A Phase I Trial and *In Vitro* Studies Combining ABT-751 with Carboplatin in Previously Treated Non-Small Cell Lung Cancer Patients

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Abstract

Background—ABT-751 is a novel antimitotic agent that confers cytotoxic effects in pre-clinical studies. Carboplatin has efficacy in treating advanced non-small cell lung cancer (NSCLC) in combination with other drugs.

Methods—Lung cancer cell lines were treated with ABT-751 and/or carboplatin to investigate their impact on cell growth. Phase I study with an expansion cohort was conducted in previously treated NSCLC patients. The primary objective was the maximum tolerated dose (MTD), secondary objectives were objective response rates, median survival, time to tumor progression (TTP), dose-limiting toxicities (DLTs), and pharmacodynamic evaluation of buccal swabs.

Results—Combining ABT-751 with carboplatin significantly reduced growth and induced apoptosis in lung cancer cell lines. Twenty advanced NSCLC patients were enrolled. MTD was ABT-751 125 mg orally twice daily for 7 days with carboplatin AUC 6. DLTs included fatigue, ileus, neutropenia and pneumonitis. Two patients had confirmed partial responses. Median overall survival was 11.7 months (95% CI 5.9–27.0). TTP was 2.8 months (95% CI 2.0–2.7). Four of 6 patients showed decreased cyclin D1 protein in post-treatment versus pre-treatment buccal swabs.

Conclusion—Combining ABT-751 with carboplatin suppressed growth of lung cancer cell lines and had modest clinical anti-tumor activity in advanced NSCLC previously treated predominantly with carboplatin. Further studies of this combination are not recommended while investigations of biomarkers in different patient populations, alternative schedules and combinations may be pursued.

Keywords

ABT-751; carboplatin; phase I clinical trial; non-small cell lung cancer

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INTRODUCTION

Lung cancer is the leading cause of cancer death globally and in the United States (U.S.) [1]. Improved outcomes in the treatment of advanced stages of lung cancer can occur with combination chemotherapy regimens. Agents that act at different phases or inhibit specific components of the cell cycle are often combined. This can enhance anti-neoplastic activity of the regimen as compared to the anti-tumor effects of each agent used in the regimen. Two-drug platinum-based combinations have emerged as a standard treatment option for patients with advanced non-small cell lung cancer (NSCLC) [2–4]. Carboplatin or cisplatin combined with a microtubule-interfering drug (paclitaxel, docetaxel or vinorelbine) or with an antimetabolite (gemcitabine, pemetrexed) were compared in several large randomized trials conducted in the U.S. and worldwide [5–8]. These studies demonstrated similar efficacy in response rates, overall and one-year survival with minor differences in toxicities. Unfortunately, all patients with advanced NSCLC relapse [9]. Once this occurs, median survival is typically between 4 and 8 months [10–13].

The U.S. FDA has approved three drugs (pemetrexed, docetaxel and erlotinib) for use in second-line treatment of NSCLC. While these agents have different mechanisms of action and toxicities, the benefits are similar, with response rates of 8% and median survival in randomized studies improving to 8 months [14]. More effective treatments are needed for relapsed NSCLC.

Microtubules are a target of multiple chemotherapy agents including vinca alkaloids and taxanes. By binding to tubulin and disrupting microtubule dynamics, these agents block the cell cycle at the G2/M phase and induce apoptosis. However, the efficacy of these agents is limited by the development of resistance [15]. ABT-751 (previously known as E7010) is a novel oral sulfonamide [16] that binds the colchicine-binding site of beta-tubulin thereby inhibiting microtubule polymerization and leading to G2/M arrest [17]. In addition to its cytotoxic effects, ABT-751 also demonstrated vascular disrupting effects by decreasing tumor perfusion in animal studies [18, 19]. Preclinical studies of ABT-751 showed anti-tumor activity against multiple rodent tumor cell lines and in xenograft models of human tumors including in gastric, colon, breast and lung cancer [20, 21]. Notably, ABT-751 is not a multi-drug resistance transporter substrate and shows activity in cell lines that are resistant to taxanes and vinca alkaloids [17, 22]. Five phase I studies of ABT-751 have been performed in pediatric and adult patients with advanced malignancies [23–27]. Study results support fixed dosing (i.e. non-body surface area-based dosing) [25] and suggest that divided dose scheduling best maintained blood levels of the drug [26]. The recommended phase II dose schedule was 150 mg orally twice daily given for 7 days every 3 weeks [25].

ABT-751 is well-tolerated, and dose-limiting toxicities (DLTs) in the adult population are peripheral neuropathy and ileus [23–27]. A phase IB study of ABT-751 and docetaxel in patients with castrate-resistant prostate cancer had DLTs of febrile neutropenia, diarrhea and nausea [28]. Interestingly, ABT-751 demonstrated limited myelosuppression, thus it may be safely combined with myelosuppressing chemotherapies [23–25].

Preclinical studies of ABT-751 in combination with other cytotoxic therapies in xenograft models of NSCLC and colon cancer have shown additive or greater cooperativity [29]. In the Calu-6 lung cancer model, ABT-751 in combination with cisplatin exhibited greater than additive responses for all three studied doses of ABT-751 tested (Abbott, data on file). In clinical practice in the US, carboplatin has become a standard drug for combination therapy for advanced NSCLC. Moreover, it has a more favorable toxicity profile than cisplatin [30, 31].

Based on their anti-tumor activity and their favorable toxicity profile, we proposed that the combination of ABT-751 and carboplatin might prove effective in treating advanced NSCLC patients. Therefore, we conducted a phase I trial with an expansion cohort of the combination of ABT-751 and carboplatin in previously treated patients with advanced NSCLC. The primary objective was to evaluate the MTD of escalating doses of ABT-751 when used in combination with carboplatin. Secondary endpoints were objective response rates after two cycles of treatment, median survival, time to tumor progression (TTP), DLTs and side effects profile, along with pharmacodynamic evaluations.

PATIENTS & METHODS

Cell Culture, Proliferation, Apoptosis and Immunoblot Assays

Human lung cancer cell lines HOP62, A549 and U1517 were each cultured in RPMI 1640 media supplemented with 10% fetal bovine serum (FBS), 100 U/ml penicillin and streptomycin at 37°C with 5% CO_2 in a humidified incubator. ABT-751 (Abbott, Abbott Park, IL) was dissolved in dimethyl sulfoxide (DMSO) and carboplatin (Sigma-Aldrich, St. Louis, MO) was dissolved in water. For proliferation assays, HOP62, A549 and U1517 cells were independently seeded at 4×10^3 per well in 6-well culture plates and treated with the indicated drugs 24 hours later. Experiments were done in triplicates and results were replicated in independent experiments. Logarithmically growing cells were assayed 72 hours following drug treatment using the CellTiter-Glo growth assay (Promega, Madison, WI) according to previously established methods [32]. For apoptosis and immunoblot assays, HOP62 and A549 were independently treated with vehicle alone, ABT-751 alone, carboplatin alone or the combination of these drugs. Apoptosis was measured by the Annexin V-FITC signal by flow cytometry using the Annexin V:FITC Assay Kit (AbD Serotec, Raleigh, NC) according to manufacturer's protocol. For immunoblot assays, cells lysates were harvested 72 hours after the indicated drug treatments and subjected to individual immunoblot analysis as previously described [33] using an antibody against cyclin B1 (H-433, Santa Cruz Biotechnology, Santa Cruz, CA), cyclin D1 (M-20, Santa Cruz Biotechnology, Santa Cruz, CA) or actin (C-11, Santa Cruz Biotechnology, Santa Cruz, CA).

Patients

Adult patients (Age >18) with histologically-confirmed diagnosis of advanced stage non-small cell lung cancer (IIIB with malignant pleural effusion or Stage IV) with at least one, but not more than two prior chemotherapy regimens were eligible for enrollment if there were at least one measurable lesion for Response Evaluation Criteria in Solid Tumors (RECIST) tumor assessments and they had adequate hematologic, renal and hepatic function, with Karnofsky Performance Score of 70–90. Prior treatment with a platinum agent was allowed. Exclusion criteria included untreated central nervous system metastases, neuropathy, GI abnormalities, concurrent colchicine therapy, and cytotoxic chemotherapy within three weeks of initiating the investigational treatment. The protocol was approved by the Institutional Review Board (IRB) at Dartmouth College. Voluntary written informed consent was obtained prior to any screening or study-specific procedures. The trial was registered on the ClinicalTrials.gov website as NCT00735878.

Treatment and Dose Escalation

ABT-751 was supplied as 25 mg and 100 mg capsules by Abbott Laboratories. To maximize the clinical tolerability of combining carboplatin with ABT-751, a twice daily regimen for 7 out of 21 days was chosen. The carboplatin dose was given on day 4 of the first cycle to allow for a detailed pharmacodynamic assessment, and on day 1 of subsequent cycles. All cycles were 21 days. For the phase I portion of the study, a dose-escalation trial design was

chosen based on prior phase I data using the rapid dose escalation model (Table 1) [34]. Cohorts of one patient each for the first 3 dose levels were treated. If one patient completed cycle 1 without experiencing grade 2 toxicity, then enrollment at the next dose level commenced. If the patient experienced grade 2 toxicity or higher, then two additional patients were enrolled at the same dose level. At dose level 4, a minimum of three patients was to be enrolled. If one of the 3 patients experienced a grade 3 or higher toxicity, the cohort was to be expanded to 6 patients. However, if 3 patients completed one cycle at the assigned dose without experiencing a grade 3 or higher toxicity, then the next cohort would begin treatment. Once the MTD was established, that dose level was planned to be expanded with an additional 10 patients to assess efficacy and potential toxicities.

Toxicities and Definition of MTD and DLTs

Toxicities were defined by the National Cancer Institute Common Toxicity Criteria version 3.0 [35]. MTD was defined as the highest dose of ABT-751 given in combination with carboplatin at which less than one third of patients experienced a DLT at the end of cycle 1. DLTs were defined as an episode of febrile neutropenia (grade 4 neutropenia (<500/µL) lasting more than six days or any episode of febrile neutropenia or neutropenia with documented infection or sepsis), grade 3 or higher thrombocytopenia (<50,000/µL) that occurred on the day of scheduled treatment, grade 3 or higher non-hematologic toxicity (except nausea/vomiting or anaphylactoid reactions) or inability to begin the next cycle of therapy within 2 weeks of its scheduled start due to delayed recovery. Any non-reversible toxicity greater than grade 2 was considered a DLT.

Patients had assessments for treatment-related toxicity on days 1, 4, and 8 of the first cycle and at the start of each subsequent cycle. Tumor assessments were performed every two cycles. Disease progression was determined using RECIST [36]. Survival information was gathered at three month intervals after the last study visit.

Pharmacodynamic Studies

Six patients treated at the MTD underwent pharmacodynamic analysis with buccal mucosa swabs on day 0 (before treatment) and days 4, 8, and 22 of treatment. The studied swabs were analyzed for cyclin B1 and cyclin D1 protein expression by immunoblot analysis, as previously described [33]. Immunoblot analyses were conducted with densitometry to determine cyclin B1 and cyclin D1 levels relative to actin expression as a loading control and as a confirmation of protein integrity.

Statistical Methods

For *in vitro* cancer cell line studies, changes in growth were evaluated by two-sample two-tailed t-test using Microsoft Excel software, and the comparison among the growth suppressive effect of etoposide, gemcitabine and carboplatin were evaluated by one-way ANOVA using Graphpad Prism. $P < 0.05$ was considered statistically significant. For the clinical trial, analyses of baseline patient characteristics, efficacy and safety were performed for all patients. Response rates along with confidence intervals were determined. Median and progression-free survival (PFS) were determined using the Kaplan-Meier method, as before [33].

RESULTS

Effects of Combining ABT-751 with Carboplatin in Lung Cancer Cell Lines

A549 and HOP62 lung cancer cell lines were independently examined for growth inhibitory responses after 3 days of treatment at drug dosages that were at the highest relative peak plasma values observed in prior clinical trials for etoposide (8.6 ug/ml), gemcitabine (114

μM) and carboplatin (39 ug/ml) [37–39]. While growth of each cell line was repressed by more than 75% as compared to controls, there was not any significant difference observed among these treatments (data not shown). Carboplatin was chosen to study in combination with ABT-751 because greater than additive anti-tumor effects with a platinum/ABT-751 combination were observed in a murine cancer model [29]. Individual and combined anti-neoplastic effects of ABT-751 and carboplatin treatments on cancer cell growth were investigated in the human lung cancer cell lines HOP62, A549 and U1571. Subtherapeutic drug concentrations were chosen for study to search for enhancing effects on growth and gene expression in these cell lines after combining ABT-751 with carboplatin. In all of these cell lines, significant growth suppression was observed after combining ABT-751 (200 nM) with carboplatin (5 μM) as compared to ABT-751 alone or carboplatin alone (Figure 1). We further studied if this growth suppression was associated with apoptosis induction and expression of cell cycle regulators in HOP62 and A549 cells. The combined treatment significantly increased apoptosis induction as compared to controls for both HOP62 and A549 cells as well as a decline in expression of cell cycle regulators cyclin B1 and cyclin D1 in HOP62 cells, but not in A549 cells.

Patient Characteristics

Between September 2004 and August 2008, 20 patients with advanced NSCLC were enrolled onto this trial. The study was administratively closed by the sponsor before it reached full accrual when a larger study of ABT 751 in combination with a diffent chemotherapy agent, pemetrexed, did not meet its efficacy objectives in previously treated lung cancer. Baseline patient characteristics are presented in Table 2. All patients had prior treatment for their lung cancer, and 80% had prior platinum-based therapy (75% carboplatin, 5% cisplatin). The median interval since the prior platinum exposure was 10 months (range 2–26 months). One patient was found to have disease progression in the brain on the same day as study enrollment and was therefore not treated with this regimen. Therefore, 19 patients were evaluable for toxicity and efficacy.

Safety and Toxicity

For the phase I portion, 12 patients were enrolled at the dose levels shown in Table 1. No DLTs were observed in the patients enrolled at the first three dose levels. Non-dose limiting toxicities beyond cycle 1 experienced by these patients included grade 2 anemia, and grade 2 thrombocytopenia. Treatment was held for two weeks for persistent grade 2 thrombocytopenia in one patient. At dose level 4 one patient experienced DLT of grade 3 fatigue with the first cycle, and had a dose-reduction for carboplatin to the first dosage level for subsequent cycles. Another patient had DLTs of a grade 3 ileus, as well as grade 4 neutropenia requiring hospitalization with the first cycle. A third patient treated at the level 4 dose, had grade 4 pneumonitis, grade 3 hypokalemia and hyponatremia. Given these findings, the MTD was proposed as dose level 3, and the institutional Safety and Data Monitoring Committee recommended that additional patients be enrolled at level 3, prior to completing the phase I portion. None of these patients had DLTs with the first cycle. Non-dose limiting toxicities that were noted at this level included grade 2 constipation in two patients, as well as single reports of grade 2 dysphagia, grade 3 hypokalemia and grade 2 thrombocytopenia. One patient in this group required a dose reduction of carboplatin for grade 2 thrombocytopenia. Another patient had two one week delays in treatment: one due to a hospitalization for pneumonia and the other for evaluation of new dyspnea. The MTD was confirmed to be dose level 3 (ABT 751 125 mg twice daily for 7 days with carboplatin AUC 6) and was chosen as the recommended phase II treatment dose for the expansion portion of the trial. Overall toxicity data are summarized in Table 3.

The median number of cycles of ABT-751 administered in the phase I portion was 2 cycles (range, 1–5) and the overall median number of cycles for the trial was 3 (range, 1–7). For the phase I portion there were 7 treatment delays due to: grade 2 thrombocytopenia, hospitalization for non-treatment related events (neurosurgical procedure and pneumonia), grade 2 fatigue and grade 2 neuropathy. Of the 12 patients who were treated on the phase I portion, 7 discontinued the study drug due to progression of disease. Another patient was advised to come off study after experiencing grade 4 pneumonitis, 1 patient came off the study for worsening performance status and a third patient discontinued treatment for worsening grade 2 neuropathy. Two patients withdrew consent without citing any specific toxicity.

Seven patients were enrolled in the expansion portion of the study at dosage level 3. They received a median of 5 cycles of treatment (range, 2–7). There were 7 delays in administration of cycles of chemotherapy due to: grade 2 thrombocytopenia, grade 2 back pain, grade 2 fatigue, grade 2 and 3 neutropenia, grade 2 neuropathy, grade 3 weakness and grade 3 nausea. The dosage of the study drug was reduced in two patients. One had grade 2 thrombocytopenia that persisted despite a reduction in the carboplatin dose. The other patient had grade 2 neuropathy. Five patients discontinued the study drug due to disease progression. Two patients completed at least 4 cycles before stopping without evidence of progression. Overall, after discontinuation of the study drug, 17 subjects (85%) went on to receive additional therapeutic regimens.

Efficacy

Two patients (11%) had partial responses after two cycles: one at dose level 2 and the other at dose level 4. The durations of responses in these subjects were 14 and 28 weeks, respectively. Both subjects had received prior platinum-containing regimens, one with cisplatin and progression of disease as best response, 19 months prior to study enrollment, and one with carboplatin and partial response as best response, 11 months prior to enrollment. There was no association between the interval from or the response to prior platinum treatment and the outcome from the study treatment. All 3 patients who had not had prior platinum agent exposure achieved stable disease as best response to the study combination. Efficacy endpoints for the 11 patients treated at the recommended phase II dose are summarized in Table 4. Seven patients had stable disease and 4 patients progressed. The median TTP was 2.8 months (95% CI 2.0–2.7). The median overall survival was 11.7 months (95% CI 5.9–27.0). The median PFS was 2.8 months (95% CI 1.3–5.2).

Pharmacodynamic Analysis of Cyclin D1 in Buccal Swabs

Cyclin D1 levels were evaluated by immunoblot analyses of pretreatment (Day 0) and paired post-treatment (Day 4, 8, and 22) buccal swabs. Relative cyclin D1 levels were obtained by normalizing cyclin D1 levels to actin levels. Six paired buccal swab samples were obtained from patients treated at the phase II dosage. All of these patients had stable disease after treatment with the study regimen. Out of these 6 paired buccal swabs, 3 showed decline in cyclin D1 expression on day 8, and 4 showed a decline on day 22, relative to baseline cyclin D1 levels (Figure 2). There was not enough protein in the samples for analysis of cyclin B1 expression.

DISCUSSION

Treatment of advanced NSCLC is a major challenge in oncology. While increasing attention to personalized therapy based on individual tumor and patient characteristics holds great promise, treatment outcomes with current agents need to be improved. This phase I trial combined a well-established agent in the treatment of advanced lung cancer, carboplatin,

with a novel antimicrotubule agent, ABT-751, which was shown in preclinical studies to be active against lung cancer cells and xenograft tumors [29]. ABT-751 is reported as less susceptible to drug resistance mechanisms described against other antimicrotubule agents [17, 22]. We studied a twice daily dosing schedule for 1 week of a three week cycle and this trial established a MTD and recommended phase II dose of ABT-751 125 mg twice a day with carboplatin at an AUC of 6. The drug combination was safe and well-tolerated. The DLTs were ileus, fatigue and neutropenia. Two patients (11%) had confirmed partial response after two cycles. The median survival was 11.7 months (95% CI 5.9–27.0) and the TTP was 2.8 months (95% CI 2.0–2.7).

In the phase I clinical trial, the most common side effects were constipation (grade 1/2 in 52% of patients), thrombocytopenia (47% grade 1/2 and 11% grade 3), anemia (63%, all grade 1/2), nausea (37% grade 1/2, 5% grade 3) and fatigue (42% grade 1/2 and 5% grade 3). Overall, this toxicity profile for ABT-751 was consistent with other trials of this agent with gastrointestinal side effects as the most prominent side effect observed [40, 41]. Previous trials of single agent ABT-751 were notable for the lack of myelosuppression [23–25, 40]. While there are few single agent carboplatin trials in lung cancer for direct comparison, the rates of myelosuppression in our trial are similar to published results [42]. This suggests that myelosuppression was likely from carboplatin and the addition of ABT-751 did not enhance this appreciably. This is in contrast to other current therapies for advanced NSCLC. For example, the combination of paclitaxel, carboplatin and bevacizumab was notable for a relatively high degree of myelosuppression (25% grade 4 neutropenia) and bleeding events, but not for gastrointestinal toxicity [43]. Thus, the toxicity profile reported here does appear distinct and makes ABT-751 a potential agent for combination therapies, especially with those which cause myelosuppression as their adverse effects.

The combination of ABT-751 and carboplatin demonstrated modest activity against NSCLC with 2 patients out of 19 (11%) achieving a confirmed partial response after two cycles. As the majority of the study patients had received prior carboplatin, this activity likely reflects the benefits of the combination, although a benefit from the rechallenge with carboplatin could not be excluded, given the relatively long interval from the prior platinum exposure. This is similar to the 8% response rate seen with the currently approved therapies beyond first line. a After the initiation of our combination study, two multicenter trials of ABT-751 with docetaxel or pemetrexed compared to chemotherapy alone in the second line setting were opened. A different schedule of ABT-751 given once daily for 14 days was chosen based on more recent data. The ABT-751/docetaxel trial was terminated early due to excess toxicities. The ABT-751/pemetrexed trial did not establish effectiveness in treating unselected NSCLC patients, but showed a trend of improved outcome for the squamous NSCLC subpopulation [41]. We could not identify any specific subset of patients with NSCLC to derive differential benefit from treatment with our drug combination. In our clinical trial, we noticed that the twice daily schedule used in our study was difficult for some patients to comply with although it was tolerated. Yet, the once daily schedule of ABT-751 was well tolerated [41]. Therefore, if future studies of ABT-751 with carboplatin in lung cancer are conducted, alternative schedules, such as once daily dosing, should be considered. Moreover, since most patients in our study had been previously treated with carboplatin, inclusion of chemotherapy naïve patients and excluding previous carboplatin treatment will allow a more precise assessment of the efficacy of the combination in this different study population.

In a search for biomarkers that would predict responses to ABT-751 and carboplatin combination treatment, we evaluated expression of cyclins since both ABT-751 and carboplatin induce cell cycle arrest. In our *in vitro* studies, combining ABT-751 with carboplatin significantly decreased human lung cancer cell growth and significantly induced

apoptosis. This drug combination led to a substantial cyclin D1 repression and a trend of cyclin B1 suppression in HOP62 cells. A549 cells did not show suppression of cyclin B1 or cyclin D1 expression after these drug treatments, suggesting that the association of growth inhibition with suppression of these cyclins may be cell context-dependent at the dosage examined and that other mechanisms including apoptosis induction may play a greater role in reducing tumor cell proliferation. In addition, since subtherapeutic dosages were used to explore cooperation between these two drugs, it is possible that cyclins would be repressed upon drug treatments at higher dosages. In our clinical study, we evaluated cyclin D1 levels in 6 paired pre-treatment versus post-treatment buccal swabs. Four of these 6 specimens showed a decrease in cyclin D1 protein expression in the day 22 post-treatment as compared to the pre-treatment swabs. To evaluate whether cyclin D1 was a clinically-relevant biomarker for this drug regimen, we compared these decreased cyclin D1 levels to the observed clinical responses. All of these 6 patients had stable disease independent of the changes in cyclin D1 levels. Based on these findings, cyclin D1 may not serve as a clinically-predictive biomarker of anti-tumor response in this trial population. Further investigations are needed to explore other candidate biomarkers, such as circulating tumor cells, squamous cell carcinoma antigen and cytokeratin 19 fragment antigen 21-1 that were highlighted in other ABT-751 clinical trials [27, 41].

In conclusion, the combination of ABT-751 and carboplatin markedly suppressed lung cancer cell growth *in vitro*, and had modest anti-tumor activity in advanced NSCLC patients previously treated with carboplatin. Our findings suggest that additional investigation of this combination in lung cancer is not recommended. ABT-751 is clinically well-tolerated and further studies in different patient populations, such as chemotherapy-naive lung cancer patients, in alternative schedules, in other tumor types, and in combination with other therapies may be pursued only after a successful search for biomarkers that reliably predict clinical responses to ABT-751 treatment and its combination with other therapies.

Acknowledgments

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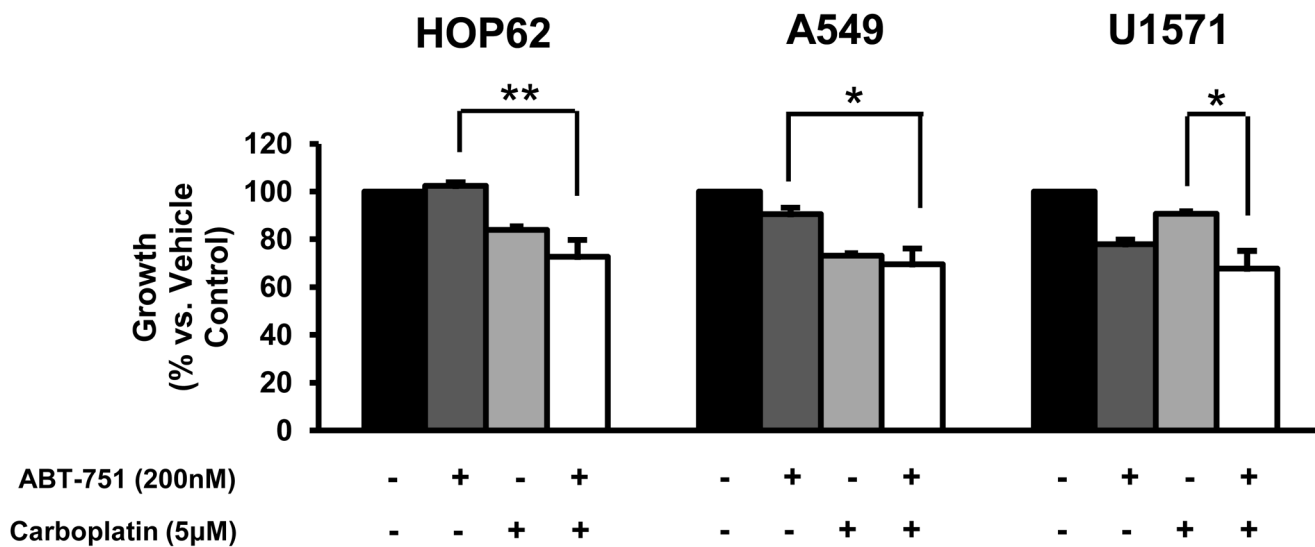
Figure 1.
Effects of ABT-751 and carboplatin on cell growth in the indicated human lung cancer cell lines. Effects of individual and combined treatments of ABT-751 and carboplatin at the indicated dosages for 3 days on cell growth of human lung cancer cell lines HOP62, A549 and U1571. The symbols indicate $P < 0.05$ (*) and $P < 0.01$(**), respectively.
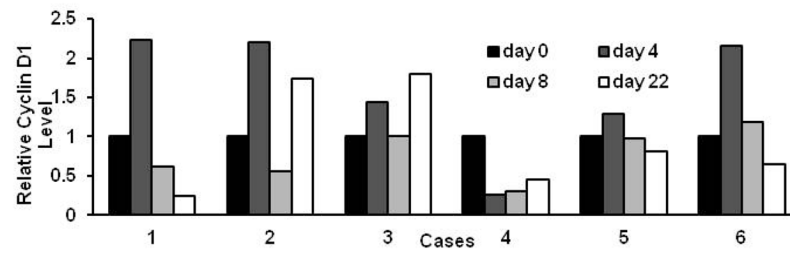
Figure 2.
Relative cyclin D1 expression (normalized to actin as a loading control) in pre-treatment (day 0) versus post-treatment buccal swabs (days 4, 8, and 22).

Table 1

Dose Escalation Scheme

Dose Level (Cohort)	ABT-751 mg BID (not mg/m^2)	Carboplatin AUC	Planned enrollment	Enrolled patients
1	100	4.5	1–6	1
2	125	4.5	1–6	1
3[##]	125	6	1–6	5
4	150	6	3–6	5
5	175	6	3–6	0
6	200	6	3–6	0
Expansion cohort	MTD	MTD	10	7

[##]After dose-limiting toxicities (DLTs) were identified at dose level 4, additional 4 patients were enrolled at the level 3 dose (patients 10, 11, 12, 13).

Table 2

Patient Characteristics

N=20	
Characteristic	**Number (Percent %)**
Age (years)	
Median	64
Range	47–73
Gender	
Male	13 (65)
Female	7 (35)
Race	
White	20 (100)
Stage	
IIIB	1 (5)
IV	18 (95)
Karnofsky Perfomance Score	
100	0
90	1 (5)
80	19 (95)
Number of Prior Lines of Therapy	
1	9 (45)
2	11 (55)
Prior Radiation Therapy	12 (60)
Histology	
Adenocarcinoma	14 (70)
Squamous cell Carcinoma	5 (25)
Poorly Differentiated Carcinoma	1 (5)
Post Study Treament	
Yes	17 (85)
No	3 (15)

TABLE 3

Overall Toxicities (19 evaluable patients)

Hematologic	Grade 1/2 (%)	Grade 3 (%)	Grade 4 (%)
Anemia	12 (63)	0	0
Leukopenia	2 (11)	0	0
Neutropenia	5 (26)	2 (11)	1 (5)
Thrombocytopenia	9 (47)	2 (11)	1 (5)
Febrile Neutropenia	0	0	0
Non-hematologic			
Confusion	1 (5)	0	0
Constipation	10 (52)	0	0
Dehydration	1 (5)	0	0
Dyspepsia	2 (11)	0	0
Fatigue	8 (42)	4 (21)	0
Hyperlipidemia	3 (16)	0	0
Hypokalemia	4 (21)	2 (11)	0
Hyponatremia	9 (47)	1 (5)	0
Hypophosphatemia	3 (16)	0	0
Ileus	1 (5)	1 (5)	0
Infection (no neutropenia)	0	2 (11)	1 (5)
Myalgia	1 (5)	0	0
Nausea/Vomiting	7 (37)	1 (5)	0
Neurological symptoms	1 (5)	1 (5)	0
Neuropathy	3 (16)	0	0
Pain	6 (32)	2 (11)	0
Pneumonitis	0	0	1 (5)
Rash	1 (5)	0	0
Transaminase elevation	1 (5)	0	0
Weakness	0	2 (11)	0

Table 4

Summary of Efficacy of Patients on Level 3 Dosages

Endpoint	Number (Percent)
Response at two cycles (RECIST criteria)	
Complete Response	0 (0)
Partial Response	0 (0)
Stable Disease	7 (64)
Progressive Disease	4 (36)
Time to Progression	
Median (months)	2.8
95% CI	(2.0–3.7)
Overall Survival	
Median (months)	11.7
95% CI	(5.9–27.0)
Progression-Free Survival	
Median (months)	2.83
95% CI	(1.3–5.2)