Survival of Patients with Limited-Stage Small Cell Lung Cancer Treated with Individualized Chemotherapy Selected by in Vitro Drug Sensitivity Testing

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ABSTRACT

Our purpose was to study the feasibility of determining individualized chemotherapy regimens by in vitro drug sensitivity testing (DST) for patients with limited-stage small cell lung cancer (SCLC) and to evaluate patient response and survival. Fifty-four previously untreated patients with limited-stage small cell lung cancer were studied. Fresh tumor specimens for DST were collected, when possible, from patients' biopsies before the start of treatment. The differential staining cytotoxicity assay was used to determine the in vitro sensitivity of the tumor cells to different drugs. From these results, an in vitro best regimen (IVBR), a three-drug combination of previously proven efficacy of seven active drugs in SCLC, was selected. Patients were initially treated with four cycles of etoposide/cisplatin and concurrent radiotherapy. This was followed by four cycles of either individualized chemotherapy regimens based on the results of DST or, when DST results were not available, four cycles of vincristine, doxorubicin, and cyclophosphamide. Eighteen patients (33%) underwent biopsy procedures that provided tissue specimens for DST. The biopsy specimens contained tumor cells in 16 of 18 patients. The median duration from diagnosis to start of treatment was 22 days (range, 4-58 days) for the 18 patients who underwent elective thoracic biopsies compared to 21 days (range, 2-74 days) for members of the group that did not (P<0.08). Time from thoracic biopsy to initiation of chemotherapy was a median of 4 days (range, 2-22 days). DST was done in 10 patients, and IVBR was administered to 8 patients. The median actuarial survival of 8 patients treated with their IVBR was 38.5 months compared to 19 months for the 46 patients treated with empiric chemotherapy. Selection of individualized chemotherapy regimens is labor intensive but feasible in limited-stage SCLC. Treatment with an individualized IVBR in our patients was associated with prolonged patient survival; however, because of the nature of our study design, other factors could have affected the results.

INTRODUCTION

Investigators have developed numerous types of in vitro DST2 for identifying drugs more likely to kill the tumor cells of a given patient, attempting to increase the therapeutic efficacy of chemotherapy. Clonogenic, colorimetric, rapid [3H]thymidine incorporation, and chemotherapy treatment of athymic nude mice with implanted tumors have all been used to assess the sensitivity of tumors and tumor cell lines (1-6). Patient chemotherapy regimens have been selected by assay results in only a few prospective studies (7-10).

Investigators in our branch initially studied tumors from patients with NSCLC and extensive-stage SCLC to select chemotherapy regimens for individual patients in 1983 (11, 12). The differential staining cytotoxicity assay was used in these studies because it is able to distinguish cytolysis of tumor cells from effects on normal cells (13, 14). This allows assessment of in vitro chemotherapeutic effect on cancer cells in a mixed population of tumor and normal cells. The study of patients with extensive-stage SCLC showed that selection of chemotherapeutic agents by DST was feasible. Patients who had no response or a partial response after 12 weeks of treatment and those who relapsed after a complete response were treated with IVBR if DST had been done. Four of 16 (25%) patients treated with a regimen selected by DST had a complete response compared to 3 of 43 (7%) patients treated with an empiric regimen of VAC (10). Therefore, administration of chemotherapy selected by in vitro was feasible in a subset of patients, and the regimens selected by DST seemed to be at least as active as an empiric regimen.

There may be a greater potential utility of using DST to select chemotherapy agents in a clinical situation in which the cancer is more sensitive. Patients with limited-stage SCLC are twice as likely to achieve a complete response after chemotherapy treatment compared to patients with extensive-stage disease (15). Therefore, we decided to use in vitro DST to select chemotherapy regimens for patients with limited-stage SCLC.

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2 The abbreviations used are: DST, drug sensitivity testing; NSCLC, non-small cell lung cancer; SCLC, small cell lung cancer; IVBR, in vitro best regimen; CDP, cisplatin; CCNU, lomustine; CTX, cyclophosphamide; DOX, doxorubicin; NM, nitrogen mustard; VCR, vincristine; VP-16, etoposide; VAC, vincristine, doxorubicin, and cyclophosphamide; MTX, methotrexate.
because the in vitro DST had proven feasible in the extensive-stage setting. Patients with limited-stage SCLC do not typically have tumor tissue available for biopsies under local anesthesia. The data collected from our patients with extensive-stage SCLC suggested that the in vitro selected regimens were more active against SCLC than the empiric regimens. Therefore, we believed it was appropriate to ask patients to undergo a biopsy procedure under general anesthesia to obtain tumor tissue for in vitro DST to select their regimens for chemotherapy treatment administered in weeks 13–24. We performed surgical procedures including supraclavicular lymph node biopsies, mediastinoscopies, and thoracotomies to obtain tumor tissue for in vitro DST. Surgical biopsies requiring general anesthesia solely for obtaining tumor tissue for DST have infrequently been used, so we believed it was important to obtain information on the safety of these procedures. Although extensive information has been collected on the safety of these procedures in patients with NSCLC, we believe it is important to collect information from patients with SCLC because it is more widely disseminated in the mediastinum and more rapidly growing (15). We prospectively collected information about the number of patients who could be biopsied and the number of specimens from which DST could be successfully performed. The time required to obtain the biopsies, surgical complications, and time elapsed from diagnosis and first clinic visit until a biopsy was performed and treatment was initiated was evaluated. Patients were initially treated with four cycles of standard-dose VP-16/CCDP with concurrent chest radiotherapy. This treatment was followed by either four courses of a three-drug combination regimen, selected by in vitro DST, or VAC, if DST results were not available. We evaluated the response to treatment and survival of patients treated with an IVBR compared with those treated with an empiric VAC regimen.

PATIENTS AND METHODS

Previously untreated patients with histologically or cytologically documented SCLC were considered for this study. The pathology or cytology specimens were reviewed by one of the reference pathologists (J. W. or A. F. G.). Patients with predominant small cell carcinoma mixed with another histological type (16) were also eligible for this study.

The protocol was approved by the local institutional review board, and written informed consent was obtained from all patients. Before the initiation of chemotherapy and chest radiotherapy, patients underwent staging evaluation as described previously (17). Information regarding dates of diagnosis, first clinic visit, biopsy, and initiation of treatment was recorded. Eligibility criteria for patient entry were as described previously (17).

Elective Biopsies of SCLC for in Vitro DST. Patients who had no evidence of lobar or mainstem airway obstruction, superior vena cava syndrome, or other need for urgent chemotherapy were identified before the start of treatment. Patients with enlarged supraclavicular lymph nodes had such nodes biopsied before starting treatment. If the nodes contained SCLC cells, patients were not considered for elective thoracotomy. If they had no enlarged supraclavicular lymph nodes and were considered acceptable candidates for an elective intrathoracic tumor biopsy by the medical oncology, radiation oncology, cardiothoracic surgery, and anesthesia staffs, they were offered an intrathoracic biopsy under general anesthesia to obtain tumor tissue for DST. Patients were still eligible for treatment on this study if they did not opt for intrathoracic tumor biopsy.

In Vitro DST. Specimens obtained were handled and cultured as described previously (10). Tumor cell numbers were multiplied by culture in selective media before DST could be performed. The dye exclusion assay described by Weisenthal et al. (13) was used for all of the DSTs (18). Tumor cell suspensions were incubated with three concentrations of seven individual drugs. These concentrations included a reference concentration as well as 10-fold higher and 10-fold lower concentrations of each drug, as described previously (10). The chemotherapeutic agents tested against the SCLC cells included VP-16, CDDP, DOX, CCNU, VCR, MTX, and NM (used in place of CTX, which requires activation in vitro). These seven drugs were selected in 1983 when the extensive-stage SCLC study was designed; the same design has been used in this study (15).

After 4 days of incubation, acetaldehyde-fixed duck red cells were added to the cell suspensions as an internal control to correct for cell proliferation during incubation. Cytocentrifuge preparations were made and stained with Fastgreen (J. T. Baker Chemical Co.) and/or nigrosin dyes (Sigma Chemical Company) and counterstained with H&E. Living tumor cells were identified by their ability to exclude Fastgreen and nigrosin. The ratio of surviving tumor cells: duck cells was compared in controls and drug-exposed samples. If the mean survival of tumor cells was less than 50% at the reference concentration, a drug was considered to be active against the cell line. The best drug or drugs for each cell line were those that had the lowest tumor cell survivals at the reference concentration(s), regardless of whether cell survival was less than 50%.

The IVBR for each patient was selected from the DST data. Of the 35 possible 3-drug combinations resulting from the 7 individual drugs tested, 13 were reported to be effective against lung cancer (10). We chose the IVBR from among these 13 combinations by determining the combination with the lowest mean cell survival at the reference concentrations. In vitro testing of drug combinations or drug-drug interactions in the in vitro system were beyond the scope of this study.

The Combined Modality Regimen. The combined modality regimen consisted of four cycles of VP-16 and CDDP with concurrent chest radiotherapy followed by four cycles of an IVBR or four cycles of VAC when DST results were not available (17). The initial 12 weeks of therapy consisted of VP-16 (80 mg/m²) administered on days 1, 2, and 3, and CDDP (80 mg/m²) on day 1. Chest radiotherapy was administered at 1.5 Gy twice daily fractions for 3 weeks to a total of 45 Gy. The VP-16/CCDP chemotherapy was repeated at week 4, after completion of chest radiotherapy, and at weeks 7 and 10. Initial therapy was given for 12 weeks to allow time for an increase in the number of tumor cells from patients' specimens required for DST (10). Selection of chemotherapy for the final 12 weeks of treatment depended upon whether DST could be performed on a cell line established from a pretreatment tumor specimen procured from the patient. If DST results were available, 1 of 13...
standard 3-drug combinations that most closely corresponded to the 3 most effective drugs in vitro was administered for 12 weeks (10). If results were not available, patients were treated on day 1 with VCR, 1.4 mg/m² (maximum dose, 2.0 mg); DOX, 45 mg/m²; and CTX, 1000 mg/m², every 3 weeks. After four cycles, chemotherapy was discontinued. Dose modifications during these treatment periods have been described (11).

Response Assessment. Response assessment after 12 weeks of combined-modality therapy was based on history and physical examination, chest radiograph, fiberoptic bronchoscopy with washings and biopsies, bilateral bone marrow aspirate and biopsy, computerized tomography scans of the chest and head, radionuclide or computerized tomography scans of the liver, and radionuclide bone scan. At the end of 24 weeks of treatment, only those tests that were abnormal at 12 weeks were repeated to determine if a new complete response had been attained. Complete and partial responses were defined as described previously (17).

Statistical Analysis. Survival duration was calculated from the date of treatment initiation until the date of last follow-up or death. Survival curves were generated using the Kaplan-Meier method (19). The Mantel-Haenszel test was used to compare the survivals between two groups of patients (20). A Cox proportional hazards model was also used to evaluate the effect of variables on survival (21). All P values are two-sided.

RESULTS

Fifty-four patients (37 men and 17 women) were treated for limited-stage small cell cancer between July 1986 and July 1993 (Fig. 1). Fifty-three patients had limited-stage SCLC, and 1 patient had limited-stage extrapulmonary small cell cancer with involvement of inguinal and retroperitoneal lymph nodes. The median age was 58 years. Fifty of 54 patients were fully ambulatory with performance status of 0 or 1. One of the patients with a performance status of 2 was in the DST treatment group. Two patients with a performance status of 2 and one patient with a performance status of 3 were in the empiric chemotherapy group.

Pretreatment Elective Biopsies. Thirty-six patients (67%) had no biopsy procedures. Of these, 8 patients refused the procedure, 11 patients had lobar or mainstem airway obstruction, 3 patients had superior vena cava syndrome, and tumors from 4 patients were not accessible by mediastinoscopy. The remaining 10 patients had poor medical status: 6 patients had a history of coronary artery disease, 2 patients had a neurologic paraneoplastic syndrome, 1 patient had inadequate pulmonary function tests, and 1 patient had a small right pleural effusion. Eighteen patients (33%) underwent a biopsy procedure to provide tumor tissue for DST. One patient underwent a left upper lobectomy because other procedures failed to reveal a diagnosis. Two patients had biopsies of a supraclavicular lymph node, and 15 patients had mediastinoscopic procedures under general anesthesia. The biopsy specimens contained tumor cells in 16 of 18 (89%) biopsied patients. There was no evidence of tumor in mediastinal lymph node specimens from two patients. One of these patients had chronic obstructive pulmonary disease, and his pulmonary reserve was judged to be inadequate to undergo an elective parenchymal biopsy or lobectomy. The other patient had a biopsy of a mediastinal lymph node that showed no cancer cells.

There were no surgical complications. One patient’s treatment was interrupted for 24 h, after he developed shortness of breath from fluid overload while receiving hydration before the initial cisplatin administration. The treatment was safely restarted the following day with no complications. The median duration from diagnosis to start of treatment was 22 days (range, 4–58 days) for the 18 patients who underwent elective thoracic biopsies compared to 21 days (range, 2–74 days) for the 36 patients who did not ($P_2 = 0.58$). The median time from first clinic visit at our institution to treatment in the group of biopsied patients was 14 days (range, 7–28 days) compared to 10 days (range, 0–62 days) for the group not biopsied ($P_2 = 0.07$). Time from thoracic biopsies to treatment was a median of 4 days (range, 2–22 days). Five of 18 patients were treated more than 7 days after the biopsy. One patient had a lobectomy and required 2 weeks to recover from the surgery, two patients had

![Fig. 1 Diagram of the number of patients entered in the protocol and their selection process. Numbers in parentheses, percentages of all patients entered in this study.](clincancerres.aacrjournals.org)
Survival of SCLC Patients Treated with IVBR

Additional chemotherapy after five cycles (two patients) and seven their second 12 weeks of chemotherapy. Fourteen patients began patients who underwent a biopsy, the second 12 weeks of therapy. This represents 44% of the 18 patients from whom tumors containing biopsies were procured or 15% of all patients. The other 6 of 18 patients had an inadequate number of tumor cells for DST. There was considerable heterogeneity in the sensitivities of the tumor cell lines. We determined the mean percentage of cell survivals for individual drugs (Table 1). VP-16 and NM showed the lowest cell survivals, with a median of 45 and 48%, respectively, for the eight patients who had DST. The median for the combined three IVBR drugs used was 46%.

Table 1: Mean in vitro cell survivals produced by individual drugs

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<tr>
<th>Variable</th>
<th>Mean</th>
<th>SE</th>
<th>Minimum</th>
<th>Median</th>
<th>Maximum</th>
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<td>13</td>
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</tr>
<tr>
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<td>8</td>
<td>9</td>
<td>33</td>
<td>73</td>
</tr>
<tr>
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<td>57</td>
<td>8</td>
<td>11</td>
<td>22</td>
<td>48</td>
</tr>
<tr>
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<td>8</td>
<td>7</td>
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<td>71</td>
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* x, mean.

<table>
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<th>Frequency</th>
<th>Percentage</th>
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<td>12</td>
</tr>
<tr>
<td>NM</td>
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<td>62</td>
</tr>
<tr>
<td>CCNU</td>
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<td>12</td>
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<td>IVBR combination</td>
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<td>37</td>
</tr>
<tr>
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<td>12</td>
</tr>
<tr>
<td>VP-16/CTX/DOX</td>
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<td>12</td>
</tr>
<tr>
<td>DOX/VCR/CTX</td>
<td>1</td>
<td>12</td>
</tr>
<tr>
<td>DOX/VP-16/CDDP</td>
<td>1</td>
<td>12</td>
</tr>
<tr>
<td>CTX/CCNU/VCX</td>
<td>2</td>
<td>25</td>
</tr>
</tbody>
</table>

* NM was used in place of CTX, which needs to be activated in vitro.

their staging evaluation completed after their biopsies, one patient developed a small pleural effusion requiring diagnostic thoracentesis, and another patient had symptomatic hypotension requiring electrolyte replacement. DST and Selection of IVBR. SCLC cells from 10 patients provided successful in vitro DST; but only 8 patients had the results within 12 weeks of starting treatment to allow for an in vitro selection of combination chemotherapy for the second 12 weeks of treatment. This represents 44% of the 18 patients from whom tumors containing biopsies were procured or 15% of all patients. The other 6 of 18 patients had an inadequate number of tumor cells for DST. There was considerable heterogeneity in the sensitivities of the tumor cell lines. We determined the mean percentage of cell survivals for individual drugs (Table 1). VP-16 and NM showed the lowest cell survivals, with a median of 45 and 48%, respectively, for the eight patients who had DST. The median for the combined three IVBR drugs used was 46%.

NM, a surrogate for CTX, was the most active antitumor agent in specimens from five of eight patients (Table 2). DOX, VP-16, and CCNU were selected one time each as the single most active drugs tested. CDDP, VCR, and MTX were not selected at all.

Of the 13 possible 3-drug combinations used previously in the therapy of lung cancer, 5 were selected as an IVBR, each selected 1–3 times (Table 2).

Combined Modality Therapy Administered. Fifty-three of 54 patients (98%) received 4 cycles of VP-16/CCDP chemotherapy and a full planned course of 4500 cGy of chest radiotherapy during their initial 3 months of therapy. Two patients died during the first 3 months of therapy. One patient died of a myocardial infarction during the 3rd week of therapy, before completing the planned course of chest radiotherapy. A second patient died of a perforated intestine after four cycles of VP-16/CCDP and chest radiotherapy.

Forty-four of 52 (85%) patients were treated with empiric VAC for their second 3 months of therapy. For 8 of 18 (44%) patients who underwent a biopsy, the second 12 weeks of treatment were based on the results of DST (Table 2).

Thirty-eight of 54 patients (70%) completed 4 cycles of their second 3 months of chemotherapy. Fourteen patients began but did not complete their fifth through eighth cycles of therapy; two patients' cancers progressed during cycles 5 and 6, and nine patients had unacceptable toxicity. Three patients declined additional chemotherapy after five cycles (two patients) and seven cycles (one patient). Of the eight patients that received IVBR, one received VAC in the first cycle (while waiting for DST results) and IVBR in the remaining three cycles. The other seven patients received four cycles of IVBR. Doses and schedules of the IVBR treatments are described in the references cited in Table 3.

Correlation of DST and Response to Chemotherapy. Fifty-two patients were evaluable for response. The other two patients died from drug toxicity during their initial 12 weeks of therapy. Of the 44 patients who eventually received VAC, 32 patients (73%) had a partial response, and 12 patients (27%) had a complete response at week 12. All eight patients who later received IVBR were partial responders at week 12. After 12 weeks of empiric chemotherapy (VAC), 21 (66%) of 32 partial responders had a complete response. Hence by week 24, 33 of 44 patients (75%) had a complete response, and 11 of 44 patients (25%) had a partial response.

By week 24, after 12 weeks of therapy with IVBR, 7 of 8 patients (87%) became complete responders, and 1 patient continued as a partial responder.

Of the 54 patients beginning treatment, 40 patients (74%) had a complete response at 24 weeks, whereas 12 patients (22%) had a partial response.

Relationship between DST Data and Patient Survival. The median potential follow-up of patients enrolled in this study is 6.1 years. Results are updated to March 1996. Eight patients are still alive and free of SCLC; 46 patients have died. The median actuarial survival was 21.4 months, with 83% survival probability at 1 year; actuarial survival was 44% at 2 years and 20% at 5 years. The median survival of patients treated with empiric chemotherapy (VAC) was 19 months, compared to 38.5 months in patients treated with their IVBR (P\text{\textsubscript{2}} = 0.035) for comparison of the entire curve (Fig. 2). In addition, the median survival of patients who underwent elective thoracic biopsy and received their IVBR was 38.5 months compared to 17.5 months in patients biopsied but not treated with IVBR (P\text{\textsubscript{2}} = 0.0074) for the difference between the curves.

The mean of the three lowest cell survivals and the mean of the agents used in treatment was formed for the eight patients who received IVBR. A Cox proportional hazards model was constructed to separately evaluate the effect of these variables.
Fig. 2 Actuarial survival of patients who were treated with their IVBR (●) compared to that of patients treated with empiric chemotherapy (NO IVBR, ○). Tick marks, patients still alive.

Table 3 Doses and schedules of the IVBR three-drug combination used in eight patients.

All patients received four cycles of chemotherapy. All regimens were given every 3 weeks except CTX/CCNU/VCR, which was given every 6 weeks. All drugs were given i.v. except CCNU, which was given p.o. Doses were modified from previously published regimens to produce similar hematological toxicity.

<table>
<thead>
<tr>
<th>Drug combination</th>
<th>No. of patients</th>
<th>CTX (mg/m²)</th>
<th>VP-16 (mg/m²)</th>
<th>CDDP (mg/m²)</th>
<th>DOX (mg/m²)</th>
<th>VCR (mg/m²)</th>
<th>CCNU (mg/m²)</th>
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<tr>
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<td>3</td>
<td>750*</td>
<td>80*</td>
<td>40*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VP-16/CTX/DOX (28)</td>
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<td>750*</td>
<td>80*</td>
<td>45*</td>
<td></td>
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<tr>
<td>DOX/VCR/CTX (29)</td>
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<td>1000*</td>
<td>80*</td>
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<td>2</td>
<td>1000*</td>
<td>45*</td>
<td>1.4*</td>
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</tbody>
</table>

* Day 1.
* Day 1–3.
* Maximum dose, 2.0 mg.
* Day 22.

on survival. Having higher values from either the mean of the three lowest cell survivals ($P_2 = 0.13$; relative risk, 1.02) or the mean of the three IVBRs used in treatment ($P_2 = 0.10$; relative risk, 1.03) conveys a slightly greater risk of death, but not significantly so. It is probably reasonable to state that there could be an association between these parameters and survival, but it is likely to be limited.

To examine these limited effects on survival, Kaplan-Meier analysis was performed. The cell survival data indicated that the three most active drugs (the chemotherapeutic agents that caused the most cell kill) had a median survival of 44%. The three chemotherapy drugs selected for the IVBR had a median survival of 47%. To compare the patients' survival, we selected 45% as the percentage to divide the patients into those whose tumor cells are more sensitive and those whose tumor cells are more resistant (which roughly corresponds to the median survival of the two values 44% and 47%). The median survival of patients whose in vitro DST showed less than 45% cell survival was 38.5 months compared to 21.4 months for patients whose in vitro DST showed more than 45% cell survival. Therefore, Kaplan-Meier analysis suggested there was a slight tendency to have a better survival with lower values, but the difference did not approach standard statistical significance ($P_2 = 0.45$).

DISCUSSION

Patients with limited-stage SCLC could be safely biopsied in a subset of patients and treated with different assay-selected regimens during the second 12 weeks of chemotherapy treatment. In our study, the 18 patients who underwent biopsies had no significant surgical complications and began treatment a median of 22 days after diagnosis, compared to 21 days for those who did not have a biopsy. The survival of our 8 patients treated with IVBR was longer than that of 44 patients treated with empiric chemotherapy ($P_2 = 0.035$). The study was not randomized, so other factors could have influenced this outcome. One potential bias was the assignment of patients with airway obstruction or superior vena cava syndrome to the empiric chemotherapy treatment group. However, a recent study showed that the survival of patients with SCLC and obstructive atelectasis was similar to that of patients without obstructive atelectasis (22). In addition, two studies of patients with SCLC and superior vena cava syndrome showed that the presence of superior vena cava syndrome had no adverse effect on treatment outcome (23, 24). Our previous study of patients with extensive-stage SCLC showed no correlation between the ability of tumor cells to be established as a cell line and survival (10). Therefore,
the fact that some of the patients treated in the empiric chemotherapy group had obstructive atelectasis or superior vena cava syndrome should not have altered the outcome of the study. The extensive selection process shown in Fig. 1 could have selected patients who would have lived longer despite the different type of chemotherapy (empiric or IVBR).

The selection of individualized chemotherapy regimens based on DST data is labor intensive but workable. However, in spite of the consistent specimen collection procedure and culture methodology, only 15% of the patients participating in the protocol were able to be treated with an IVBR (Fig. 1).

Three other groups of investigators outside our institution have prospectively studied patients with DST to select their chemotherapy regimens in solid tumors and have encountered similar problems. This includes a single prospectively randomized study of 133 patients with advanced cancer, some of whom had had previous chemotherapy (8). In this study, 65 patients were randomly assigned to receive chemotherapy selected by a clinician, and the other 68 patients were randomized to receive chemotherapy selected by an in vitro capillary cloning system. Only 19 of 68 patients (28%) randomized to receive DST-selected therapy were actually treated with those agents. Although 4 of 19 patients (21%) had a partial response to DST-selected therapy compared to 1 of 36 (3%) treated empirically, there was no difference in survival between the 2 groups.

Two other uncontrolled studies selected chemotherapy for patients with lung cancer and a variety of solid tumors. A study by Wilbur et al. (9) showed 35 of 45 patients with advanced NSCLC were able to have DST of their tumor performed by the differential staining cytotoxicity assay if their tumor reached the laboratory, and 25 patients were treated with an IVBR. The patients treated with their IVBR had a response rate of 36% (9). Yamaue et al. studied 78 patients with solid tumors (n = 54) and malignant ascites (n = 24) with the (3-(4,5-di-methylthiazol-2-y1)-2,5-diphenyltetrazolium bromide (MTT) assay. Sixty-eight of 78 patients were able to have DST performed on their tumor, and 31 patients were treated with an IVBR. Ten of 31 (32%) patients treated with assay-directed therapy had a clinical response, 5 complete and 5 partial responses (7). In our study of patients with NSCLC, 36 of 161 (22%) patients were able to have DST performed on their tumor, and 21 patients (58% of those who had DST) were treated with an IVBR. Twenty of 21 (9%) patients treated with IVBR had a clinical response compared to 10 of 69 (14%) patients treated with empiric chemotherapy (12).

In our previously published studies and these other prospective studies, investigators were able to perform DST in 22–75% of the tumor samples that reached the laboratory (7–10, 12). However, only a minority of patients entering a prospective trial were able to have a tumor specimen obtained for DST (9, 10, 12). In this study and in our study of patients with extensive-stage SCLC, only 16 of 54 patients (30%) and 60 of 80 patients (75%), respectively, had a tumor specimen reach the laboratory. In the study by Wilbur et al. (9), only 45 tumor specimens were obtained from 180 patients seen in consultation (25%). Therefore, only 13–25% of patients with lung cancer evaluated for participation in these trials were able to be treated with a regimen selected by in vitro DST.

We observed considerable heterogeneity in the sensitivities of tumor cell lines to the drugs tested, similar to what other authors have found (Table 1). The DST selected five different three-drug combinations for the treatment of eight patients. Our other studies showed that 6 different regimens were selected for 36 patients with NSCLC, and that 8 different regimens were selected for 26 patients with extensive-stage SCLC (10, 12). The other prospective study of patients with NSCLC showed that 20 different regimens were selected for 25 patients with NSCLC (9). Therefore, the in vitro DST selected different combinations of chemotherapy for different patients. In vitro testing of drug combinations or drug-drug interactions in the in vitro system were beyond the scope of this study.

In our study of patients with extensive-stage SCLC, we found a relationship between in vitro tumor sensitivity and patient response (10). In the present study, we had inadequate numbers to evaluate this issue. Other investigators have performed DST and compared the in vitro tumor sensitivity to patients’ response rates and survival. A prospective study of 45 patients with NSCLC, a retrospective analysis of 30 patients with non-Hodgkin’s lymphoma, and a prospective study of 7 patients with SCLC showed a correlation between the in vitro sensitivity and complete remission and prolonged survival (4, 6, 9).

Based on this data showing that cancer cells have heterogeneous sensitivity to chemotherapeutic agents and the close relationship between in vitro and in vivo sensitivity of different cancers to chemotherapeutic agents (25), we believe it is important to continue working on DST for the screening of new chemotherapy agents that are now available as taxanes, gemcitabine, vinorelbine, and topoisomerase inhibitors.

The success rate of this study makes it difficult to evaluate DST in a randomized trial. Only 15% of the patients entering our limited-stage SCLC trial could be treated with an IVBR. A trial comparing patients randomized at the beginning of treatment to either empiric chemotherapy or an IVBR selected by DST would require a large number of patients entering the trial. If the trend in prolonged survival observed in our patients treated with an IVBR continued, a study would require 91 patients per arm to demonstrate a 20% difference in 2-year survival at the two level with 80% power. However, because only 15% of the patients entering our study were able to be treated with an IVBR, we would need to enter 1229 patients to find a 20% difference in survival. We do not believe such a trial is justified at the current time.

However, this is the first published prospective study of in vitro selected chemotherapy for patients with limited-stage SCLC. The major achievements from this study have been to demonstrate that the selection of individualized chemotherapy based on an IVBR is feasible and safe in patients with limited-stage SCLC and that the regimens selected by DST were clinically active. In addition, this study provided tumor cell lines of limited-stage SCLC patients for biological studies, including oncogene amplification and its association with survival (26).

This study provides information about the safety and feasibility of elective biopsies under general anesthesia for obtaining tumor tissue for laboratory investigations for selecting therapeutic agents. The continuing introduction of agents active against lung cancer with different in vitro sensitivity patterns and improving technologies for evaluating in vitro drug sensi-
tivity may make a randomized trial evaluating in vitro drug testing possible in the future. We suggest that investigators plan future trials to be able to obtain tumor tissues from a large number of patients entering a study.

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