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Correlation of In Vitro Drug Sensitivity Testing Results With Response to Chemotherapy and Survival: Comparison of Non-Small Cell Lung Cancer and Small Cell Lung Cancer

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Abstract Clinical protocols for small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC) were devised to prospectively select individualized chemotherapy based on in vitro drug sensitivity testing (DST) of cell lines derived from the patient's SCLC tumor cell lines or the patient's fresh NSCLC tumor. DST data derived from SCLC tumor cell lines were available for 33/115 (29%) patients. The DST-selected chemotherapy regimen was administered to 21 (18%) patients, or 64% of patients with DST. In SCLC, the DST-selected chemotherapy was administered either during weeks 13–24 following 12 weeks of etoposide/cisplatin, or at relapse after complete response to etoposide/cisplatin. Several parameters of in vitro drug sensitivity were significantly associated (two-sided $P < 0.05$) with clinical response to primary therapy and also with response to the DST-selected chemotherapy regimen, but were not associated with survival ($P = 0.24$). Five patients treated with their DST-selected chemotherapy attained a complete or partial response, compared to 5 of 68 who received an empiric regimen ($P = 0.057$). A total of 36/165 (22%) NSCLC patients had DST successfully completed. These results directed management for 21/96 (22%) patients who eventually received chemotherapy, or 58% of patients with DST. Response to chemotherapy for the patients treated prospectively with their DST-selected chemotherapy regimen (2/21; 9%) was not significantly different than the response rate for patients treated empirically with etoposide/cisplatin (10/69; 14%) in the absence of in vitro results to direct chemotherapy ($P = 0.73$). There was no difference in survival by treatment group for the NSCLC patients. The correlation between in vitro and clinical response was not significant for any individual drug or for all drugs considered together, illustrating the poor predictive value of in vitro testing with currently available chemotherapy in NSCLC.

Key words: non-small cell lung cancer, small cell lung cancer, drug resistance, cell survival

INTRODUCTION

Although small cell lung cancer (SCLC) usually responds to initial combination chemotherapy, metastatic non-small cell lung cancer (NSCLC) demonstrates only modest responsiveness to cytotoxic drug programs [1–3]. A minority of SCLC patients are resistant to initial chemotherapy, and most who initially respond eventually relapse. It is possible that chemotherapy might be more effective if individualized treatment based on in vitro drug sensitivity

Abbreviations used: ADR, doxorubicin; CCNU, lomustine; CDDP, cisplatin; CR, complete response; CTX, cyclophosphamide; DST, drug sensitivity testing; ECOG, Eastern Cooperative Oncology Group; IVBR, in vitro best regimen; MTX, methotrexate; NM, nitrogen mustard; NSCLC, non-small cell lung cancer; PR, partial response; RPMI, Roswell Park Memorial Institute; VAC, vincristine, doxorubicin, cyclophosphamide; VCR, vincristine; VP-16, etoposide.

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testing (DST) of the patients' tumor samples could be administered. Therefore, parallel trials in patients with extensive stage SCLC and all stages of NSCLC were designed to evaluate the utility of this concept. Our objectives included (1) determination of the feasibility of DST-based protocols in the clinical management of lung cancer patients, (2) administration of individualized chemotherapy selected by DST completed in a timely fashion, and (3) evaluation of whether DST results predict response to chemotherapy or outcome. These protocols also allowed us to conduct studies of lung cancer biology using fresh tissue and immortalized cell lines for clinicopathologic correlation. The major problems in conducting a DST-based trial are obtaining adequate numbers of viable tumor cells and the low clonogenic potential of lung tumors and cultures [4–6]. Since SCLC is seldom surgically resected, only small tumor samples are usually available from accessible metastatic sites. Tumor obtained from metastatic sites during staging procedures was used, and was cultured using previously reported methods for the selective in vitro propagation of SCLC cells [7,8]. For NSCLC, DST was performed on fresh tissue obtained from surgical resections of the primary tumor or biopsies or aspirations of metastatic sites. This report summarizes the experiences of two trials in which 115 patients with SCLC and 165 patients with NSCLC were entered.

METHODS

Protocol Design

Both prospective clinical trials were approved by the appropriate institutional review boards, and all patients entered on these studies gave informed consent. All patients with newly diagnosed extensive-stage SCLC were eligible for the SCLC protocol. Pathologic and cytologic material from routine staging and initial diagnostic procedures were routinely submitted for tumor cell culture. All patients with NSCLC undergoing surgical resection or biopsy of sites of known metastatic disease were eligible for the NSCLC protocol. One hundred fifteen patients with pathologically confirmed extensive-stage SCLC and 165 patients with any stage or histologic subtype of pathologically confirmed NSCLC entered these studies at the National Cancer Institute-Navy Medical Oncology Branch, National Naval Medical Center, Bethesda, Maryland.

Tumor specimens from patients undergoing diagnostic or therapeutic procedures requiring general anesthesia were procured only during procedures performed as part of standard clinical management. Biopsies for the purpose of tissue procurement for protocol entry were allowed only if performed under local anesthesia after the patient's informed consent. Peritoneoscopic liver biopsies were done if imaging studies showed parenchymal lesions in patients without other sites of more easily accessible tumor. Effusions were collected in preservative-free heparin. Bilateral bone marrow specimens were processed, evaluated, and cultured separately, but each pair was counted as a single specimen. Because bronchial biopsies and washings obtained by fiberoptic endoscopy usually contain microbial contaminants and seldom can be cultured [9], these specimens were not used for DST studies. The schematic design of the protocols is presented in Figure 1.

Extensive-stage SCLC was defined as previously reported [10]. After completion of initial staging procedures, patients were treated with etoposide (VP-16) and cisplatin (CDDP) for 12 weeks [9]. During the initial 12 weeks of therapy, tumor cells from tumor-containing specimens were increased in number by selective cell culture, DST performed, and the in vitro best regimen (IVBR) of combination chemotherapy determined [9]. After 12 weeks, patients were restaged to assess response. Complete response was defined as the lack of clinical, radiologic, or pathologic evidence of residual tumor. Partial response was defined as greater than 50% reduction of the sum of measurable or assessable tumor lesions. Patients in CR or PR were considered responders, whereas those with no response or development of tumor progression with or without a prior brief response were considered nonresponders. Patients dying within the first 12 weeks of chemotherapy without evidence of tumor progression were not assessed for response. Subsequent therapy for the SCLC patients depended on tumor response and the availability of DST data. Complete responders continued VP-16-CDDP therapy for 12 more weeks. If DST data were available, partial or nonresponders received their individualized three-drug IVBR during weeks 13–24. If in vitro data were unavailable, patients received the empiric regimen of vincristine, doxorubicin, and cyclophosphamide (VAC). After another 12 weeks, patients' responses were evaluated and chemotherapy was discontinued. Patients relapsing after attaining a complete response from

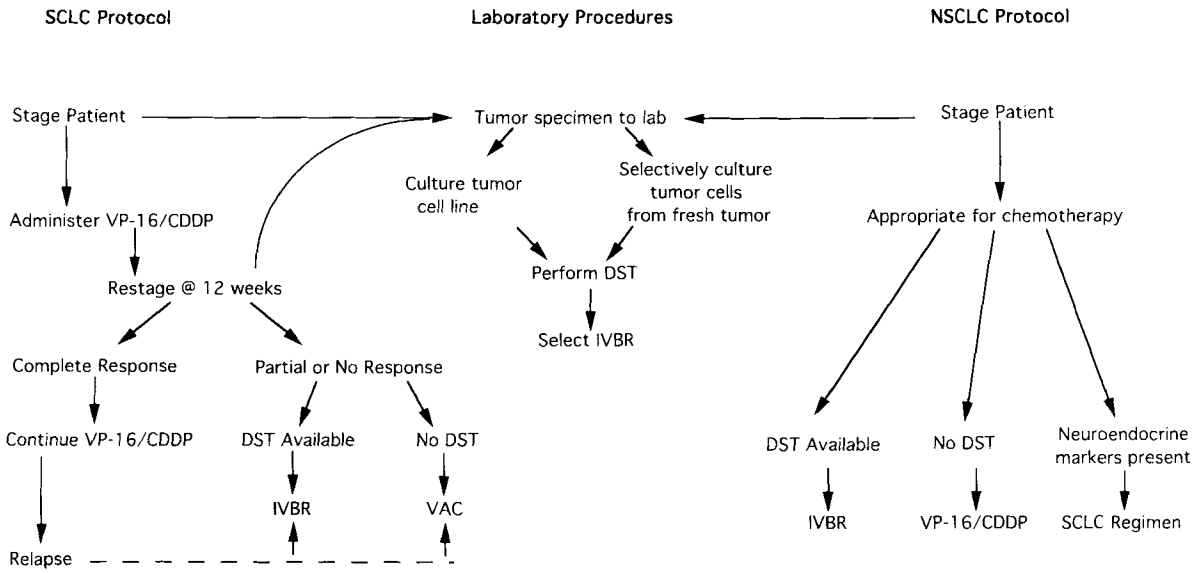

Fig. 1. Schematic outline of protocols. Specimens were obtained prior to therapy. Drug sensitivity testing (DST) was attempted for both small cell (SCLC) and non-small cell lung cancer (NSCLC) specimens and used to select the in vitro best regimen (IVBR) of combination chemotherapy. The eventual prospectively assigned treatments of patients receiving chemotherapy on these studies are indicated. Abbreviations used are: VP-16/CDDP, etoposide and cisplatin; VAC, vincristine, doxorubicin, and cyclophosphamide.

initial VP-16-CDDP therapy were given their IVBR if DST data were available, and VAC if data were not available. For all SCLC patients studied, the first chemotherapy regimen administered was VP-16-CDDP, and the second was either an IVBR or VAC. Specimens obtained at restaging following the initial 12 weeks of therapy also were sent to the laboratory for cell culture and DST. Data obtained from cell lines initiated from posttherapy samples from two patients are included in the relevant analyses because DST data were available early enough to permit administration of the IVBR at week 13. Data from laboratory studies of other post-therapy specimens are not presented.

All NSCLC patients were staged according to the international system of the American Joint Committee on Cancer guidelines adopted in 1987 [11]. Patients were stratified into two groups. The potentially curative group (N = 67) included Stage I, II, and IIIA patients who underwent definitive primary therapy with surgical resection, chest irradiation, or both procedures. On relapse, any patient with good performance status who did not require immediate palliative radiotherapy was offered chemotherapy on this study. The palliative treatment group (N = 98) consisted of patients with locally advanced unresectable NSCLC (Stage III) who were not thought to be candidates for curative radiation therapy, or patients with metastatic (Stage IV) or recurrent NSCLC after prior treatment not administered on this protocol. Patients who were ambulatory over half the day and had evaluable tumor lesions which did not require immediate palliative radiotherapy were offered chemotherapy.

Results of in vitro DST were used to select chemotherapy for NSCLC whenever possible. The three treatment options consisted of (1) the combination chemotherapy regimen being offered to contemporaneous SCLC patients if L-dopa decarboxylase activity was elevated as determined by enzymatic assay [12,13], (2) the most active in vitro combination determined by DST (the IVBR) if NE markers were not detected or were unknown, or (3) VP-16-CDDP when no vitro analyses were available. Combination VP-16-CDDP was chosen as the empiric chemotherapy because of acceptable toxicity and documented response rates in large cooperative group studies of chemotherapy for NSCLC [14,15]. Response to chemotherapy was assessed following restaging of tumor extent at 12 weeks and chemotherapy was continued up to 24 weeks in patients who responded. Therapy was stopped at the time of progressive disease. Preliminary survival results have been previously reported [16–18].

Pathology Review

All pathology specimens were prospectively reviewed by two of the authors (A.F.G. and R.I.L.). Two carcinoid tumors were identified and were excluded from the group prospectively treated with a SCLC regimen, since carcinoid tumors have a different clinical course [19,20] as well as markedly more resistant in vitro DST profiles than NSCLC with NE markers [21]. When chemotherapy was indicated, patients with carcinoid tumors received either their IVBR or VP-16-CDDP.

Cell Culture

Specimen processing methods have been previously described [9,17]. Briefly, fresh tumor specimens were processed for in vitro culture to increase tumor cell numbers for DST on SCLC specimens and if possible, to establish permanent cell lines for both SCLC and NSCLC specimens. SCLC samples were cultured in RPMI-1640 supplemented either with 10% fetal bovine serum or with HITES medium (RPMI-1640 plus 10^{-8} M hydrocortisone, 5.0 μg/ml insulin, 10 μg/ml transferrin, 10^{-8} M 17 β-estradiol, and 3×10^{-8} M sodium selenite [7,8]), plus 2% fetal bovine serum. Media used for culture of NSCLC specimens were those felt most appropriate for each histologic type of NSCLC as determined by the preliminary histologic diagnosis. Adenocarcinomas were cultured in a modification of ACL3 [22,23]. The medium used for squamous cell carcinomas was that reported by Ervin et al. [24]. RPMI-1640 was supplemented with 10% fetal bovine serum for the large cell carcinomas [6]. When sufficient quantities of tumor cells were available, cells were cultured in all three media.

Cytologic examination of an aliquot of the cultured preparation and examination of the structure of the cultured cells by two independent reviewers (A.G. and R.I.L.) using phase-contrast microscopy determined the presence of tumor cells in the specimen [8,25]. For the SCLC protocol, the presence of any tumor cells in the cytospin preparation implied a tumor-containing specimen. A cell line was considered to exist when sufficient in vitro amplification of tumor cell number had occurred to permit DST. Cultures were passaged whenever adequate cell growth had occurred. NSCLC tumor specimens were considered adequate for in vitro analysis, including attempted cell line propagation, if at least 1×10^6 trypan-blue-excluding (viable) tumor cells were identified on the cytospin preparation of the processed tumor. A NSCLC culture was considered to be an established permanent cell line when it had been passaged continuously for 6 months and could be recovered after cryopreservation.

Drug Sensitivity Testing

Methodology for DST was a modified version [9,17] of the dye exclusion assay which does not require clonogenic capacity described by Weisenthal et al. [26,27]. DST was performed on fresh tumor tissue from NSCLC patients and on cell lines from SCLC patients. Briefly, twelve chemotherapeutic agents included in the combination regimens thought to have clinical activity in NSCLC were tested at three concentrations ranging from 10-fold above to 10-fold below the reference concentration specified for each individual drug [28]. Reference concentrations were those determined by Weisenthal et al. extrapolating from the approximation of clinical drug exposures calculated from the pharmacokinetics literature to reflect use of the drugs in clinical experience [28]. Seven of these agents, all used in reported effective drug regimens for SCLC, were tested on the SCLC cell lines. Nitrogen mustard was substituted as an alkylating agent for cyclophosphamide, which requires in vitro activation, and carmustine (BCNU) was substituted for orally administered lomustine (CCNU). After 4 days incubation, acetaldehyde-fixed duck red blood cells were added to the cell suspensions as an internal control for cell proliferation during incubation; cytocentrifuge preparations were made and stained with fast green and/or nigrosin dyes, and counterstained with hematoxylin and eosin. Living tumor cells were identified by their ability to exclude fast green and nigrosin. The proportions of surviving tumor cells to duck cells were compared in control 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 tumor cell population. The best drug or drugs for each tumor specimen were those that had the lowest tumor cell survival at the reference concentration, regardless of whether cell survival was less than 50%. The DST results for the single agents at the reference concentrations were used to select the IVBR, defined as the combination regimen with the lowest mean in vitro cell survival from among

8 combination regimens commonly used in NSCLC and 13 three-drug combinations with demonstrated efficacy in SCLC for treatment on the respective protocols.

Statistical Analyses

Methods for statistical comparisons included the Wilcoxon rank-sum procedure and Fisher's exact test. Response on the three NSCLC treatment arms was compared using Mehta's version of Fisher's exact test [29]. Spearman rank correlation was used to assess the association between pairs of drugs tested with respect to percent cell survival. Survival was calculated from the date of protocol entry to the date of death or last known date alive and also from date of chemotherapy treatment for NSCLC patients to date of death or last known date alive. Death from any cause was treated as the main outcome event in all survival analyses. The Kaplan-Meier method was used to calculate the probability of survival as a function of time, and the entire survival distributions were compared using the Mantel-Haenszel procedure [30,31]. All cited P values are two-sided.

RESULTS

Patient Characteristics

One hundred fifteen patients with SCLC were entered on study between November 1983 and December 1991. One hundred sixty-five patients with NSCLC were entered on study between May 1984 and August 1990. Table I outlines the characteristics of patients entered on these studies. The median age of patients entered on the SCLC protocol was 61 years, compared to 57 years for the patients entered on the NSCLC protocol. Men comprised 58% of SCLC and 69% of NSCLC patients entered on protocol. The NSCLC protocol patients were more likely to be fully ambulatory (ECOG performance status 0–1) (80%) than the SCLC patients (68%). The majority of NSCLC cases (72%) were classified as adenocarcinoma including bronchioloalveolar carcinoma, consistent with the trend that has been observed at our institution in recent years [32]. A schematic outline of the studies is presented in Figure 1. Specimens were obtained from all patients at protocol entry, and specimens from 161 of 165 (98%) NSCLC patients and 79 of 115 (69%) SCLC patients contained viable tumor.

TABLE I. Patient Characteristics at Study Entry

	Non-small cell carcinoma		Small cell carcinoma	
Characteristic	Number	%	Number	%
Age				
≤50	46	28	17	15
51–60	59	36	40	35
>60	60	36	58	50
Median age	57		61	
Gender				
Male	114	69	67	58
Female	51	31	48	42
ECOG performance status				
0	30	18	4	4
1	103	62	74	64
2	21	13	20	17
3	7	4	12	10
4	4	3	5	4
Histologic diagnosis				
Small cell carcinoma			115	100
Adenocarcinoma	118	72		
Epidermoid carcinoma	22	13		
Large cell carcinoma	16	10		
Other	9	5		

Specimen Accrual and Cell Culture

The outcome of specimens by patients entered on both protocols is presented in Figure 2. Thirty-six cell lines were established from 33 patients from 102 SCLC tumor containing specimens (35%). In many cases only the largest or most viable sample from a patient with multiple tumor-containing specimens was cultured. SCLC tumor-containing specimens from subcutaneous nodules (2/2) and lung (2/3) were most likely to yield cell lines, although the number of specimens was small. Specimens from lymph nodes (43%) and from bone marrow (39%) comprised the majority of specimens yielding SCLC cell lines, as shown in Table II. Mediastinal lymph nodes were frequently crushed from mediastinoscopic biopsy in SCLC patients but the success rate for cell line formation (33%) was similar to ones from NSCLC patients (46%) which were primarily surgically resected specimens.

Thirty-nine cell lines from 39 patients were established from 161 NSCLC tumor-containing specimens (24%). Twenty-three of the patients with cell lines also had DST performed on fresh

tumor. Among NSCLC specimens, tumor from pleural effusions yielded DST results most frequently (55%). Specimens from lymph nodes were more likely to yield DST data (33%) and establish cell lines (44%) than specimens from lung (7% for each) as shown in Table II. Tumor from lung from patients with advanced disease established immortalized cell lines for 2 of 8 (25%) patients, compared to only 3 of the 61 (5%) lung specimens from patients who underwent resection with curative intent ($P = 0.099$). Immortalized NSCLC cell lines were more likely to be established from specimens from metastatic sites (34/92; 37%) than from specimens from lung (5/69; 7%) ($P < 0.0001$). No tumor resected from a patient with Stage I NSCLC (N = 32) established a cell line compared to the 39 cell lines from 133 patients with Stage II-IV disease ($P = 0.0001$). The presence of NE markers in NSCLC tumors did not significantly influence the likelihood of cell line propagation, with 3 of 12 (25%) specimens expressing NE markers establishing cell lines compared to 25/100 (25%) without NE markers.

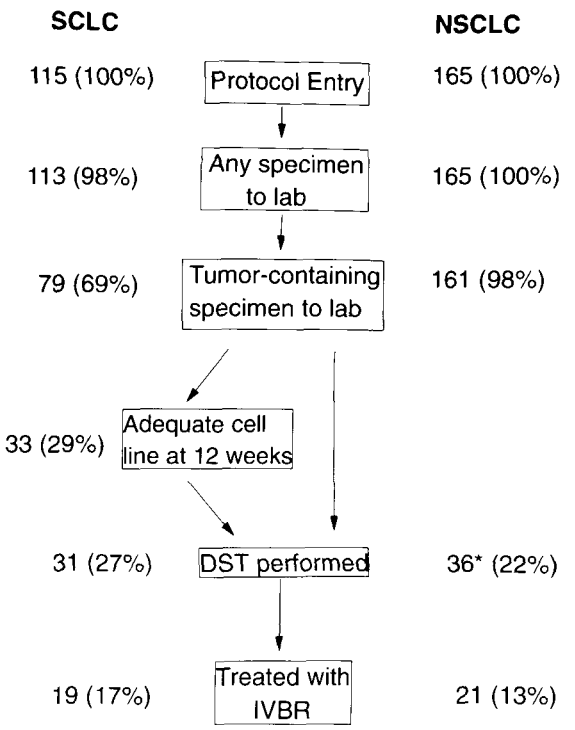

Fig. 2. Outcome of specimens by number of patients entered on the small cell (SCLC) and non-small cell lung cancer (NSCLC) protocols, with the percentage of total patients at each stage of specimen processing indicated in parentheses. Processing of SCLC tumors required establishing a cell line for drug sensitivity testing (DST) but DST was performed on cells from fresh NSCLC tumors. Number of patients eventually treated with their in vitro best regimen (IVBR) of combination chemotherapy is indicated. *Twenty-three of the patients with NSCLC who had DST also had NSCLC tumor cell lines established and an additional 16 patients had cell lines but never had DST, resulting in 24% of total NSCLC patients establishing cell lines.

Drug-Sensitivity Testing and Selection of the In Vitro Best Regimen

DST was performed on cell lines established from pretherapy specimens from 31 of 33 SCLC patients. In addition, DST was performed on two cell lines established from previously treated patients expeditiously enough to permit administration of the IVBR. Thus, DST data were available from 33 patients (29% of all patients). In vitro testing demonstrated considerable heterogeneity in SCLC tumor cell sensitivity to the drugs tested as shown in Table III. VP-16 and CCNU were frequently selected as the single best drug, and VCR and MTX were seldom selected. VP-16 was considered active in 15 (47%) of specimens tested, and the single most active agent in 10 (30%). DST of the 33 cell lines resulted in the selection of 9 of the 13 potential IVBR combinations at frequencies ranging from 24% to 3% (Table IV). The drugs most fre-

TABLE II. Source of Pre-Treatment Tumor Containing Specimens and Establishment of Cell Lines and Drug Sensitivity Testing

	NSCLC cell lines established		NSCLC fresh tumors with DST		SCLC cell lines with DST	
Specimen source	Number	%	Number	%	Number	%
Lung	5/69	7	5/69	7	2/3	33
Peripheral lymph nodes	13/30	43	9/30	30	9/21	43
Mediastinal lymph nodes	6/13	46	5/13	38	2/6	33
Pleural effusion	12/31	39	17/31	55	2/15	13
Liver	1/3	33	0/3	0	2/10	20
Bone marrow	0/3	0	0/3	0	17/44	39
Other	2/12	17	1/12	8	2/3	66
Total	39/161	24	37/161	23	36/102	35

TABLE III. Frequency of an Individual Chemotherapeutic Agent Yielding the Lowest Tumor Cell Survival and Being Selected as the Single Most Active Drug In Vitro Among 36 NSCLC Patients and 33 SCLC Patients With Drug Sensitivity Testing Data*

	NSCLC	SCLC
	Number (%)	Number (%)
CDDP	8 (22)	4 (12)
VP-16	8 (22)	10 (30)
NM	6 (16)	6 (18)
ADR	4 (11)	2 (6)
VCR	2 (5)	1 (3)
BCNU	3 (8)	8 (24)
MTX	1 (3)	2 (6)
VDS	1 (3)	
VBL	1 (3)	
MMC	1 (3)	
5-FU	2 (5)	
PROC	0 (0)	

*Nitrogen mustard (NM) was used as a surrogate for cyclophosphamide (CTX) and carmustine (BCNU) was substituted for orally administered lomustine (CCNU). Sums to greater than 100% due to one patient with equal cell survival for cyclophosphamide and doxorubicin for the most active agent.

TABLE IV. Frequency of Selection of Chemotherapy Combinations as the In Vitro Best Regimen From 36 NSCLC and 33 SCLC Patients With Drug Sensitivity Testing Data

	Number (%)
NSCLC regimens	
CDDP/VP-16	16 (45)
CAP	7 (19)
CMC	6 (16)
FOMi	1 (3)
FAM	4 (11)
VDS-PLAT	2 (5)
SCLC regimens	
CTX/CDDP/VP-16	8 (24)
CTX/ADR/VP-16	6 (18)
CTX/ADR/CDDP	3 (9)
ADR/CDDP/VP-16	3 (9)
CTX/VP-16/MTX	4 (12)
VAC	2 (6)
CTX/VCR/CCNU	4 (12)
CTX/MTX/CCNU	2 (6)
ADR/VCR/VP-16	1 (3)

quently selected in these combinations were CTX, VP-16, ADR, and CDDP (29, 22, 15, and 14 times, respectively).

DST was performed on 37 fresh tumor specimens from 36 NSCLC patients. In vitro testing also demonstrated considerable heterogeneity in NSCLC tumor cell sensitivity to the drugs tested as shown in Table III. CDDP and VP-16 were the most active agents against tumor cells each in 8 of 36 NSCLC patients. CDDP was an active drug against one-third of the tumor specimens tested with cell survival below 50%. While CDDP and VP-16 were most frequently selected as the single most active drugs tested, carmustine (BCNU), methotrexate, vincristine, vindesine, vinblastine, mitomycin C, and 5-fluorouracil were seldom selected as shown in Table III. Based on the use of nitrogen mustard (NM) as surrogate, CTX was considered an active agent in 27% of specimens tested and the single most active agent for 6 (16%) specimens. Of the eight combination chemotherapy regimens commonly used in NSCLC, six were selected as IVBRs for at least one patient as shown in Table IV, with VP-16-CDDP being the most active combination for 16/36 (44%) patients.

We also examined the correlation between in vitro cell survival of pairs of the seven individual drugs tested for both SCLC and NSCLC specimens as shown in Tables V and VI, respectively. Sensitivity among SCLC cell lines to VP-16 was well correlated with sensitivity to NM ($P = 0.0001$) and to MTX ($P = 0.002$). However, the fresh NSCLC tumor exhibits the greatest correlation between sensitivity to CDDP and NM ($P = 0.0001$). Sensitivity of NSCLC tumor to Mitomycin-C was also well correlated with sensitivity to NM ($P = 0.0005$) and Procarbazine ($P = 0.0006$).

For SCLC, two other drug pairs were also correlated with $P < 0.05$, but these correlations are not clearly significant when the standard Bonferroni correction is applied [33]. However, the large number of positive correlations (18 of 21, $P < 0.001$ by binomial distribution) suggests that small cell lines from untreated patients exhibit broad multidrug-sensitive or multidrug-resistant phenotypes. In NSCLC, DST results for 11 drug pairs were also correlated with $P < 0.01$, but as with SCLC, these correlations are not clearly significant when the standard Bonferroni correction is applied [33]. Again, the large number of positive correlations (64 of 66), suggests that NSCLC tumor from untreated patients also exhibits broad multidrug-sensitive or multidrug-resistant phenotypes.

Clinical Response to Administration of the IVBR-Selected Therapy

The IVBR was administered to 21 of 33 (64%) SCLC patients having DST data. Twelve other

TABLE V. Comparisons Using Spearman Correlations Between Pairs of Chemotherapeutic Agents Tested In Vitro in Small Cell Lung Cancer Cell Lines (r/p_2)

Drug	CDDP	ADR	NM	VCR	CCNU	MTX
VP16	0.30/0.13	0.24/0.19	0.67/0.0001	0.27/0.15	0.19/0.32	0.53/0.0022
CDDP		0.28/0.16	0.31/0.13	−0.016/0.94	0.14/0.49	0.21/0.31
ADR			0.38/0.033	0.12/0.51	0.12/0.52	0.21/0.26
NM				0.25/0.17	−0.013/0.94	0.48/0.0052
VCR					0.10/0.58	0.25/0.19
BCNU						−0.018/0.92

patients did not receive their IVBR because 7 died before week 13, 3 did not have the results of DST available until after week 13, one complete responder did not receive any therapy at relapse, and one remains in initial complete response. Administration of the IVBR induced a complete or partial response in 5 of 21 (24%) patients. None of the other 16 patients had a partial response when restaging was performed after 12 weeks of treatment. The three patients with DST data available only after week 13 who received VAC chemotherapy had no response to that therapy. An additional 65 patients (8 after relapse from CR and 57 at week 12) for whom DST data were not available received VAC chemotherapy. Five of the 68 (7%) had complete or partial responses after 12 weeks of treatment. Patients receiving their IVBR had marginally better response rates ($P = 0.06$) than those receiving VAC.

Of the 36 NSCLC patients with DST, 21 (58%) received their IVBR, 2 also had NE markers and were treated with a SCLC regimen, 6 had been treated empirically with VP-16-CDDP before DST data were available, and 7 were not treated. The 7 patients not treated included 3 Stage IIIA patients who initially underwent definitive chest radiotherapy, and 4 patients with metastatic disease. There was no significant difference in response rate between patients treated with their IVBR (2/21; 9%) and patients treated with empiric VP-16-CDDP in the absence of DST or NE markers (10/69; 14%) ($P = 0.73$) or in the comparison of all three treatment arms, including the 50% response rate among 6 patients treated with a SCLC regimen ($P = 0.08$).

Association Between Drug-Sensitivity Testing Data and Response to the In Vitro Best Regimen

The DST data of SCLC patients receiving their IVBR was compared with their response to this therapy as well as their response to initial therapy with VP-16-CDDP. The correlations between in vitro sensitivity to individual drugs and clinical response to primary therapy for the SCLC patients demonstrated significantly lower mean cell survivals to all 7 drugs tested ($P < 0.001$), the best three drugs ($P < 0.001$), as well as to VP-16, NM, and VCR (each $P < 0.02$), as shown in Table VII. SCLC patients responding to initial therapy with VP-16-CDDP had a mean cell survival of 32.9 ± 3.3% to their three best drugs compared to 69.9 ± 7% mean cell survival for patients with no response. The response to primary therapy in SCLC patients represents the relationship of the clinical responsiveness of the tumor in untreated patients to the in vitro sensitivity of the cell line immediately after the primary tumor was obtained for culture. This relationship was more impressive than the association between in vitro sensitivity and the clinical response to the IVBR which was administered after VP-16-CDDP therapy. Nevertheless, the fraction of active drugs (Fig. 3) and the combined mean percent cell survivals for all drugs (as a group) were significantly different for SCLC patients showing responses to their IVBR than for those with no response ($P = 0.018$ and 0.029, respectively). Neither the fraction of active drugs (Fig. 3) nor the combined mean percent cell survivals for all drugs were significantly different for clinically responding and non-responding NSCLC patients ($P = 0.86$ and 0.76, respectively). SCLC patients treated with their IVBR who responded did not have significant differences in mean in vitro cell survival to the drugs in the IVBR compared to non-responders (32.1 ± 6.3% vs. 47.8 ± 5.1%, respectively, $P = 0.15$). Similarly, NSCLC patients treated with their IVBR who responded did not have significant differences in mean in vitro cell survival to the drugs in the IVBR compared to non-responders (51.2 ± 0.7 vs. 52.0 ± 3.7%, respectively, $P = 0.86$). Among patients responding to their IVBR, the mean percent cell survival to each of the 7 drugs tested for both SCLC and NSCLC was lower for SCLC patients than for

TABLE VI. Comparisons Using Spearman Correlations Between Pairs of 12 Chemotherapeutic Agents Tested In Vitro in Non-Small Cell Lung Cancer Tumor Cells (r/p_2)

	VP-16	CTX	ADR	VCR	CCNU	MTX	VDS	VBL	MM-C	5FU	PROC
CDDP	0.50/0.0024	0.70/0.0001	0.35/0.05	0.51/0.0043	0.09/0.62	0.043/0.81	0.47/0.024	0.49/0.0050	0.47/0.0066	0.34/0.08	0.56/0.0041
VP-16		0.14/0.44	0.22/0.21	0.50/0.005	0.10/0.58	0.35/0.044	0.37/0.07	0.26/0.14	0.18/0.32	0.36/0.05	0.35/0.09
CTX			0.42/0.017	0.36/0.06	0.21/0.26	0.05/0.79	0.31/0.16	0.26/0.16	0.59/.0005	.22/.27	.45/.03
ADR				.39/.04	.09/.63	.36/.04	.21/.35	.50/.0044	.51/.0026	.27/.17	.48/.02
VCR					.13/.50	.17/.38	.58/.007	.45/.02	.49/.008	.43/.03	.50/.02
CCNU						.36/.05	.46/.04	.06/.74	.11/.56	−.16/.42	.22/.34
MTX							.28/.22	.04/.82	.28/.12	−.017/.93	.11/.64
VDS								.36/.11	.39/.06	.15/.53	.54/.03
VBL									.51/.0032	.40/.04	.49/.02
MM-C										.44/.02	.66/.0006
5FU											.19/.42

NSCLC patients, but these differences were not statistically significant.

In both SCLC and NSCLC, the chemosensitivity data for single drugs reveals a wide range of drug sensitivity among both responders and nonresponders to the IVBR. In the SCLC specimens, the mean cell survival is less than 50%, our definition of an active agent in this protocol, among responders for 6 of 7 drugs tested, as well as for VP-16 among nonresponders. In contrast, the NSCLC specimens have mean cell survival less than 50% among the responders only for CDDP and NM, reflecting a more drug resistant phenotype overall.

Drug Sensitivity Testing and Survival

The relationship between DST results and survival was compared using three approaches. In SCLC patients the mean percent cell survival to all agents tested was examined using a cutpoint value of 60% mean cell survival which approximated the median value. A cutpoint of 40% was used for the mean cell survival to the best three drugs, and a cutpoint of 30% was used for the mean fraction of active drugs. None of the differences between each two levels were significant ($P = 0.24$, 0.29, and 0.24 for all drugs tested, the three best drugs, and the fraction of active drugs, respectively). Preliminary data from the SCLC trial [9] had suggested that there was a trend toward longer survival among patients with lower in vitro cell survival; with further follow-up, this relationship is not significant. The relationship between survival and the mean percent cell survival of the tumor cells for all drugs tested is shown in Figure 4. Among SCLC patients having viable tumor reach the laboratory, growth of a tumor cell line was not associated with poorer survival ($P = 0.83$).

The relationship between DST results and survival in NSCLC patients was also examined in a similar fashion comparing survival probability by mean percent survival of all agents tested, mean cell survival of the best three drugs, and the mean fraction of active drugs. Cutpoints used to approximate the median values were 70, 50, and 16%, respectively. None of these differences were significant ($P = 0.38$, 0.50, and 0.49, respectively). The relationship between survival and the mean percent cell survival of the tumor cells for all drugs tested is shown in Figure 5.

Among the NSCLC patients treated with chemotherapy, survival measured from the beginning of treatment of patients treated with

TABLE VII. Relationship Between Drug Sensitivity Testing as Determined by Mean Tumor Cell Survival and Response to Primary Therapy With Etoposide/Cisplatin in Small Cell Lung Cancer Patients*

Drugs	Mean cell survival (%)		Wilcoxon rank sum (P_2)
	Responders (n = 20)	Non-responders (n = 6)	
VP-16	37.8 ± 5.4 (20)	74.8 ± 11.4 (5)	0.012
CDDP	59.7 ± 7.5 (15)	67.0 ± 8.0 (5)	0.60
ADR	58.2 ± 5.1 (20)	81.4 ± 5.6 (5)	0.062
NM	41.7 ± 5.1 (19)	77.3 ± 8.4 (6)	0.0034
VCR	66.5 ± 6.7 (19)	97.8 ± 1.7 (5)	0.0134
CCNU	56.0 ± 7.1 (20)	76.0 ± 4.5 (5)	0.23
MTX	73.5 ± 5.1 (19)	91.2 ± 3.7 (6)	0.06
Three best	32.9 ± 3.3 (20)	69.9 ± 7.0 (6)	0.0009
All 7	55.6 ± 2.6 (20)	82.1 ± 4.3 (6)	0.0009

*Values given are the mean ± standard error of the mean, with the number of patients in each category indicated in parentheses.

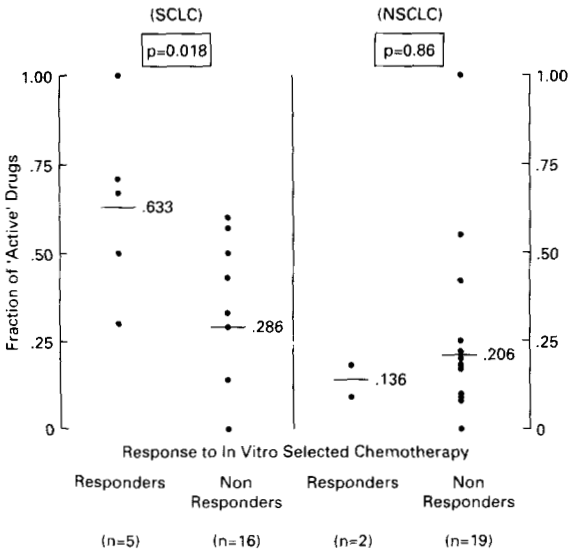

Fig. 3. The fraction of all chemotherapeutic agents tested which were considered "active," i.e., having less than 50% cell survival in vitro, is plotted by the patient's clinical response to treatment with the in vitro selected combination regimen for small cell lung cancer (SCLC) specimens and non-small cell lung cancer (NSCLC) specimens. The difference in the fraction of active drugs between patients responding to treatment and those not responding is significant for SCLC patients but not NSCLC patients.

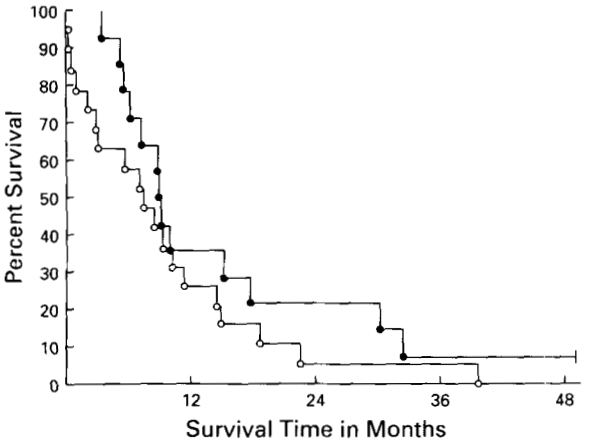

Fig. 4. Small cell lung cancer (SCLC) patient survival presented by survival of tumor cells in vitro. The median value of the mean percent SCLC tumor cell survival in vitro to all 7 drugs tested was 60%. Survival was not significantly improved in patients with lower mean percent cell survival in vitro ($P = 0.24$). ●, cell survival <60%; ○, cell survival >60%.

their IVBR was not different from patients treated with empiric chemotherapy ($P = 0.34$) or the SCLC regimen ($P = 0.83$) [18]. However, the ability to obtain DST data, which was associated with establishment of a cell line, was associated with a median survival from study entry of 7.2 months compared to 11.7 months for those patients who were unable to have DST performed ($P = 0.0018$). Having DST data did not affect survival after treatment among chemotherapy treated patients ($P = 0.56$). Immortalized cell lines were eventually obtained from 23 of the 37 specimens with DST data (62%) compared to only 16 of the 128 (12%) specimens in which DST could not be performed ($P < 0.0001$).

DISCUSSION

We designed protocols for treatment of lung cancer patients with individualized combination chemotherapy selected by in vitro DST of a sample of each patient's tumor. The decision to treat patients with chemotherapy was based on standard clinical practice. We determined that it was feasible to perform in vitro tumor analysis in a timely fashion, and that chemotherapy could be prospectively selected by these methods. The

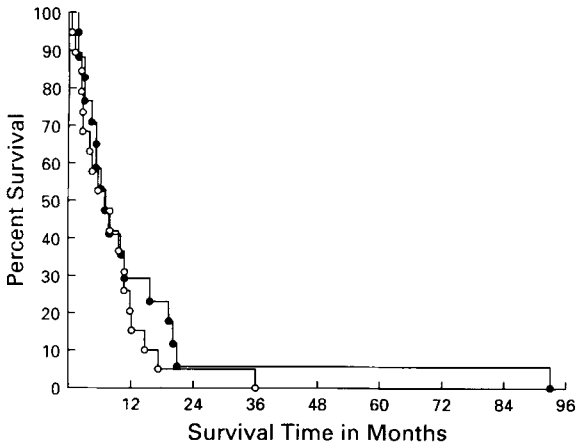

Fig. 5. Non-small cell lung cancer (NSCLC) patient survival presented by survival of tumor cells in vitro. The median value of the mean percent NSCLC tumor cell survival in vitro to all 12 drugs tested was 70%. Survival was not significantly improved in patients with lower mean percent cell survival in vitro ($P = 0.38$). ●, cell survival < 70%; ○, cell survival > 70%.

procedures involved are labor intensive and require close coordination of efforts between the medical oncologists, thoracic surgeons, pulmonologists, nurses, pathologists, and research laboratory personnel. Although we had in place an extremely efficient specimen collection system, state-of-the art culture methodology, and an in vitro assay that permitted tumor specimens to be tested, only 22% of all NSCLC patients and 18% of all SCLC patients entered on these protocols received individualized chemotherapy based on DST.

In vitro DST is limited by the requirement for an adequate number of tumor cells and short-term incubation in culture to obtain results. For the SCLC specimens, most of which were small biopsies or bone marrow aspirates, DST was performed by necessity on cell lines. The presence of any SCLC tumor cells was considered a tumor-containing specimen. In contrast, 10^6 tumor cells were required for an adequate NSCLC specimen. Selective culture of fresh NSCLC tumor cells was employed to obtain sufficient cells for testing. Subculturing and media which did not include matrix factors specifically excluded fibroblasts and benign epithelial cells. Consequently, harvesting a large tumor mass from a lung cancer resection or from a metastatic lesion sometimes yielded only a limited number of tumor cells. Since our objective was to test the activity of the chemotherapeutic agents against only tumor cells, this limited the number of specimens from which we were able to obtain DST results. Possible reasons for our ability to obtain DST on only 22% of the tumor-containing NSCLC specimens and 39% of the pretreatment tumor-containing SCLC specimens may include the use of media systems which may be suboptimal for the specific requirements of particular tumors.

Twenty-one of the 36 NSCLC patients (58%) with DST received their IVBR, and 7 patients (19%) were never treated with chemotherapy, demonstrating that it is possible to deliver individualized chemotherapy to 72% of patients treated with chemotherapy who have this data available. Of the 33 SCLC patients with DST data, 21 (64%) received their IVBR. Even with the small numbers of SCLC patients, the difference in response rates to treatment with an IVBR compared to treatment with empiric VAC achieved borderline significance. However, therapy was not randomized between these two patient groups. Modification of DST procedures to obtain results more quickly from small tumor samples could allow the benefit of individualized chemotherapy to be assessed in a larger patient population.

Among SCLC patients whose tumor reached the lab, there is no survival difference between patients who had tumor specimens from which cell lines grew vs. specimens which did not yield cell lines [34]. However, even though 62% of the NSCLC specimens with DST also eventually yielded cell lines, the ability of these tumor cells to survive short term, i.e., just long enough for DST to be performed, was associated with decreased survival in NSCLC patients. This is in concordance with our earlier findings of decreased survival among NSCLC patients from whom tumor cell lines were established [17]. This difference between SCLC and NSCLC cell line growth and patient survival may be related to the fact that cell culture techniques have been more successful with SCLC tumors than NSCLC. In addition, all of the SCLC patients had disseminated disease while 41% of NSCLC patients had localized disease. The finding that NSCLC tumor cell lines were more likely to be established from tumor from metastatic sites and that none were established from patients with pathologic Stage I NSCLC suggests that factors associated with the ability to survive in cell culture may contribute to the metastatic potential of NSCLC.

Multiple efforts are in progress to attempt to identify a subset of early stage NSCLC patients who are at the greatest risk for dissemination of

disease and to devise more effective treatments for SCLC resistant to therapy. DST revealed heterogeneity of response of individual cell cultures to the drugs tested, which supports a theoretical basis for administration of individualized therapy. The greater ease of obtaining SCLC tumor during routine diagnostic and staging procedures as well as the greater success of obtaining DST results supports further investigation of this approach in SCLC. However, the clinical activity of the chemotherapeutic agents currently used in NSCLC is so marginal that the distinction between more sensitive and less sensitive tumors in vitro is of little predictive value. This markedly limits the potential clinical benefit of an in vitro assay selected chemotherapy approach employing currently available agents. In contrast, the DST data for SCLC appears to predict clinical response to combination chemotherapy, particularly in previously untreated patients. The clinical response to primary therapy with VP-16-CDDP was associated with in vitro sensitivity to VP-16, but also with sensitivity to NM and VCR. This suggests that SCLC is generally sensitive or generally resistant to most of the drugs studied. Such clinical correlations support the use of unselected cell lines, including the ones derived from these studies, for in vitro drug screening and studies of drug resistance mechanisms. SCLC patients identified as having a drug resistant phenotype could be selected for investigational studies to attempt to improve their response rate. Unfortunately, it is not clear that this distinction is meaningful in NSCLC with currently available chemotherapeutic agents.

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