 pt 1):374-381.

90. Cortesina G, Sacchi M, Galeazzi E, et al. Immunology of head and neck cancer: perspectives. *Head Neck.* 1993;15:74-77.

91. Grandis JR, Falkner DM, Melhem ME, et al. Human leukocyte antigen class I allelic and haplotype loss in squamous cell carcinoma of the head and neck: clinical and immunogenetic consequences. *Clin Cancer Res.* 2000;6:2794-2802.

92. Spafford ME, Koch WM, Reed AL, et al. Detection of head and neck squamous cell carcinoma among exfoliated oral mucosal cells by microsatellite analysis. *Clin Cancer Res.* 2001;7:607-612.

93. Gottschlich S, Folz BJ, Goerogeh T, et al. A new prognostic indicator for head and neck cancer—p53 serum antibodies? *Anticancer Res.* 1999;19:2703-2705.

94. Ralhan R, Nath N, Agarwal S, et al. Circulating p53 antibodies as early markers of oral cancer: correlation with p53 alterations. *Clin Cancer Res.* 1998;4:2147-2152.

95. Brennan JA, Mao L, Hruban RH, et al. Molecular assessment of histopathological staging in squamous-cell carcinoma of the head and neck. *N Engl J Med.* 1995;332:429-435.

96. Nathan CA, Sanders K, Abreo FW, et al. Correlation of p53 and the proto-oncogene eIF4E in larynx cancers: prognostic implications. *Cancer Res.* 2000;60:3599-3604.

97. Clayman GL, El-Naggar AK, Lippman SM, et al. Adenovirus-mediated p53 gene transfer in patients with advanced recurrent head and neck squamous cell carcinoma. *J Clin Oncol.* 1998;16:2221-2232.

98. Kirn D, Hermiston T, McCormick F. ONYX-015: clinical data are encouraging. *Nat Med.* 1998;4:1341-1342.

99. Ganly I, Kirn D, Eckhardt G, et al. A phase I study of Onyx-015, an E1B attenuated adenovirus, administered intratumorally to patients with recurrent head and neck cancer. *Clin Cancer Res.* 2000;6:798-806.

100. Nemunaitis J, Khuri F, Ganly I, et al. Phase II trial of intratumoral administration of ONYX-015, a replication-selective adenovirus, in patients with refractory head and neck cancer. *J Clin Oncol.* 2001;19:289-298.

101. Khuri FR, Nemunaitis J, Ganly I, et al. A controlled trial of intratumoral ONYX-015, a selectively-replicating adenovirus, in combination with cisplatin and 5-fluorouracil in patients with recurrent head and neck cancer. *Nat Med.* 2000;6:879-885.

102. Gleich LL. Gene therapy for head and neck cancer. *Laryngoscope.* 2000;110:708-726.

103. Gleich LL, Gluckman JL, Nemunaitis J, et al. Clinical experience with HLA-B7 plasmid DNA/lipid complex in advanced squamous cell carcinoma of the head and neck. *Arch Otolaryngol Head Neck Surg.* 2001;127:775-779.

104. Rocco JW, Li D, Liggett WH, Jr, et al. p16INK4A adenovirus-mediated gene therapy for human head and neck squamous cell cancer. *Clin Cancer Res.* 1998;4:1697-1704.

105. Clayman GL, Liu TJ, Overholt SM, et al. Gene therapy for head and neck cancer. Comparing the tumor suppressor gene p53 and a cell cycle regulator WAF1/CIP1 (p21). *Arch Otolaryngol Head Neck Surg.* 1996;122:489-493.

106. Robert F, Ezekiel MP, Spencer SA, et al. Phase I study of anti-epidermal growth factor receptor antibody cetuximab in combination with radiation therapy in patients with advanced head and neck cancer. *J Clin Oncol.* 2001;19:3234-3243.

107. Shin DM, Charuruks N, Lippman SM, et al. p53 protein accumulation and genomic instability in head and neck multistep tumorigenesis. *Cancer Epidemiol Biomarkers Prev.* 2001;10:603-609.