Cellular Studies Guide Scheduling of Ara-C Treatment for Patients with Acute Leukemia

Leukemia cell reactions to high-dose cytarabine (Ara-C) infusion may soon be used to direct treatment in patients with acute leukemia at UT MDAH, according to results of a recent investigation conducted by William K. Plunkett, Jr, PhD, Elihu H. Estey, MD, Michael J. Keating, MD, and Emil J Freireich, MD, all of the Department of Developmental Therapeutics.

The purpose of the investigation was to determine, at the cellular level, the basis for success or failure of high-dose Ara-C treatment so that this information might be used to improve treatment results. As Dr Plunkett explained, "We wanted to determine the cellular pharmacokinetic parameters important to clinical response to maximize the likelihood of attaining those favorable characteristics in treating patients."

Investigators followed the metabolism of Ara-CTP, the active metabolite of the prodrug Ara-C, in the leukemia cells of patients during treatment. Although a single mechanism for its cytotoxicity was not discovered, Ara-CTP was found to inhibit directly the synthesis of DNA, thereby causing chromosomal aberrations and breaks. To determine the clinical implications of this cellular activity, Ara-CTP concentrations in leukemia cells of individual patients, as well as degrees of leukemia cell DNA synthesis after high-dose Ara-C administration, were correlated with clinical results. From these correlations, researchers determined the minimum level of Ara-CTP concentration required to produce cytotoxic activity sufficient for response. They were then able to alter treatment schedules for selected patients to maintain this concentration for the duration of therapy.

The study involved 34 evaluable patients suffering relapse after treatment with combination chemotherapy, which included low-dose Ara-C. Concentrations of Ara-CTP in leukemia cells drawn from the bone marrow and blood of each patient were measured at various intervals, using high-pressure liquid chromatography, during and after high-dose Ara-C administration (3g/m² of body surface area over 2 hours every 12 hours for nine doses). These measurements were then correlated with treatment results, determined by bone marrow biopsy and blood tests beginning approximately four weeks after treatment.

Potent Noncardiotoxic Drug Proves Effective for Metastatic Breast Cancer

by Hwee-Yong Yap, MD, Department of Internal Medicine; Boh-Seng Yap, MD, Department of Developmental Therapeutics; George R. Blumenschein, MD, Department of Internal Medicine; Brian C. Barnes, MD; Frank C. Schell, MD; and Gerald P. Bodey, MD, Department of Developmental Therapeutics

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A recent phase II study at UT MDAH suggests that bisantrene [CL 216, 942; 9,10-anthracenedicarboxaldehyde bis(4,5-dihydro-1H-imidazole-2-yl) hydrazone] dihydrochloride, a new anthrancene compound, may be as effective as doxorubicin for treating patients with metastatic breast cancer but does not exhibit the same potential for cardiotoxicity. Our findings indicate that bisantrene, without this dose-limiting side effect, may prove to have great therapeutic value for patients with metastatic breast cancer.

We studied forty-four such patients between 29 and 74 years of age. All had measurable or evaluable metastatic disease and had previously been treated with conventional chemotherapeutic agents, including combination chemotherapy with doxorubicin.

The duration of prior systemic therapy was 3 to 73 months (median 27 months), and the number of prior therapeutic regimens ranged from one to eight (median three).

We chose a single-dose schedule of 250 mg/m² of body surface area administered every three weeks. Patients with poor bone marrow reserve or bilirubin greater than 2 mg/dl but less than 5 mg/dl were given a 220 mg/m² dose. Because the myelosuppression associated with the 250 mg/m² dose was mild to moderate, the starting dose of bisantrene was subsequently escalated to 300 mg/m² for the last 14 patients in the study.

Prior to therapy, all patients had adequate blood cell counts (absolute granulocyte counts equal to or greater than 1500/mm³ and platelet counts greater than 100,000/mm³). Blood cell counts, differential counts, and platelet counts were obtained before therapy and measured at weekly intervals after therapy. Blood chemistry profiles (including serum creatinine, blood-urea nitrogen, and liver function tests), tumor measurements, and appropriate radiologic and radionuclide studies were obtained prior to therapy and were repeated at least every three to nine

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weeks after therapy.

We defined partial response as a 50% or greater decrease in the sum of the products of the two largest diameters of each lesion without simultaneous increase in the size of any lesion or the appearance of new lesions. Evidence of blastic repair of previously known lytic lesions indicated response in the bone. Partial response in the liver was defined as either a 3-cm reduction in hepatomegaly, a 50% reduction in the size of the portion of the enlarged liver below the costal margin in both midclavicular and epigastric lines, or a substantial improvement in liver scan, ultrasound, or computed tomographic scan results. An unequivocal increase of at least 25% in the size of any major lesion or the appearance of new lesions was the criterion for increasing disease. Stable disease was defined as a steady state of response but less than that considered to be partial response, or progression less than that of increasing disease, for a minimum of eight weeks. Inclusion in the stable disease category required that the patient have no new lesions.

Of the 44 patients, 4 were considered inevaluable for response. One patient died unexpectedly within the first 14 days after initiation of chemotherapy from a presumed pulmonary embolism, and three patients had inadequate trials. All three patients had received only one course of bisantrene and were either without evidence of progressive disease or did not return for reevaluation. Among the 40 evaluable patients, 9 had partial response, yielding a response rate of 22%. Disease was stable in 19 patients and progressive in 12 patients in spite of treatment. The median time from the onset of bisantrene therapy to evidence of progressive disease for responding patients was 28 weeks (range 22 to 39 or more weeks). This median time is significantly better ($P = 0.02$) than that of patients with stable disease, who had a median time to disease progression of 16 weeks (range 8 to 26 or more weeks). The median time to progression for all patients in the increasing disease category was six weeks. Responses were seen in all major sites of disease including the chest wall, lymph nodes, bone, liver, and lung. The median time from the onset of bisantrene therapy to response was 6 weeks (range 3 to 12 weeks).

Although patients who had responded to prior doxorubicin therapy or those not known to be refractory to prior doxorubicin therapy were more likely to respond to bisantrene, several patients refractory to prior doxorubicin therapy or who had previously failed to exhibit a response to doxorubicin showed response to bisantrene.

Myelosuppression, principally granulocytopenia, was the most frequent toxic effect of bisantrene. Other side effects included mild nausea and vomiting in 20% to 30% of patients. General malaise and low-grade fever occurred in 20% of patients with documented infection. One patient suffered a urinary infection.
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Correlations indicated that cellular retention of Ara-CTP was of primary importance in the achievement of complete remission (Figure 1a), Dr Plunkett said. According to clinical test results, 11 patients achieved complete remission, 11 developed hypoplastic bone marrow but soon regrew leukemia cells, and 12 failed to respond. Seven of 11 patients who achieved complete remission retained greater than 75 µM Ara-CTP over a 12-hour period. Of the 23 patients with resistant disease, only three were able to maintain this level of Ara-CTP concentration over 12 hours.

These findings were used to establish the cellular pharmacokinetic parameters that significantly correlated with clinical results: 1) The total cellular exposure to Ara-CTP, 2) the rate of drug degradation or the ability of leukemia cells to retain Ara-CTP, and 3) the cellular Ara-CTP concentration at 12 hours just before administration of a subsequent dose. Because all patients attained substantial peak cellular concentrations of Ara-CTP (33 to 1300 µM), no correlation was found between clinical results and peak cellular concentration.

Investigators then determined the level of Ara-CTP cellular concentration required during treatment to sustain cytotoxic activity, or inhibition of DNA synthesis. Measurements of extent of inhibition of DNA synthesis in 17 of the 34 original patients were made before and at two-hour intervals after treatment by separating leukemia cells from the blood and pulse treating the cells in vitro with [H]thymidine. Measurements before and after treatment were then compared. Twelve hours after high-dose Ara-C infusion, DNA synthesis recovered to greater than 10% of pretreatment levels in 10 of 11 patients who subsequently failed to respond; DNA synthesis was inhibited to greater than 90% of pretreatment values in four of six patients who achieved complete remission (example, Figure 1b).

When these results were coordinated with correlations of Ara-CTP retention and clinical response, the investigators found that a critical Ara-CTP cellular concentration of approximately 75 µM should be maintained in leukemia cells throughout treatment to ensure maximum cytotoxicity and to enhance clinical response.

Researchers attempted to maintain this level for the duration of therapy in four patients by altering the 12-hour interval between Ara-C infusions. "In each patient, we changed the interval between doses to maintain the successful DNA inhibitory concentration of Ara-CTP in the cells," Dr Estey explained. Treatment schedules were tailored to each patient after a single test dose of Ara-C (3g/m² of body surface area over two hours) was administered and cellular Ara-CTP retention measured. In accordance with these measurements, the interval between drug dose administration was altered to every 8 hours in two patients, every 10 hours in one patient, and every 36 hours in another patient who had exceptionally high Ara-CTP cellular retention. In three of the four patients, adequate levels of Ara-CTP were maintained. To date, one patient (on the 36-hour-interval schedule) has achieved a complete remission, according to Dr Estey.

Although the hypothesis that cellular pharmacokinetic studies might be used to improve the results of treatment is not new, new techniques for measuring cellular activity, a suitable patient population, and collaboration between researchers and clinicians have enabled investigators to test this hypothesis. According to Dr Plunkett, "All the elements have come together. Now we can determine the cellular pharmacokinetic characteristics of a particular patient and have those characteristics mean something to the subsequent treatment of the patient."

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Factors Affecting Accurate Reading Of

by Herbert A. Fritsche, Jr, PhD, Chief, Section of Clinical Chemistry, Department of Laboratory Medicine

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Accurate interpretation of tumor marker test results is essential when these tests are used to aid in the diagnosis and monitoring of patients with cancer. Such interpretation requires a thorough knowledge of the production and clearance factors of these substances in the body, as well as other variables that may affect tumor marker levels. Unfortunately, this type of information is not always known, even for the most well-studied markers, and must often be obtained from clinical experience with the tumor marker test.

Because the carcinoembryonic antigen (CEA) is the most widely used tumor marker, it is presented as the primary example to illustrate those factors that must be considered to insure the accurate clinical interpretation of tumor marker tests. Considerations presented here in the interpretation of the CEA test are applicable to other markers as well and should be included in the clinical evaluation protocols of all newly developed tumor marker tests.

Tumor Markers in Diagnosis

The limitations of the CEA test for establishing an early diagnosis of entodermal cancer are well known. The serum or plasma CEA level is not always elevated in patients with these cancers. Only 40% to 60% of patients with adenocarcinomas of the gastrointestinal tract, breast, and lung will have an elevated plasma CEA value. Therefore, a negative CEA test does not rule out the presence of neoplastic disease. In addition, elevated CEA levels are more frequently observed in the circulation of patients with extensive disease than in patients with limited disease. In patients with metastatic disease, high CEA levels are often present, and in those with liver or bone metastases CEA levels are highest. In patients with localized disease, however, CEA levels generally are not significantly elevated. Therefore, the use of CEA for establishing an early diagnosis of cancer is not warranted.

Elevated CEA levels observed in the circulation of patients with noncancerous disease may again cause misinterpretation of test results. High CEA levels have been reported in patients with a variety of inflammatory and benign disorders, such as viral infections. Generally, the CEA elevations that occur in nonmalignant disorders do not exceed four to five times the upper limit of normal. A CEA value greater than this value would be suggestive of cancer. Also, the rise in the CEA level seen in noncancerous disorders is generally accompanied by a fall in CEA levels after the acute phase of the disease. Therefore, a false-positive CEA value in detecting cancer can sometimes be readily identified by this transient elevation. Significantly elevated CEA levels that continue to rise in serial samplings are highly suggestive of neoplastic disease.

The diagnostic limitations of the CEA test are observed with other tumor markers as well. For example, elevation of prostatic acid phosphatase (PACL) levels in the serum of patients with prostatic cancer occurs in 65% to 92% of patients with bone metastasis, in 10% to 40% of patients with other metastatic sites, and in only 5% to 10% of patients with localized disease. Also, PACP elevations may occur in nonprostatic malignancies and benign disorders of bone. False-positive PACP values produced by nonmalignant diseases may result from the lack of test specificity, causing the measurement of some other nonprostatic acid phosphatase. Similar crossreaction problems have been observed with the beta chain human chorionic gonadotrophin (B-HCG) assay when B-HCG has been used as a tumor marker for germ cell tumors of the testis and ovary. A recent report indicates that the crossreaction of the luteinizing hormone (LH), even with beta-chain-specific antiserum, may cause falsely elevated values for B-HCG.

It should also be noted that while many tumor tissues may actually contain significant amounts of a marker such as CEA or B-HCG, either these substances are not secreted by the tumor or the marker is rapidly cleared and undetectable in the circulation. In any case, the high false-positive and false-negative rates in testing for current tumor markers severely limit their use for diagnostic purposes.
Detection of Recurrent Disease

While many would agree that serial tumor marker testing is useful in patient monitoring, there is no general consensus with regard to the optimal frequency of such tests. This may depend on the clinician's experience with the marker, as well as the type of tumor, status of the patient, current therapy, and metabolic decay rate of the marker.

It is generally agreed, though, that a marker baseline value should first be established for each patient to be monitored and that subsequent marker values be interpreted with respect to the baseline values. This type of pattern or trend analysis helps to distinguish a significant change in the marker value from the expected variation that is related to the test precision.

When tumor markers are used for the detection of recurrent disease, the run-to-run precision of the tumor marker test at values near and below the upper limit of normal should be determined. A significant change of the marker, i.e., a change greater than two times the standard deviation, will identify those patients who require closer follow-up examination. This may consist of more frequent marker testing or further diagnostic workup.

The usefulness of CEA for the early detection of disease recurrence is demonstrated in Figure 1. This is the CEA pattern of a breast cancer patient with choroidal, pleural, and pericardial metastases who responded to chemotherapy and was in complete remission during the period of June 1976 through November 1977. During this period, the CEA values remained constant at 6.0 ng/ml. By June 1978, the CEA value had risen to 20 ng/ml, but due to the lack of any other clinical signs and symptoms, the patient was determined to be in complete remission. At the next follow-up visit in December 1978, the CEA value was 54.0 ng/ml, and an abdominal recurrence was observed. In this patient, the rise of the CEA level indicated the recurrence of disease six months before it became clinically evident. This CEA pattern emphasizes the necessity of following a monitoring protocol as opposed to collecting follow-up samples on an irregular basis. Had blood samples been collected from this patient on a monthly basis, it is highly likely that the rise of the CEA level would have been detected during the period of November 1977 through June 1978. Certainly, the significant change in the CEA level observed in June 1978 should have identified this patient for additional diagnostic workup before January 1979.

Figure 2 shows the usefulness of pattern analysis in identifying a false rise of CEA levels related to an inflammatory process. The transient rise in CEA level in this breast cancer patient, who was on maintenance chemotherapy, correlated with a severe influenza infection. The CEA values returned to the baseline level with subsidence of the influenza symptoms.

Figure 3 emphasizes the fact that not all patients with recurrent or progressive disease will demonstrate CEA abnormalities. This breast cancer patient had extensive bone metastases and a recurrence to the chest wall and never demonstrated a significant change in CEA level. This patient did not have an elevated CEA value before therapy was initiated, which raises the question as to the usefulness of monitoring patients with previously negative CEA levels.

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CEA values. Experience suggests that only a few patients who have not previously demonstrated elevated CEA levels with their primary disease will benefit from monitoring with this marker. However, recent data from other groups suggest that as many as 50% of patients in this category may have elevated CEA levels associated with the recurrence of their disease.

Slope analysis of serial CEA data has been suggested as a method for differentiating local tumor recurrence from metastatic spread of disease. While several investigators have confirmed that a faster rate of CEA increase is more closely associated with a metastatic process than with a local recurrence, caution must be exercised in the interpretation of these slope data. In the case of CEA, decreased clearance resulting from hepatobiliary disease may cause the serum level of the CEA to rise, thereby implying a faster rate of production than that actually present. Such a situation is demonstrated in Figure 4. This breast cancer patient with an initially elevated CEA level responded to therapy. The falling CEA values indicated the response to the chemotherapy until January 1979, when the CEA value increased significantly and bone roentgenograms were suggestive of metastatic disease. During the following two months, liver metastases became apparent, and the serum CEA values were markedly elevated. The change in the slope of the CEA pattern was significantly greater during the month of March 1979, when the bilirubin values were elevated, than in the previous month. In this case, it is highly likely that the serum level in March reflected both CEA production and decreased clearance.

Fig. 4. CEA pattern of a breast cancer patient demonstrating elevated CEA level resulting from progressive disease and decreased clearance due to impaired liver function. (PLE FF = pleural effusion; BILI = total bilirubin; Tamox = tamoxifen; FAC = 5-fluorouracil, Adriamycin, cyclophosphamide; CMF = cyclophosphamide, methotrexate, and 5-fluorouracil.)

It is obvious that clinical experience with the CEA test for the early detection of recurrent disease varies from one institution to the next. The type of patient being monitored, the timing of follow-up blood collections, and the criteria used to define a significant change in CEA values are all critical factors affecting the usefulness of this marker for the detection of recurrent disease.
reason for this apparent physiologic variation is not known. It has also been observed that some patients may have considerable variation in daily marker values during therapy. When this type of variation occurs, clinical interpretation of single point determinations are meaningless, and the necessity for assessment of the marker trend is emphasized.

Since patients with active disease may have marker values ranging from borderline to greatly elevated, the test method must be capable of providing an accurate test value with known precision over the entire range of values that can be encountered. Again, using the CEA pattern shown in Figure 4, it can be seen that the decrease from 600 to 480 ng/ml in June 1978 and the following rise from 100 to 400 ng/ml in January 1979 were both clinically significant changes. Therefore, there is a need for accurate and precise quantitation of greatly elevated marker values.

The CEA pattern shown in Figure 4 also demonstrates a paradox increase in the CEA level during the month of May 1978. That is, the CEA values increased while the patient responded to therapy. A transient increase in the CEA level may result from the release of tumor cell contents into the circulation as a result of cell death or drug-related membrane damage. The chemotherapy-induced peak appears to vary from one patient to the next, in that the rise in CEA level may occur after one or more courses of chemotherapy and last for a variable period. The CEA values may have lasted for at least four months in this breast cancer patient. When the CEA was at its highest value, the lung scans showed significant improvement in the lung metastases.

The paradox rise in CEA level can be differentiated from a rise associated with progressive disease, since its occurrence correlates with the start of therapy; in addition, the paradox rise in CEA level is generally greater than that observed with metastatic spread of the disease. A chemotherapy-induced peak has also been reported for B-HCG and alphafetoprotein (AFP) during therapy of patients with testicular cancer. Patients subjected to radiation therapy may also demonstrate a transient rise in CEA levels caused by cell death. The radiation-induced rise in CEA levels may occur soon after therapy is initiated and last for the duration of therapy.

False-negative tumor marker values also present a problem when markers are used to assess therapeutic response. When patients are responding to therapy but are not yet in complete remission, marker values may fall to constant baseline values within the normal range. In some patients, this false-negative response may be due to the reduction of tumor mass to a size not capable of significant marker production. In other cases, tumor mass may still be quite large and clinically evident. In these cases, tumor heterogeneity appears to be the basis for the false-negative phenomenon. In germ cell tumors, in which the tumor markers HCG and AFP are associated with specific cell types, discordant behavior of the markers can be explained on the basis of the sensitivity of selected cell types to the chemotherapeutic agent. It has not yet been firmly established that various cell types of adenocarcinoma with differing capabilities for marker production have varying sensitivities to chemotherapeutic agents. However, such tumor heterogeneity could explain the false-negative results obtained in tests for PAcP and CEA that are observed during the chemotherapy of prostatic cancer and breast cancer patients, respectively.

Finally, when the CEA test is used to monitor patients after surgery, it is recommended that the first follow-up value be determined two to four weeks after surgery to avoid false interpretation of the postsurgical transient CEA rise associated with the healing process. A CEA follow-up study of patients after complete removal of their gastrointestinal tumors has indicated that a period of two to six months may be required before CEA values return to "normal" levels. Postoperative CEA values that continue to rise during follow-up study are suggestive of incomplete resection, tumor recurrence, or metastatic disease. Transient postsurgical increases of AFP, B-HCG, and PAcP have not yet been reported to occur.

The usefulness of tumor marker testing in the clinical management of the patient with cancer is becoming more apparent. However, tumor markers cannot be used independently to assess the status of the patient or to evaluate the response to therapy. Tumor marker test results should be evaluated in light of other clinical data with reference to previous marker values obtained on a regular basis. If possible, a strict monitoring protocol should be followed. The frequency and timing of the follow-up specimens should be determined after consideration of the type of marker, the status of the patient, and the nature of the disease.

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and another a catheter site infection. Phlebitis was encountered in all seven patients who received the drug via the peripheral vein, and in all but one of these seven the drug was subsequently administered through an indwelling central venous catheter for the duration of therapy. An acute anaphylactic reaction, probably related to bisantrene, occurred in one patient. This patient achieved a significant response in disease of the soft tissue following therapy with bisantrene but subsequently died of infection, which developed at the site of a previous tracheostomy.

The results of our study indicate that bisantrene, like its congener dihydroxyanthracenedione, appears to be one of the most active single antitumor agents we have tested in the treatment of patients with metastatic breast cancer. The 22% response rate and a 28-week median duration of progression-free disease observed in these 40 heavily pretreated patients indicates the significant activity of bisantrene in breast cancer. This response rate is identical to that obtained with doxorubicin in a similar group of heavily pretreated patients; doxorubicin is presently considered the most active single agent for advanced breast cancer. In addition, the lack of crossresistance between bisantrene and doxorubicin, further indicates the potential value of this drug.

In this phase II study, unlike our previous phase II evaluation of dihydroxyanthracenedione, no patient suffered congestive cardiac failure after treatment with bisantrene. This finding supports our phase I study results, indicating that bisantrene is substantially less cardiotoxic than dihydroxyanthracenedione or doxorubicin; the minimal cumulative cardiotoxic doses in rat models for bisantrene, dihydroxyanthracenedione, and doxorubicin were 300 mg/kg of body weight, 18 mg/kg, and 11 mg/kg, respectively. Additionally, in phase I cardiotoxicity studies with beagle dogs, myocardial lesions were seen only in the sequential endocardial biopsies obtained from dogs receiving doxorubicin and were not seen in dogs receiving bisantrene.

On the whole, bisantrene was generally well tolerated and relatively free of acute toxicity, especially when compared to doxorubicin. The development of an acute anaphylactic reaction in one patient was disturbing. We are unable to ascertain whether this was truly an allergic reaction to bisantrene, but would, however, recommend that all patients receiving bisantrene be kept under close observation.

Nevertheless, the observed antitumor activity in this group of heavily pretreated patients should make bisantrene an important new antitumor agent in the treatment of patients with metastatic breast cancer.

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