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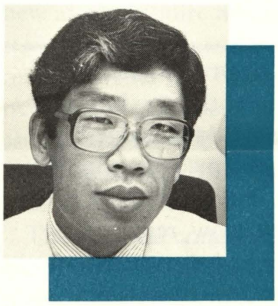
Chemotherapy for Brain Metastasis Sees Progress in the Last Decade



Jin S. Lee

Cancer researchers have traditionally had little confidence in chemotherapy for brain tumors. Because of the "blood-brain barrier," the consensus was that systemically administered agents would never reach the tumor or, if they did, only in such small quantities that the tumor would not respond.

Several reports over the past decade, however, suggest that the blood-brain barrier may not be so formidable.

"The existence of a blood-brain barrier does not necessarily mean that a blood-tumor barrier exists," said Jin S. Lee, M.D., Department of Medical Oncology, The University of Texas M. D. Anderson Cancer Center. "Even a brain tumor needs blood to survive, and if it is receiving a supply of blood from the general circulation, it stands to reason that systemic chemotherapy would reach the tumor."

Last year, Lee and his colleagues established just such a possibility in a report that showed that brain metastases in patients with small cell lung cancer do respond to chemotherapy. The study was important because it was "the first prospective study to demonstrate that chemotherapy given as initial treatment for metastatic small cell lung cancer could produce an objective tumor response in the brain," Lee said.

The key words are "initial treatment." Studies of other types of primary tumors have concluded that systemic chemotherapy reduces brain tumors. But though the results were highly suggestive, they were not conclusive because the patients had received or were receiving other therapy, such as radiation, corticosteroids, or intrathecal injections.

Some Studies Promising but Not Conclusive

In 1983, Dutzu Rosner, M.D., and his colleagues from the Roswell Park Memorial Institute gave 66 breast cancer patients with brain metastasis (42 of whom had been previously treated) various combinations of cyclophosphamide, 5-fluorouracil, methotrexate, vincristine, and prednisone. Thirty-four patients achieved objective tumor responses, but the addition of prednisone led some members of the research community to question the study. Since prednisone reduces swelling, it may have skewed the brain scan results.

The existence of a blood-brain barrier does not necessarily mean that a blood-tumor barrier exists.

Three years later, another promising study was published. Gordon Rustin, M.D., and his team at Charing Cross Hospital in London, England, treated 147 patients who had nonseminomatous germ cell tumors. Of the 10 patients who had brain metastases, eight were alive at the time of the report (1985). But because the regimen included intrathecal meth-

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otrexate, the efficacy of the drugs administered intravenously could not be established.

Though not conclusive, these investigations deserved attention. By the time of Rustin and coworkers' report, Lee and his team had already set out to evaluate the efficacy of systemic intravenous chemotherapy in metastatic small cell lung cancer. This cancer was chosen because of its chemosensitivity and because 10% of the patients have brain metastasis at initial diagnosis. Lee's study was the first "clean" study, since it examined patients who had not yet been treated and who were given no other concurrent treatment during the first three courses of intravenous chemotherapy.

Nine of Eleven Patients Achieve Objective Tumor Response

Fourteen patients were initially entered on the study. Eight patients had a single metastatic brain lesion, and six had multiple lesions, as documented by computed tomographic (CT) scans. Two died early because of sepsis, and one had radiation after the first course of chemotherapy when the primary lung lesion progressed. Eleven patients therefore were evaluable for response after initial chemotherapy with no other concurrent treatment.

The chemotherapy consisted of 600 mg/m^2 of cyclophosphamide and 50 mg/m^2 of doxorubicin on day 1, 60 mg/m^2 of etoposide on days 3 through 5, and 1.5 mg of vincristine on days 1 and 5, all of which were administered intravenously. Of the 11 evaluable patients, eight achieved a partial remission, defined as a 50% reduction in all measurable lesions for at least four weeks, and one achieved a complete remission, defined as the absence of all signs and symptoms of tumor for four weeks. The median survival was 34 weeks (range, 1 to 93), whereas for untreated patients it is generally less than eight weeks.

Of 11 patients, 8 achieved a partial remission.

"This study was important not only because it showed that systemic chemotherapy alone can induce the objective regression of metastatic brain lesions, but also because the regression occurred rather promptly after chemotherapy," Lee said. Such promptness in tumor regression, if corroborated by future studies, may force investigators to reevaluate the conventional methods of treating brain metastasis. Currently, most protocols rely on radiation.

Current Strategies Must Be Reevaluated

"Knowing that chemotherapy is effective, we can hold off on radiation treatment initially. We would want to order a CT scan immediately to determine if a metastatic brain lesion is present, but even if it is, we have the option of waiting to see if the chemotherapy will take care of the primary tumor and the brain metastasis," Lee said.

This treatment strategy may be better than current strategies for two other reasons, Lee said. First, radiation, when given concurrently with chemotherapy, increases the already significant myelosuppression caused by chemotherapy. And second, most patients also have disease in areas other than the brain, and though radiation can effectively induce local tumor responses of up to six months, it does not affect systemic disease. "Ultimately, cure will most likely require a systemic component," Lee said.

Radiation Exacerbates Myelosuppression

Radiotherapy's exacerbation of chemotherapy-induced myelosuppression has not always been acknowledged. Irradiation of less than 25% of the bone marrow generally was considered to be well tolerated, and because the skull contains less than 10% of the bone marrow, researchers believed that whole-brain irradiation had no significant myelosuppressive sequelae. This may have been true for radiation given alone, but Lee and his colleagues noticed that radiation seemed to increase the myelosuppressive effects of combination chemotherapy. In the early 1980s, Lee and his team set out to examine this issue, and their results, like those of their more recent study, challenged conventional thinking.

Their study of 48 patients with metastatic small cell lung cancer was published in 1986 and compared 24 patients who received three courses of combination chemotherapy (etoposide, cyclophosphamide, doxorubicin, and vincristine) with 24 patients who received the chemotherapy and concurrent irradiation (3000 rad in 10 fractions over two weeks).

They found that the irradiated patients indeed had more severe suppression of white blood cell counts and platelet counts and, not surprisingly, a higher incidence of infection. In addition, the increased severity of myelosuppression occurred in spite of the fact that 11 of the irradiated patients had to have chemotherapy dose reductions, as opposed to three in the control group.

Nevertheless, Lee by no means advocates eliminating radiation as therapy for brain metastasis. In the more recent

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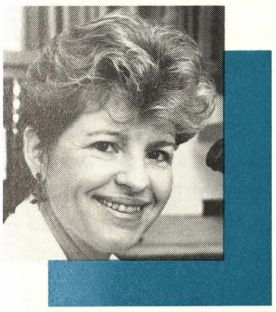
Immunocytochemical Assays for Steroid Receptors: A New Application for Fine Needle Aspiration



Ruth L. Katz

At The University of Texas M. D. Anderson Cancer Center, breast cancer is routinely diagnosed by examining tumor tissue aspirated by needle. If the tumor is malignant, then other assays, such as those for estrogen receptors, are performed. But until recently, determination of estrogen receptor status could only be performed on surgically obtained tissue. Though medically routine, an excisional biopsy is an additional stress for patients trying to cope with the diagnosis. Fortunately, new immunocytochemical assays for estrogen receptors make surgical biopsy unnecessary. Because the new assays require much less tissue than that needed for standard biochemical assays, they can be performed on tissue obtained by fine needle aspiration. Better still, the new assays may prove more accurate than the biochemical assays, said Ruth L. Katz, M.D., chief of the Section of Cytology at The University of Texas M. D. Anderson Cancer Center.

The medical community has long known that assays of estrogen receptor status provide useful prognostic and therapeutic information, said Katz. For more than 20 years, biochemical assays using radioimmunoassay methods have been the standard for determining estrogen receptor status. But the assays require 0.5 to 1 gram of tissue, which can only be obtained surgically. Moreover, though the assays help predict the potential for recurrence of stage I breast cancer, they are only 55 to 60% accurate in predicting a patient's response to hormonal therapy.

New Assay Requires Less Tissue

The estrogen receptor immunocytochemical assay (ERICA), however, can be performed on as few as 100 cells, which can easily be obtained by fine needle aspiration. In addition, one study suggests that the new assay may be a better predictor of disease-free survival than the biochemical assay, Katz said. "Whether ERICA is better than the biochemical assays in predicting response to hormonal therapy still has to be substantiated, but I think it will surpass the 55 to 60% accuracy of the biochemical assays."

Both types of assays identify the estrogen receptor, but each assay identifies a different aspect of the molecule. The biochemical assays determine the amount of radioactively labeled estradiol that binds to the active site of the receptor. Estrogen receptor status can then be quantified by tracking the radioactive estradiol. Though the assay is useful, it can be affected by endogenous estrogen production. If endogenous estrogen occupies the receptors, the labeled estradiol has no place to bind; thus estrogen receptor content may be underestimated. "Such is often the case in younger women," Katz said, "since they produce a lot of estrogen."

New immunocytochemical assays for estrogen receptors make surgical biopsy unnecessary in some cases.

Instead of determining the patient's estrogen-binding capacity, the immunocytochemical assay uses a monoclonal antibody, H222 Spγ, that identifies a different, nonactive part of the receptor located away from the site that actively binds the estrogen. Even if the active site is occupied, the monoclonal antibody is able to bind to the nonactive site. Because the immunocytochemical assay is relatively new, most studies have been designed to compare it with the biochemical assay. In making these comparisons, investigators focus on two measures: sensitivity (the probability that an estrogen receptor-positive tumor will be detected by the test) and specificity (the probability that an estrogen receptor-negative tumor will be negative by the test). A good test is both highly sensitive and specific.

"It is crucial that false negatives be minimized," Katz said, "since a test that incorrectly identifies the tumor as negative for estrogen receptors may preclude the patient from getting beneficial nontoxic hormonal or antiestrogen therapy. The extremely high specificity of ERICA ensures that hormonal or antiestrogen treatment can be correctly planned for an overwhelming majority of patients."

Though specificity and sensitivity of the immunocytochemical assay vary among institutions, they consistently

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compare favorably with the biochemical assays. At M. D. Anderson, the sensitivity on cytological specimens is 94%.

Aspiration Is Ideal for New Assay

Needle aspiration is ideal for obtaining tissue for the immunocytochemical assay. The procedure is routinely performed at the M. D. Anderson Fine Needle Aspiration Clinic, which is directed by Nour Sneige, M.D., associate director of cytology.

Aspiration may be preferable to surgical biopsy for a number of other reasons. First, the procedure is less expensive and easier to perform. Most patients undergo the procedure as outpatients. Second, aspiration can sample very small tumors for the estrogen receptor assay, and at the same time tissue can be procured for microscopy. Third, aspiration may be the only practical method available when tumor size or accessibility preclude surgical biopsy or if patients present with metastases that need to be investigated for the presence of the receptor. And finally, if the patient is to receive preoperative chemotherapy or irradiation, aspirates are an ideal means to determine estrogen receptor status before these therapies alter the biochemistry of the tumor or eradicate it altogether.

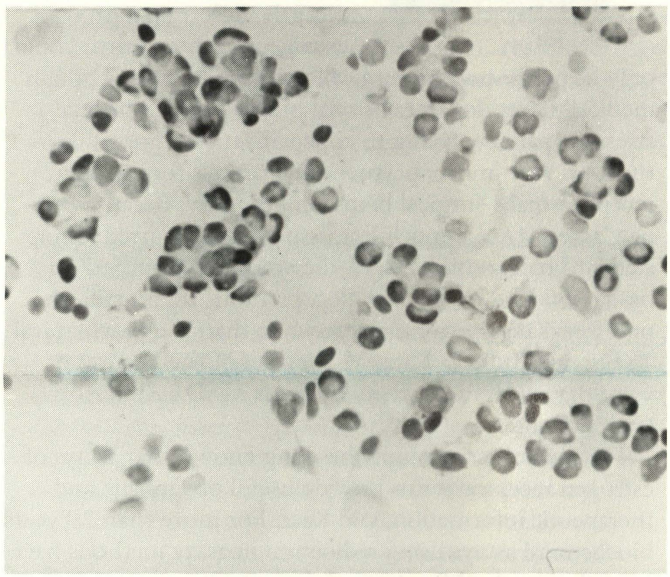
The immunocytochemical assay can be performed on as few as 100 cells.

Fine needle aspirates also allow multiple samplings of tumor, which, according to Katz, is an important advantage because breast cancer is morphologically and biochemically heterogeneous. By sampling multiple areas, the pathologist can get a more accurate sense of the distribution of estrogen-binding cells in the tumor. Such is not the case with the biochemical assays, which require the tissue sample to be homogenized before processing; consequently, any attempt at identifying the source and distribution of cells is very difficult.

Aspirates Less Likely To Allow Receptor Degradation

An additional advantage is that aspirates can be processed immediately after procurement, whereas tissue procured from surgical specimens such as mastectomies may be devascularized for up to 45 minutes during surgery before being sent to the pathologist. "Since estrogen receptors are very labile, the timeliness of processing is very important," Katz said. "Even if the pathologist processes a mastectomy specimen immediately, the estrogen receptors may still have undergone some degradation during the period of devascularization."

Receptor degradation can also be prevented by immediately freezing the surgical biopsy. "Before fine needle aspiration, frozen section diagnosis would be made during surgery. If the tumor was malignant, frozen-block tissue



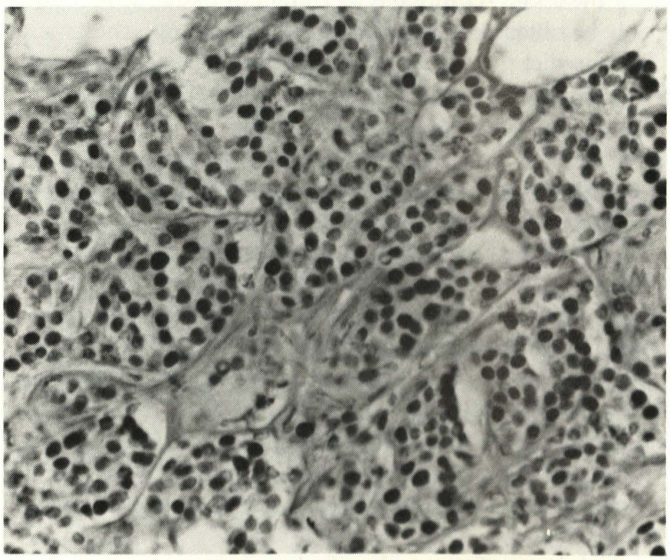


Figure 1. Breast cancer tissue stained with a monoclonal antibody that identifies estrogen receptors. Cells are positive for the receptors, as indicated by the dark nuclear staining. Top, fine needle aspirate. Bottom, histological section.

would be submitted to the pathologist for estrogen receptor assays; hence, in that situation the tissue would be fresh and receptor degradation would be minimized," Katz said.

At M. D. Anderson, however, frozen section diagnosis is less frequently used. "In years past, patients would have a general idea of the diagnosis based on clinical and radiographic findings, but a definitive diagnosis would have to be made during surgery. The patient would undergo the operation with the understanding that, if the tumor were malignant, then a mastectomy would be performed. With fine needle aspiration, the patient doesn't have to be subjected to surgery without a definitive diagnosis."

Assay Also Used for Histology

Researchers at the M. D. Anderson Cancer Center are focusing their efforts on refining the estrogen-receptor immunocytochemical assay. (M. D. Anderson is currently implementing a similar test for progesterone receptors.) The assay can be applied to histological sections of tumor, as well as to the whole-cell aspirates examined cytologically. Katz and her colleagues, in a study of 55 patients published in the March-April issue of *Breast Cancer Research and Treatment* (15:103-115, 1990), for the most part confirmed the immunocytochemical estrogen-receptor results of other investigators. However, in contrast to other studies, Katz and her team found that sensitivity and specificity of the assay performed on cytological material (94% and 100%, respectively) were higher than those obtained using cryostat (histological) sections (67% and 90%, respectively). In most previous studies, the sensitivity and specificity of the histological sections were equal to or higher than the cytological aspirates.

Fine needle aspirates allow multiple samplings of tumor.

"The surprising thing initially was that the cytology was more sensitive; however, after reviewing the literature, we found several reports in which even the biochemical assays, if performed promptly on biopsies, resulted in higher estrogen receptor values than if the same tissue was procured following mastectomy," said Katz.

Since estrogen receptors are very labile, the timeliness of processing is very important.

She emphasized that because of the small study sample, the results can only be considered preliminary, but if the data are confirmed, then immunocytochemical analysis of aspirates may emerge as the diagnostic procedure of choice.

Continued Research on Prognostic Factors Needed

Katz is encouraged by the developments in fine needle aspiration and the immunocytochemical assay, but she stressed that prognostic assay research has a long way to go. "In addition to estrogen receptors, a lot of work is being done nationwide on other prognostic factors, like the *neu* oncogene, the multidrug resistance gene, progesterone receptors, flow cytometrically derived ploidy, and proliferative indices, but long-term studies will have to be performed before we can tell whether they mean anything, since a significant percentage of breast cancer patients may live from 10 to 15 years after diagnosis and still have recurrence. Nevertheless, as far as fine needle aspiration's involvement in performing these assays is concerned, the writing is on the wall. It's here to stay." ■

Aspiration can sample very small tumors.

Physicians who desire additional information may write Ruth L. Katz, M.D., Department of Pathology, Box 85, The University of Texas M. D. Anderson Cancer Center, 1515 Holcombe Boulevard, Houston, Texas 77030, or call (713) 792-3140.

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between placebo and therapy effects.

The clinical reversal of leukoplakia by 13-*cis*-retinoic acid was the most important finding, but in a more general context the histologic findings ultimately are more important, Hong said, for they can "show the degree of dysplasia—severe, moderate, mild—or hyperplasia, with dysplasia being more serious than hyperplasia. This information is important when predicting whether a lesion will progress to squamous cell carcinoma."

Though encouraged by the treatment's effectiveness, Hong and his colleagues still had to address one obstacle: duration of remission. After therapy stopped, lesions recurred in nine patients.

Maintenance Therapy Prevents Relapse

The relapses indicated that the vitamin A analogue would have to be given in a long-term chemoprevention regimen. To that end, Hong and his team, in an ongoing study, have treated 55 patients. As in the above-mentioned study, patients received 1 to 2 mg/kg/day of 13-*cis*-retinoic acid for three months. "This was quite effective in the first study," Hong said, "except that the remissions achieved did not last after therapy was halted. So we considered how we could maintain these remissions."

The researchers added nine months of maintenance therapy consisting of low-dose retinoic acid (0.5 mg/kg/day). They also administered 15 to 30 mg/day of beta-carotene to another group of patients, since "some reports indicated that beta-carotene was as effective as retinoic acid against leukoplakia. And the beauty of beta-carotene is that it has no side effects, except for inducing some yellowing of the skin," said Hong.

Retinoic Acid versus Beta-carotene

From results with the first 20 patients who completed the three-month induction and nine-month maintenance therapy, "we found that low-dose retinoic acid appears to be more effective than beta-carotene in terms of maintaining remission," Hong said. So far, no relapses have been seen among the nine patients who have completed maintenance therapy with the low-dose retinoic acid, whereas 6 of 11 who completed maintenance therapy with beta-carotene have had leukoplakia relapses. Side effects from low doses of the vitamin A analogue are fairly minimal and at least tolerable, he said.

The advantage of using an analogue of vitamin A is that the analogue is less toxic to the liver than is vitamin A itself. The major toxicity associated with retinoic acid is to the skin, Hong said. The mucocutaneous and lipid toxicities seen with the higher dose induction therapy were significantly lower during maintenance therapy, and the side effects of low-dose therapy were mild, reversible, and similar to those associated with beta-carotene. Some research has been done with other vitamin A analogues, including tretinoin and etretinate, but the availability of these often is limited.

Most leukoplakia patients are men, by a 3-to-1 margin. That made it something of a surprise, Hong indicated, that women account for 35 of the first 55 patients in the current M. D. Anderson trial. Hong was not sure why more women than men were enrolled in this particular trial. The 12-month study, which Hong called an intermediate-term investigation, should be completed by year's end. He noted the need for additional longer-term evaluation of low-dose vitamin A analogue administration, as vitamin A toxicity is cumulative.

Low-dose retinoic acid appears to be more effective than beta-carotene.

Lessons in Reversing Carcinogenesis

In addition to treating leukoplakia itself, "our goal is to reverse carcinogenesis and to discover how retinoic acid effects that reversal. Retinoic acid can reverse the carcinogenic process, but how? We are looking at some specific biomarkers—K1 keratin, transglutaminase, involucrin (a differentiation marker), and micronuclei (genotoxic markers), as well as proliferating cell nuclear antigen."

Micronuclei, which can be measured in tissue scrapings, are formed from chromosome fragments in proliferating cells and are created by such events as carcinogenic damage. Elevated counts appear to correlate with cancer risk in oral and other cancers. The low-dose retinoic acid maintenance therapy in Hong and colleagues' ongoing study further reduced micronuclei counts already lowered by the induction therapy in three of nine patients, whereas beta-carotene did not, according to a preliminary analysis by Scott Lippman, M.D., and colleagues at M. D. Anderson.

The measurement of drug effects may be confounded by dietary factors. In fact, in some countries, dietary deficiencies are a major cause of leukoplakia. Hong noted that whether some patients have better outcomes because they eat more broccoli, for example, or take vitamin supplements is not known, although he cautions against routine use of vitamin A supplements because of the vitamin's cumulative toxicity.

Leukoplakia usually is caught early, often during routine dental exams, Hong said. Research aimed at heading it off or reversing it with retinoic acid, other vitamin A analogues, or other micronutrients such as beta-carotene likely will also shed light on how squamous cell carcinomas develop, and may eventually provide clues to preventing or reversing this cancer. ■

Physicians who desire additional information may write Waun Ki Hong, M.D., Box 80, Department of Medical Oncology, The University of Texas M. D. Anderson Cancer Center, 1515 Holcombe Boulevard, Houston, Texas 77030, or call (713) 792-6363.

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study, even though chemotherapy was the primary focus, all patients received radiation after the third course of chemotherapy. In fact, objective tumor regression was observed after whole-brain irradiation in all three patients whose disease progressed during the initial chemotherapy period.

"Since we know that certain cancers have a high probability of metastasizing to the brain, whole-brain irradiation can be used prophylactically. By and large, radiation still has a very important place in treatment," Lee said.

We're still a long way from establishing a truly effective systemic treatment for brain tumors.

More Effective Chemotherapy Is Needed

The challenge at this point is to identify an even more effective yet less toxic chemotherapeutic regimen. If such a treatment can be identified, it may be possible to delay or even omit radiation therapy to the brain, thereby reducing the risk of severe myelosuppression and infection morbidities. To this end, Lee and his team are evaluating a combination of vincristine, etoposide, cisplatin, and cyclophosphamide. (They have dropped doxorubicin because of its cardiotoxicity.)

"We still need to analyze the data," Lee said. "My feeling is that this protocol is as effective as previous ones, but we're still a long way from establishing a truly effective systemic treatment." If such a regimen is identified, then researchers can safely place the blood-brain barrier behind them and begin to focus on new challenges in the treatment of cancer patients. ■

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Researchers Hope Chemoprevention Will Reverse Tumorigenic Process

Waun Ki Hong

Unlocking the secret of how retinoic acid prevents leukoplakia, a squamous cell carcinoma precursor, may well reveal a means of reversing carcinogenesis, said Waun Ki Hong, M.D., who helped establish that the vitamin A analogue actually works to prevent or reverse leukoplakia.

Hong is chief of the Section of Head, Neck, and Thoracic Medical Oncology at The University of Texas M. D. Anderson Cancer Center. He and his colleagues are trying to refine a novel chemoprevention strategy for the precancerous lesions. Leukoplakia progresses to oral squamous cell carcinoma in 5 to 15% of patients from 1 to 20 years after it appears, and it spontaneously regresses in only 10%. The lesion affects 6 to 8% of Americans, is usually asymptomatic, and most often is caused by tobacco smoking or chewing or, less often, by alcohol abuse. Spontaneous regression or risk reduction may occur in tobacco users after they kick the habit.

Investigators have been looking for an alternative to surgery, the traditional treatment, for several reasons: The lesions often recur, and surgery is impractical when the condition spreads across the inner surface of the lip or through other areas of the mouth in multiple lesions. In 1986, Hong and his colleagues reported a trial in which they examined an alternative treatment: chemoprevention with the vitamin A analogue 13-*cis*-retinoic acid (*New England Journal of Medicine* 315:1501-1505).

Twenty-four patients who received the vitamin A analogue (1 to 2 mg/kg/day) were compared with 20 patients who received a placebo. Sixteen of the treated patients (67%) achieved a complete (2 patients) or partial (14 patients) objective clinical response, compared with two patients in the placebo group (10%), who each achieved a partial response. Histologic analysis of pre- and posttreatment biopsy specimens revealed dysplasia reversal in 13 treated patients and in 2 patients who received the placebo. The trial was halted early because of the striking differences

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