

A Randomized Phase II Pharmacokinetic and Pharmacodynamic Study of Indisulam as Second-Line Therapy in Patients with Advanced Non-Small Cell Lung Cancer

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Abstract Purpose: The primary aim of this study was to measure the objective tumor response rate following treatment with indisulam [E7070; *N*-(3-chloro-7-indolyl)-1,4-benzenedisulfonamide] as second-line therapy in patients with advanced non-small cell lung cancer. The secondary aims were to determine progression-free survival, to assess the safety and tolerability of indisulam, and to study its pharmacokinetic and pharmacodynamic profile.

Experimental Design: Patients were randomized to receive indisulam every 3 weeks either as a single i.v. dose of 700 mg/m² on day one (dx1) or 130 mg/m² given on days 1 to 5 inclusive as a daily infusion (dx5). All patients had previously received platinum-based chemotherapy.

Results: Forty-four patients were randomized. Only minor responses were seen. Myelosuppression, gastrointestinal symptoms, and lethargy were the most common toxicities and were more frequent in the dx1 arm. The pharmacokinetics of indisulam in each treatment schedule were adequately described using a previously developed population pharmacokinetic model and were mostly consistent with the results of the phase I program. Flow cytometric analysis of endobronchial and metastatic disease revealed a reduction in the fraction of cycling cells and an increase in apoptosis following indisulam compared with pretreatment levels.

Conclusions: We conclude that, despite evidence of tumor-specific indisulam-induced apoptosis, neither of these treatment schedules has single-agent activity as second-line treatment of non-small cell lung cancer.

Lung cancer is the leading cause of cancer death in the world and is associated with a 5-year survival of only 15% (1). For non-small cell lung cancer (NSCLC), surgery is the only curative option for early-stage disease. However, the majority of patients present with advanced disease, for which treatment is palliative. First-line platinum-based chemotherapy results in significant, although modest, improvement in clinical outcome (2–4). Second- or third-line therapies prolong survival when compared with supportive care alone but have objective response rates of only 7% to 9% (5, 6). There is clearly a

clinical need to develop effective second-line therapies that deliver improved response, survival, and toxicity profiles.

Indisulam (E7070) is a chloroindolyl sulfonamide that has activity in a wide range of human tumor cell lines and the PC-9 lung cancer xenograft in mice (7). Structurally, indisulam is similar to chloroquinoxaline sulfonamide, an antiproliferative compound for which clinical development was discontinued because of cardiac dysrhythmias and hypoglycemia (7). The exact molecular target of indisulam remains to be defined. In human NSCLC cell lines, treatment with indisulam induced G₁-S cell cycle arrest with up-regulation of p53 and p21 and subsequent apoptosis (8). Proteomic analysis suggests that indisulam may affect glucose metabolism and the malate-aspartate shuttle by inhibition of cytosolic malate dehydrogenase, although the relationship between this and the effect of indisulam on the cell cycle are unclear (9).

Phase I studies confirmed that reversible neutropenia and thrombocytopenia were the dose-limiting toxicities of indisulam. Mucositis and fatigue were also observed (10–14). In view of the structural similarity of indisulam to other sulphonamide agents, serum glucose and electrocardiogram monitoring were included in this study. Preclinical models suggested that the anticancer activity of indisulam is schedule dependent. The two i.v. schedules selected for evaluation were one dose of 700 mg/m² given on day one (dx1) or five doses of 130 mg/m² given on days 1 to 5 (dx5) repeated at three weekly intervals (11, 12). This multicenter randomized phase II study

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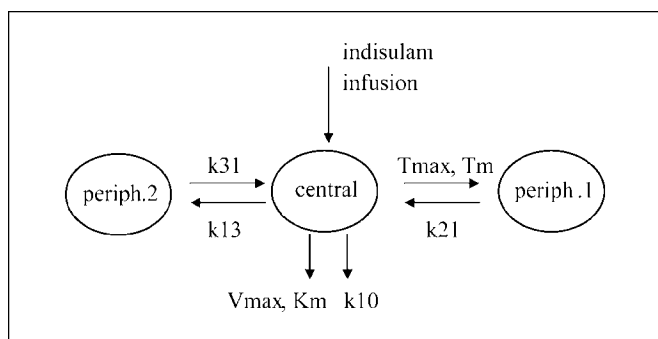


Fig. 1. Population pharmacokinetic model for indisulam. The model comprises three compartments (central, periph.1, and periph.2) with saturable distribution to one of the peripheral compartments and a linear and saturable pathway of elimination from the central compartment. V , central volume of distribution; T_{max} , maximal distribution rate; T_m , Michaelis Menten constant of distribution; k_{21} , distribution rate constant periph.1 \rightarrow central; k_{13} , distribution rate constant central \rightarrow periph.2; k_{31} , distribution rate constant periph.2 \rightarrow central; K_m , Michaelis Menten constant of elimination; V_{max} , maximal elimination rate; k_{10} , elimination rate constant.

was done to explore the efficacy and toxicity of indisulam and to identify which schedule was more active in platinum pretreated NSCLC. Sequential endobronchial and metastatic tumor samples were taken to analyze the effects of indisulam on the cell cycle *in vivo*.

Materials and Methods

Patient selection. Patients over the age of 18 years with pathologically confirmed stage IIIB or IV NSCLC who had relapsed following or progressed during treatment with a first-line platinum containing chemotherapy regimen were included. Patients could not have received chemotherapy, radiotherapy (other than palliative radiotherapy to a nonmeasurable lesion), or investigational treatment within 4 weeks of study entry (or 6 weeks for nitrosoureas, mitomycin C, or melphalan). Criteria for inclusion were as follows: bidimensionally measurable disease outside a radiotherapy field, Karnofsky performance status of 70% or higher, life expectancy of >4 months, adequate hematologic function (hemoglobin >9 g/dL, neutrophils $>1.5 \times 10^9/L$, and platelets $>100 \times 10^9/L$), renal function (serum creatinine $<1.5 \times$ upper normal limit or creatinine clearance ≥ 60 mL/min), and hepatic function [serum bilirubin $<2.5 \mu\text{mol/L}$ and alanine transaminase or aspartate transaminase $\leq 2.5 \times$ upper normal limit ($\leq 5 \times$ upper normal limit in the presence of liver metastasis)]. Patients with central nervous system metastases, uncontrolled infection, cardiac dysfunction, or serious concomitant medical conditions were excluded. In view of possible drug interactions with indisulam, patients were excluded if they had received any of the following drugs within 2 weeks of enrollment: coumarin anticoagulants, terfenadine, cisapride, cyclosporin, tacrolimus, theophyllines, diazepam, sulfonylurea hypoglycemic agents, phenytoin, or carbamazepine. Patients were also excluded if they were pregnant or breast-feeding. Adequate contraception was required where appropriate. The study was given approval by local research ethics committees. All patients were required to give written informed consent. At one center (Oxford, United Kingdom), patients with endobronchial tumors who consented to two additional bronchoscopies for tumor sampling could be entered into a corresponding pharmacodynamic study.

Study design and end points. This study was an open label, multicenter, randomized, comparative phase II study of indisulam administered by two different dosing regimens. The primary objective of the study was to measure the objective tumor response rate within

each dosing regimen. The design of the study incorporated a single-stage testing procedure as described by Gehan (15). With 44 patients randomized, the probability of the study leading to an erroneous rejection of the drug having a true second-line response rate of at least 10% is 0.098. Within each dosing regimen, the hypothesis that the true response rate is at least 10% would be rejected if there were fewer than 2 responses in 22 patients.

Secondary objectives of the study were duration of response, progression-free survival, median survival, and the safety and tolerability of indisulam. The pharmacokinetics of each schedule of indisulam were evaluated from a previously described population model (16, 17). Treatment-related changes in cell cycle progression and apoptosis were assessed before and after treatment with indisulam in a subgroup of patients.

Treatment plan. Patients were screened within 14 days of enrollment and randomized on a 1:1 basis to receive indisulam according to the dx1 or dx5 schedule. Indisulam was administered i.v. over 60 min. Toxicity was evaluated weekly using the National Cancer Institute Common Toxicity Criteria, version 2. Treatment was delayed if the neutrophil count was $\leq 1 \times 10^9/L$ or the platelets were $\leq 100 \times 10^9/L$ on day 1 of treatment or until treatment-related toxicity had returned to baseline or \leq grade 2. A maximum delay of 2 weeks was permitted. A 25% dose reduction was made to all subsequent cycles following grade 4 toxicity, except for anemia or lymphopenia. Serum glucose was measured before, at completion of, and 2.5 h after the first indisulam infusion. Tumor size was assessed by computed tomography or magnetic resonance imaging every two cycles, and response was documented using WHO criteria by independent review. Treatment was continued until disease progression or unacceptable toxicity.

Pharmacokinetics. Pharmacokinetics were evaluated in all patients during the first three cycles of treatment. Sampling time points were identified from an established population pharmacokinetics model using the D-optimality algorithm (16, 17). For the dx1 regimen, a total of six blood samples were collected: predose; at the end of infusion; and 2.5, 7, 94, and 168 h after the start of infusion. For the dx5 regimen, a total of six blood samples were collected: predose on days 1 and 5; at the end of the infusion and 1.5 h after the start of infusion on day 5; and 72 and 168 h after the start of the fifth infusion. The concentration of indisulam was determined in plasma as described previously (16).

Pharmacokinetic analyses were done using NONMEM software (version V, level 1.1; GloboMax LLC, Hanover, MD). The population model described previously (16) comprises three compartments with saturable distribution to one of the peripheral compartments and a linear and saturable pathway of elimination from the central compartment shown in Fig. 1. The pharmacokinetic variables describing this model were reestimated using the data obtained from this study in combination with the pharmacokinetic data from the phase I program. To establish whether pharmacokinetic variables differed significantly, a covariate indicating that data were obtained from this study was evaluated for each pharmacokinetic variable. Data were logarithmically transformed and analyzed using the first-order method. Individual Bayesian pharmacokinetic variables were calculated using the post hoc option in NONMEM.

Table 1. Patient characteristics

Schedule	dx1	dx5
Males	18 (78%)	17 (81%)
Females	5 (22%)	4 (19%)
Median age (range), y	58 (35-76)	61 (44-74)
Karnofsky performance status	80 (70-100)	80 (70-100)
Median (range)		
70-80%	12 (55%)	11 (52%)
90-100%	10 (45%)	10 (48%)
NSCLC stage IIIB	10 (45%)	10 (48%)
stage IV	12 (55%)	11 (52%)

Table 2. Grade 3 and 4 toxicities

Schedule	dx1 (%)	dx5 (%)
Hematologic toxicity		
Neutropenia	5 (23)	0
Thrombocytopenia	4 (18)	0
Febrile neutropenia	1 (5)	0
Anemia	4 (18)	1 (5)
Biochemical toxicity		
Hyperbilirubinemia	2 (9)	0
Hyponatremia	7 (32)	0
Hypokalemia	1 (5)	0
Hyperkalemia	1 (5)	0
Raised γ -glutamyl transferase	4 (18)	3 (14)
Hypoalbuminemia	2 (9)	0
Hyperuricemia	2 (9)	0
Other toxicity		
Fatigue/lethargy	2 (9)	2 (9)
Asthenia	2 (9)	1 (5)
Injection site reaction	1 (5)	0
Mucositis	2 (9)	0
Hemorrhagic stroke	1 (5)	0
Pulmonary septic syndrome	1 (5)	0
Candidiasis	1 (5)	0

Pharmacodynamic studies. Cells from bronchial brushings and washings were collected from 14 untreated patients with NSCLC as a control group and from three patients before and 48 h after indisulam treatment. Samples were taken from the endobronchial tumor and from the contralateral bronchus as controls. Samples were centrifuged and washed in PBS. For accessible soft-tissue metastasis, fresh core biopsy and fine-needle aspiration samples were disaggregated in PBS, trypsin, and collagenase before fixation. Cell pellets were fixed in cold ethanol, washed, and resuspended in PBS containing propidium iodide and RNase A. Samples were analyzed by flow cytometry (FACSCalibur, Becton Dickinson, San Jose, CA) using a 488 nm laser for excitation. Red fluorescence data, indicative for DNA content, were collected for

up to 10,000 cells per sample and analyzed using CellQuest software (Becton Dickinson). A gate was set using forward light scatter to exclude subcellular fragments, and the proportion of cells undergoing apoptosis in each sample was estimated by quantification of cells with an apparent DNA content less than that of diploid cells in G₁. This value was determined by reference to the sample from the unaffected bronchus.

Results

Forty-four patients from eight centers were entered into the study (23 to the dx1 schedule and 21 to the dx5 schedule). Patient characteristics are shown in Table 1. The groups were well balanced with respect to age, stage, gender, and performance status. Twenty-one (48%) patients had responded to previous platinum-based chemotherapy and 9 (20%) patients had progressed during prior treatment. Patients received a median number of two cycles on the dx1 schedule (range, 1-10) and two cycles on the dx5 (range, 1-4).

Toxicity. Eight patients experienced treatment-related serious adverse events (seven on the dx1 schedule and one on the dx5 schedule). In general, adverse events were more common among patients receiving the dx1 schedule. Two patients withdrew from the study as a result of adverse events. One patient (dx1 schedule) had a combination of asthenia, herpetic stomatitis, and anemia. The second patient (dx5 schedule) developed seizures due to cerebral metastases. Three patients on the dx1 arm and two patients on the dx5 arm had dose reductions based on grade 4 toxicity. The most common grade 1-2 toxicities were nausea, vomiting, constipation, diarrhea, asthenia, and injection site reactions. Eight patients on the dx1 schedule had an increase in serum bilirubin immediately following treatment with indisulam, of whom two experienced grade 3 hyperbilirubinemia. In all but one case, hyperbilirubinemia was transient, occurred ~2.5 h after indisulam

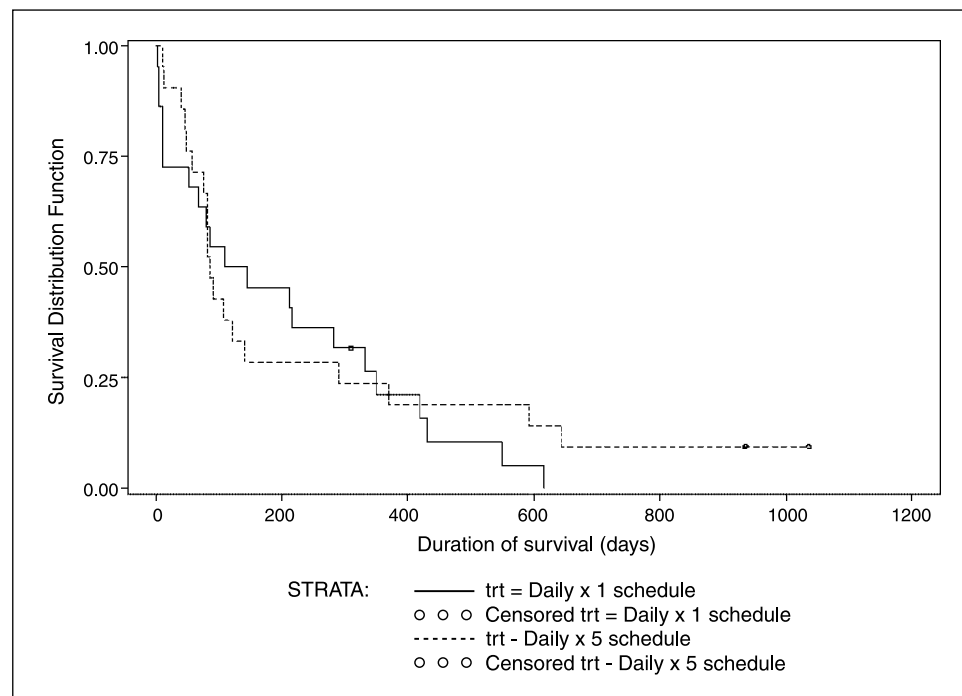
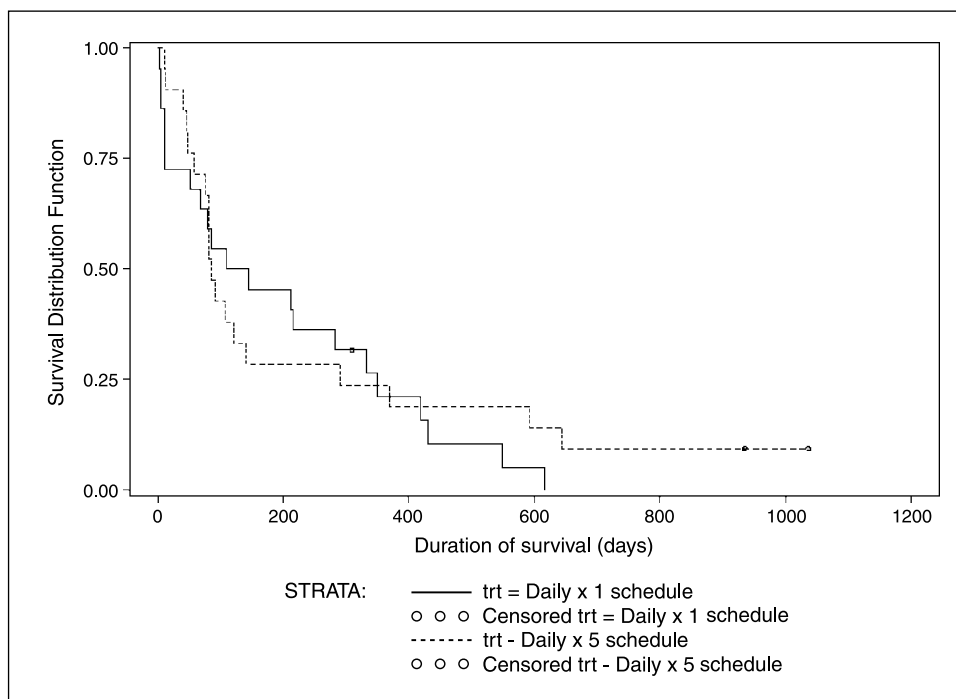


Fig. 2. Progression-free survival. Kaplan Meier plot of progression-free survival following indisulam given as a daily \times 1 schedule (—) or as a daily \times 5 schedule (- - - -).

Fig. 3. Overall survival. Kaplan Meier plot of overall survival following indisulam given as a daily \times 1 schedule (—) or as a daily \times 5 schedule (- - - -).



administration, was not accompanied by elevations in other liver function tests, and had resolved by day 8. There were no episodes of tachyarrhythmia or hypoglycemia (blood sugar \leq 3 mmol/L), although three cases of grade 3 hyperglycemia were seen. Data on grade 3 and 4 toxicity are presented in Table 2.

Response and survival. Only minor responses were seen on independent radiological review. The best response to treatment based on WHO criteria were as follows: (a) dx1 schedule: stable disease 8 (36%) and progressive disease 14 (64%) and (b) dx5 schedule: stable disease 5 (23%) and progressive disease 17 (77%). Two patients treated on the dx1 schedule had disease stabilization for $>$ 12 weeks (5.7 and 6.2 months). Survival at 6 months was 45% for patients on dx1 and 29% on the dx5 schedule. Kaplan-Meier estimates of progression-free and overall survival are presented in Figs. 2 and 3, respectively.

Pharmacokinetics. Data from all patients were included in the analysis. Variable estimates for this study were not significantly different from those of the phase I studies, except for the value of k_{13} , which was \sim 32% lower (Table 3). For a typical individual with a body surface area of 1.8 m², this would result in a 19% and 16% increase in drug exposure for patients receiving the dx1 and dx5 schedule, respectively. Plasma concentration versus time profiles for individual patients treated with the dx1 and the dx5 schedule are depicted in Fig. 4. In Fig. 5, the model predicted and the individual predicted concentrations are plotted against the observed plasma concentrations. The symmetrical distribution around the line of identity indicates that the population pharmacokinetic model adequately describes the pharmacokinetic profile of indisulam in both treatment schedules. Median area under the concentration time curve was 2.9×10^3 h mg/L (interquartile range, 1.9×10^3 - 3.4×10^3 h mg/L) and 1.8×10^3 h mg/L (1.0×10^3 - 2.2×10^3 h mg/L) for the dx1 and dx5 schedules, respectively.

Pharmacodynamic studies. Data from control patients who did not receive indisulam showed that NSCLC cells *in vivo* exhibit a large variability in $>G_1$ (aneuploidy or active cycling) compared with the contralateral unaffected lung (Table 4). In some endobronchial tumor samples, an aneuploid tumor population was clearly evident and distinguishable from the diploid epithelial component based on its increased DNA content. There was also a trend toward a higher proportion of $<G_1$ (apoptotic) cells in the affected lung, although the difference was not statistically significant. Three patients consented to flow cytometric analysis before and after indisulam treatment. Of these, two patients had bronchial brushings and washings and one patient had fine-needle aspiration and biopsy of a cytologically confirmed 3 cm diameter subcutaneous metastasis. Following treatment with indisulam, endobronchial

Table 3. Summary of estimated pharmacokinetic variables

PK variable	Variable estimate	Interindividual variability (%)
V (L)	7.3	26
T_{max} (mg/h)	25.7	43
T_m (mg/L)	2.18	127
k_{21} (h ⁻¹)	0.0735	60
k_{13} (h ⁻¹)	0.889* 0.607 [†]	31
k_{31} (h ⁻¹)	0.387	20
k_m (mg/L)	0.712	61
V_{max} (mg/h)	3.39	130
k_{10} (h ⁻¹)	0.0241	26

Abbreviation: PK, pharmacokinetic.

*Phase I.

[†]Current study.

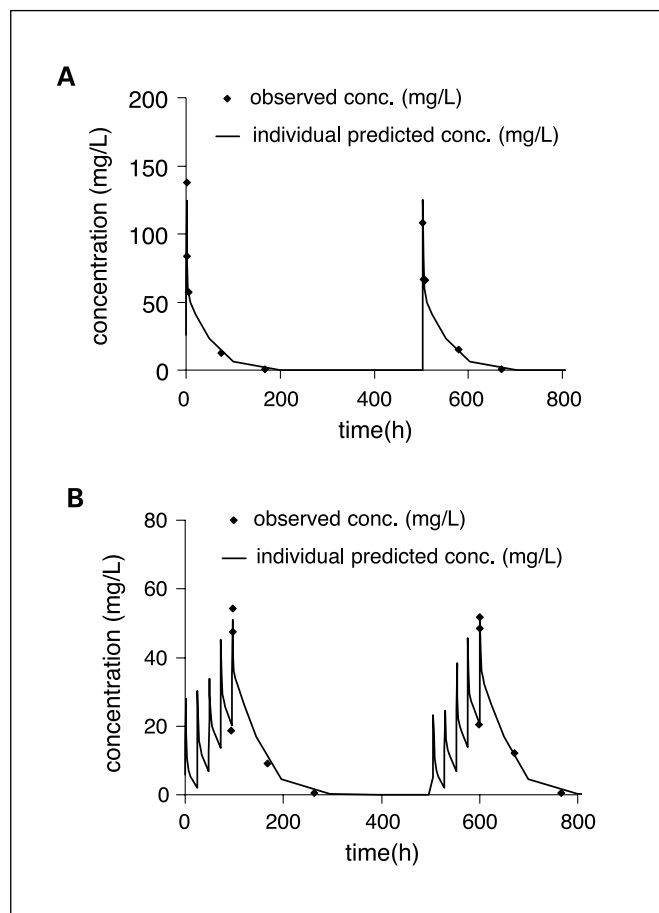


Fig. 4. Indisulam plasma concentration versus time profiles for individual patients (◆) and the profile predicted (16) by the population pharmacokinetic model (—). *A*, daily × 1 schedule. *B*, daily × 5 schedule.

tumor cells showed a large decrease in the >G₁ fraction and an increase in the <G₁ apoptotic fraction (Fig. 6). Fine-needle aspiration of the metastasis yielded insufficient tissue for flow cytometry. However, cells derived from core biopsies could be analyzed by flow cytometry. This showed that s.c. metastases had a low proportion of normal diploid cells and high levels of presumably apoptotic cells present before treatment. The apoptotic fraction of tumor cells in metastatic deposits increased substantially 48 h after indisulam treatment. A substantial decrease in the >G₁ fraction was also noted. All three patients had stable disease on radiological response criteria.

Table 4. Flow cytometry of bronchial washings and bronchial brushings

Tissue sample	Mean <G ₁ , % (range)	Mean >G ₁ , % (range)
BW-normal	5.9 (1.65-18.17)	9.3 (3.47-16.89)
BW-tumor	11.1 (1.87-24.99)	13.0 (5.37-37.73)
BB-normal	4.6 (0.84-8.1)	12.9 (4.01-17.28)
BB-tumor	9.2 (3.44-19.67)	29.0 (11.98-66.75)

Abbreviations: BW, bronchial washings; BB, bronchial brushings; tumor, samples from endobronchial tumor; normal, samples from contralateral, unaffected bronchus.

Discussion

The results of this study suggest that indisulam as a single agent at the doses and schedules investigated has limited ability to induce objective responses for patients with NSCLC treated previously with platinum-based chemotherapy. Although several patients experienced a reduction in the volume of their disease, this was usually transient and benefit was short lived. Disease stabilization beyond 5 months was observed in two patients receiving the dx1 schedule. The low response rate is consistent with other agents used in second-line therapy (5, 6). In general, treatment was well tolerated. Two patients withdrew from the study as a result of adverse events. These were more common on the dx1 schedule, although as only five patients on the dx5 schedule received more than two courses, this may reflect the number of courses administered. Myelosuppression was moderate with 23% of patients experiencing grade 3 or 4 neutropenia. One patient died during an episode of neutropenic sepsis. Although transient episodes of hypoglycemia were noted during the phase I program, hypoglycemia and arrhythmias were not a feature of indisulam therapy in this study. The dx1 schedule was associated with mild injection site reactions, which were less pronounced when indisulam was made up in 1,000 mL, rather than 500 mL, of saline. Several biochemical abnormalities were recorded, particularly hyponatremia. This may have been due to NSCLC, a worsening nutritional state or indisulam-induced inhibition of specific carbonic anhydrase isoenzymes, including those in the kidney (18).

The pharmacokinetic profile of indisulam was adequately described by the previously developed and validated population pharmacokinetic model (16). Although the value of the

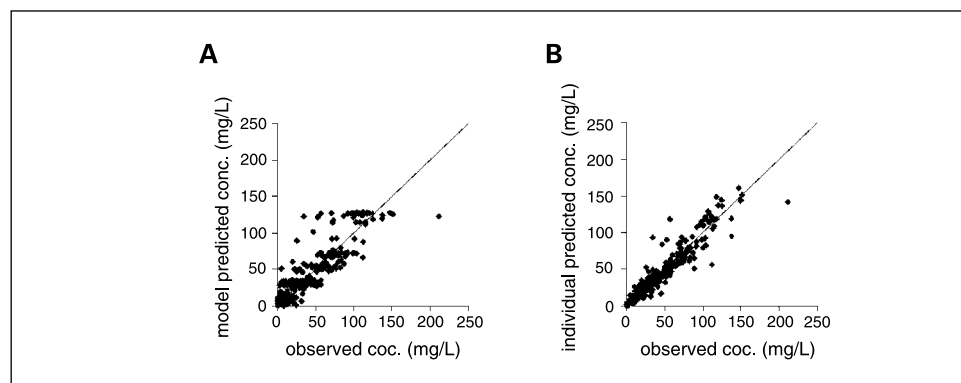
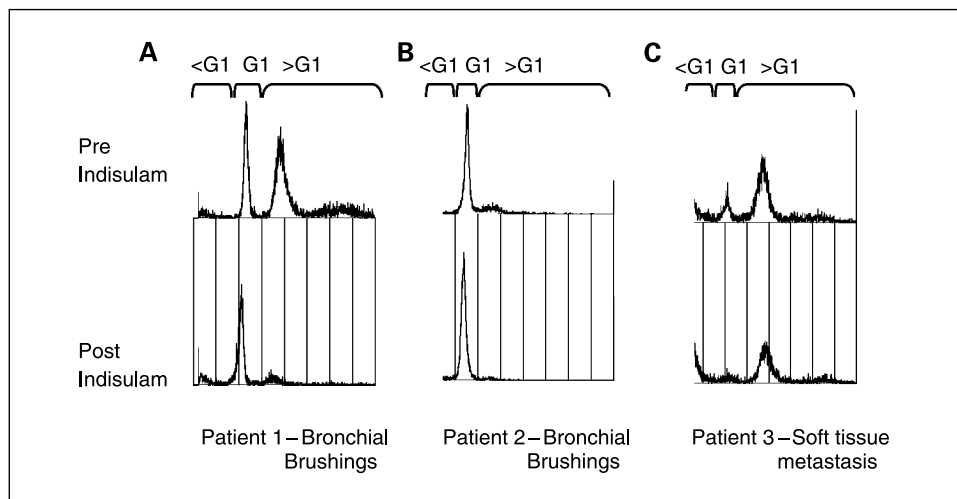


Fig. 5. Goodness-of-fit plots of the population pharmacokinetic data. Model predicted concentrations (*A*) and individual predicted concentrations (*B*) are plotted against the observed values for all treatment cycles and for both regimens. The symmetrical distribution around the line of unity indicates that these data are adequately described by the model.

Fig. 6. Flow cytometry of tumor cells before, and following, indisulam treatment. Distribution of cellular DNA: <math><G_1</math> (apoptotic); A and *B*, endobronchial tumor cells pretreatment (*top*) and following indisulam (*bottom*) for two individual patients. *C*, biopsy from soft tissue metastases pretreatment (*top*) and following indisulam (*bottom*).



microconstant k13 was $\sim 32\%$ lower in this study compared with the value obtained from the phase I studies, the relevance of this finding is difficult to interpret. The data from this study were sparsely sampled; therefore, it is difficult to establish distribution microconstants with high precision. However, the reduction in k13 was significant and has a clinically relevant effect on the overall exposure to indisulam. Nonetheless, these effects were minimal compared with the large interpatient variability in exposure.

We believe that this is the first study to report flow cytometry of serial endobronchial tumor samples in NSCLC. This study suggests that bronchial brushings are more sensitive than bronchial washings as an indicator of drug effect. There was considerable overlap in terms of the results from bronchial washings taken from healthy control lung and the tumor-affected lung. The proportions of cycling and apoptotic cells were markedly different between primary bronchial samples and metastatic tumor. Tumor samples from three patients obtained pre-indisulam and post-indisulam showed an increase in $<G_1$ fraction and a reduction in $>G_1$ cycling (aneuploid) fraction. This was not accompanied by a decrease in radiological size of the tumors. One possible explanation for this apparent discrepancy may be that these changes are transient and were not maintained long enough following treatment to produce tumor response. Due to the limited amount of material

available, the only criterion used for the assessment of apoptosis in these samples was the measurement of sub- G_1 DNA content. Use of phospho-specific anti-retinoblastoma antibodies to evaluate phosphorylated retinoblastoma in patients with head and neck cancer treated with indisulam has been reported (19). Three of five patients had informative results where a reduction in posttreatment retinoblastoma phosphorylation occurred. As in our study, this pharmacodynamic end point did not correlate with clinical tumor response. Apparently, lack of persistence in the pharmacodynamic effect may account for the absence of relationship to clinical outcome.

Although no objective responses to indisulam were documented in this setting, this study has provided valuable information about the effects on the cell cycle of indisulam *in vivo*. It also shows that it is possible to use flow cytometry of bronchial brushings from NSCLC patients to monitor the effect of novel therapies on the cell cycle. Further phase II studies of indisulam in a range of tumor types as a single agent and in combination with other therapies are ongoing. No further exploration of the dx5 schedule is planned.

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