Therapeutic Synergy of Oral Taxane BMS-275183 and Cetuximab versus Human Tumor Xenografts

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ABSTRACT

Purpose: Combination therapy consisting of an oral taxane, BMS-275183, and the anti-epidermal growth factor receptor monoclonal antibody, cetuximab, was assessed for enhanced therapeutic benefit in preclinical tumor models.

Experimental Design: Mice bearing human tumor xenografts, either L2987 lung or GEO colon carcinoma, were administered the aforementioned treatments singly or in combination regimens. Delays in tumor growth and tumor-free status were evaluated and combination treatments were assessed relative to optimal solo treatments.

Results: Combination therapies with the oral taxane plus cetuximab were tolerated and therapeutic synergistic outcomes obtained. The therapeutic enhancements were >1 log cell kill greater than the antitumor effect caused by either solo agent applied optimally. For example, at the maximum-tolerated dose of BMS-275183, 60 mg/kg/administration, given p.o. once every 3 days for a total of six administrations (q3d/H115476), 1.0 gross log cell kill was achieved in mice bearing well established (100 to 200 mg) s.c. implanted L2987 tumors. Cetuximab, at an optimal dose of 1 mg/mouse, given i.p. q3d×6, produced 1.3 log cell kill. When cetuximab, 1 mg/mouse, i.p., plus BMS-275183, 25 mg/kg/administration, p.o., were both given q3d×6, the result was 2.6 log cell kill with three of eight mice cured (P < 0.01). Similar efficacy benefits were obtained in the GEO tumor model.

Conclusions: The combination of oral taxane BMS-275183 plus cetuximab provided therapeutically synergistic antitumor activity in two different human tumor xenograft models. Clinical evaluation of this combination is recommended.

INTRODUCTION

The taxanes are best known by the first two marketed representatives of this chemical class, Taxol and Taxotere. Neither drug is orally bioavailable without being coadministered with some facilitating agent or processed into nanoparticles. BMS-275183 is the first of several orally active taxanes to enter clinical trial (1–4). It has been found in phase I testing to have notable activity in heavily pretreated patients with non–small-cell lung cancer (NSCLC), including those pretreated with Taxotere and/or Iressa (gefitinib), a small molecule anti-epidermal growth factor receptor (EGFR) inhibitor (3, 4).

Several phase II studies are planned for BMS-275183, including one or more involving NSCLC patients. The prospect of combining the oral taxane with other drugs approved for the treatment of NSCLC or with the potential for such approval was of particular interest. One such combination involved the anti-EGFR monoclonal antibody, cetuximab.

Herbst and Bunn (5) recently reviewed the potential utility of EGFR inhibitors in the clinical management of NSCLC. They concluded that small molecule (e.g., gefitinib) EGFR inhibitors have shown single agent activity against advanced, chemorefractory NSCLC. However, in clinical trials comparing these EGFR inhibitors combined with chemotherapy to chemotherapy alone, no additional benefit was discerned.

With regard to cetuximab, preclinically, it has been shown to potentiate the antitumor effects of many cytotoxic agents in vivo with tumor models sensitive to EGFR inhibition (6–10). Clinically, the combination of irinotecan and cetuximab in irinotecan-refractory EGFR-expressing metastatic colorectal cancer patients was quite effective (11, 12), and cetuximab has recently been approved for use in combination with irinotecan for just this indication. Cetuximab, in combination with gemcitabine, has shown promising activity against advanced pancreatic cancer (13). Response rates in several phase II NSCLC studies involving cetuximab plus platinum-containing chemotherapy have been characterized as encouraging, and the question posed whether an anti-EGFR antibody such as cetuximab would be more synergistic with chemotherapy than small molecule EGFR inhibitors (14).

Accordingly, in vivo antitumor testing of BMS-275183 in combination with the anti-EGFR monoclonal antibody, cetuximab, was performed in mice bearing EGFR-expressing human tumor xenografts.

MATERIALS AND METHODS

Compounds. BMS-275183 was synthesized by Bristol-Myers Squibb chemists. It was dissolved initially in equal portions of Cremophor EL and ethanol, followed by aqueous dilution to yield final ethanol and Cremophor EL concentrations of 10%. Cetuximab (also known as Erbitux) was a gift of ImClone Systems. The antibody was dissolved in PBS for i.p. injection to mice. BMS-275183 was administered to mice in a volume of 0.01 mL/g body weight based on the average weight of the mice in each group at the time of treatment. Cetuximab was administered in 0.5 mL on a per mouse basis.

Received 5/28/04; revised 7/14/04; accepted 7/21/04.
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Animals. Athymic (nude) mice, 5 to 6 weeks of age, purchased from Harlan Sprague Dawley (Indianapolis, IN), were quarantined for ~2 weeks before their use for tumor propagation and drug efficacy testing. They were fed food and water ad libitum. All studies involving these animals were conducted in accordance with NIH (Bethesda, MD) and BMS animal care and use guidelines.

Tumors. Human L2987 lung and GEO colon carcinomas were maintained in nude mice by serial s.c. passage. All efficacy testing involved tumors implanted s.c. in nude mice. Treatment was initiated when tumors had become well established at between 100 to 200 mg.

The L2987 human lung carcinoma has been characterized as positive for the EGFR and borderline levels of antitumor activity for cetuximab have been described previously (10). Similarly, GEO human colon carcinoma is also known to be EGFR positive and among the responsive tumor models to cetuximab (7, 15). The EGFR-positive characterization of the tumors used in the present studies was confirmed by immunohistochemistry (data not shown).

The L2987 is a lung adenocarcinoma isolated from a pleural effusion and was developed at BMS/Oncogene Co (16). GEO is a colon carcinoma obtained as a gift from Michael Brattain (17).

Antitumor Testing. Therapeutic results are presented in terms of either cures (see definition below) and/or primary tumor growth inhibition determined by calculating the relative median times for drug-treated (T) and control (C) or groups of mice to grow tumors of 1-g target size and expressed as T-C values (in days). Delays in tumor growth are also converted to gross log cell kill values by dividing the T-C value by the tumor volume doubling time multiplied by 3.32 [i.e., T-C value/tumor volume doubling time] × 3.32]. Weight in milligrams was estimated by the formula, weight in mg = a × b², where “a” is the length of a tumor and “b” is the width expressed in millimeters. Statistical evaluations of data were performed with Gehan’s generalized Wilcoxon test for comparisons of median time to reach tumor target size (18). Statistical significance was declared at P < 0.05. Group sizes typically consisted of eight mice. Activity was defined as ≥1 log cell kill. Cured mice are defined as those whose tumors are ≤35 mg when assessed 10 days after termination of treatment. Histologic confirmation of cures was not performed.

A regimen was described as toxic if more than one mouse died during or within 10 days after the final drug treatment or anytime after the initiation of drug therapy and whose tumor size at the time of death was <1 g. No control mice died bearing tumors < 1 g. Groups of mice with more than one death attributable to drug toxicity were considered to have had excessively toxic treatments, and their data were not used in the evaluation of a compound’s antitumor efficacy. A maximum-tolerated dose (MTD) was defined as one whose toxicity approached but did not attain the degree of lethality just described as being excessive. Therapeutic results were reported at the optimal dose, i.e., that yielding the best effect without exceeding the MTD.

Therapeutic synergy represents a therapeutic effect achieved with a tolerated regimen of a combination treatment that exceeds the optimal effect achieved at any tolerated dose of monotherapy associated with the same drugs used in the combination (19–21).

When BMS-275183 and cetuximab were both administered to mice, they were given essentially simultaneously, with no attempt at any particular sequence applied.

RESULTS

L2987 Human Lung Carcinoma. In the first of two experiments, BMS-275183 was administered p.o. to mice bearing established L2987 tumors. The highest dose administered, 60 mg/kg/administration, every third day for four administrations (i.e., q3d×4) was tolerated and did not challenge the historical MTD of ~300 mg/kg cumulative exposure established with a similar schedule (22). The treatment produced 0.6 log cell kill, an effect not quite in the active range. Cetuximab administered at 1 mg/mouse, i.p., q3d×4, was well tolerated and produced 0.6 log cell kill, similar to the borderline effect achieved with the oral taxane. The results of this initial study, as well as the combination treatment data to be described, are summarized in Table 1.

Therapeutic enhancement was observed with the combination of oral taxane plus cetuximab. Whereas each highest dose monotherapy was associated with 0.6 log cell kill, combinations of 1 mg/mouse of cetuximab with either 25 or 40 mg/kg/administration of the oral taxane yielded 1.3 and 1.9 log cell kill, respectively. The latter combination also produced two of eight mice cured and was significantly better (P < 0.01) than the best

<table>
<thead>
<tr>
<th>Treatment*</th>
<th>Average body weight change, g†</th>
<th>T-C (days)‡</th>
<th>Log cell kill (cures/total)§</th>
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</thead>
<tbody>
<tr>
<td>BMS-275183</td>
<td>Cetuximab</td>
<td></td>
<td></td>
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<tr>
<td>Initial study</td>
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<td>60</td>
<td>1.0</td>
<td>14.0</td>
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<td>5.5</td>
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<td>14.3</td>
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<td>40</td>
<td>0.9</td>
<td>43.0</td>
<td>1.9 (2/8)</td>
</tr>
<tr>
<td>25</td>
<td>1</td>
<td>2.7</td>
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<tr>
<td>Confirmatory study</td>
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<tr>
<td>60</td>
<td>−0.9</td>
<td>28.0</td>
<td>1.0</td>
</tr>
<tr>
<td>40</td>
<td>1.5</td>
<td>14.5</td>
<td>0.5</td>
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<td>36.5</td>
<td>1.3</td>
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<td>25</td>
<td>1.2</td>
<td>57.8</td>
<td>2.0 (1/8)</td>
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<tr>
<td>25</td>
<td>1.1</td>
<td>71.0</td>
<td>2.6 (3/8)‡</td>
</tr>
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* In mg/kg/administration, p.o., for BMS-275183 and mg/mouse, i.p., for cetuximab. In the initial study, BMS-275183 and cetuximab were administered q3d×4. All treatments began on day 22 after tumor implant. In the confirmatory study, BMS-275183 and cetuximab were given q3d×6, and all treatments began on day 20 after tumor implant. Group sizes = eight mice
† Determined from beginning to end of treatment period. Controls gained 2.0 g in the initial study and 1.8 g in the confirmatory study.
‡ T-C values are based on relative median times (in days) for treated (T) and control (C) mice to reach tumors of 1.0 g.
§ Log cell kill and cures/total, if any, the definitions of which are explained in Materials and Methods.
¶ P < 0.01 versus best monotherapy.
monotherapies evaluated. Additionally, there was no body weight loss associated with the combination therapy.

In the confirmatory experiment, oral taxane plus cetuximab were evaluated on the same intermittent administration schedule, but a more protracted treatment regimen, q3d×6, was used to achieve clear monotherapy and combination MTD levels and to evaluate and simulate the more protracted, chronic applications envisioned clinically. A cumulative exposure of 360 mg/kg (60 mg/kg/administration, q3d×6) of BMS-275183 was tolerated with minimal body weight loss but represents a MTD. A summary of the optimal effects of both monotherapies and combination treatments is shown in Table 1.

Cetuximab monotherapy produced 1.3 log cell kill, a better result than in the initial study and expected given the extended treatment regimen applied and the tolerability of the therapy. The oral taxane, BMS-275183, produced an active 1.0 log cell kill at its MTD, 60 mg/kg/administration. Combinations of oral taxane plus cetuximab produced enhanced delays in tumor growth compared with either optimal monotherapy of the component drugs. The best combination regimen involved 1 mg/mouse/administration cetuximab with 25 mg/kg/administration oral taxane, representing 2.6 log cell kill with three of eight mice cured (P < 0.01). Combinations containing higher doses of oral taxane were also well tolerated but provided no greater activity than the effect just described (an inexplicable inversion in anticipated dose response). The advantage of this particular combination regimen over the optimal monotherapies is illustrated in Fig. 1. The synergies produced with BMS-275183 plus cetuximab were not accompanied by enhanced toxicity as reflected by severe body weight loss but rather showed no enhanced weight loss whatsoever compared with comparable monotherapy.

![Fig 1 Synergistic combined modality therapy with oral taxane, BMS-275183, plus the anti-EGFR antibody, cetuximab, versus the human L2987 lung carcinoma xenograft. Optimal regimens for each therapy evaluated and their associated log cell kill (LCK) effects are shown in the legend.](image)

<table>
<thead>
<tr>
<th>Treatment*</th>
<th>Average body weight change, g† (dead/total)</th>
<th>T-C‡ (days)¶</th>
<th>Log cell kill§</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMS-275183</td>
<td>Cetuximab</td>
<td></td>
<td></td>
</tr>
<tr>
<td>90</td>
<td>1</td>
<td>1.4</td>
<td>10.0</td>
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<tr>
<td>60</td>
<td>−1.8</td>
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</tr>
<tr>
<td>40</td>
<td>1</td>
<td>1.4</td>
<td>10.0</td>
</tr>
</tbody>
</table>

* In mg/kg/administration, p.o., for BMS-275183 and mg/mouse, i.p., for cetuximab. BMS-275183 and cetuximab were administered q3d×6. All treatments began on day 11 after tumor implant. Treatment resulting in more than one death is described as TOX (i.e., toxic). Group sizes = eight mice.
†–§ See footnotes †–§ in Table 1. The same information applies to the relevant item in Table 2. Control mice gained 0.2 g. There were no cures in any group. If any mice died during therapy or within 10 days after the end of therapy, the incidence is shown in parentheses. ¶ P < 0.001 versus best monotherapy.

**GEO Human Colon Carcinoma.** The protracted schedules of treatments used in the second L2987 experiment were applied to our one study with the GEO tumor model. Established GEO tumors were moderately sensitive to cetuximab, reflected by a borderline-active 0.7 log cell kill after treatment with 1 mg/mouse/administration, i.p., q3d×6, beginning on day 11 posttumor implant. There was no body weight loss associated with the therapy. The oral taxane, BMS-275183, was also only capable of producing a borderline active effect of 0.7 log cell kill at its MTD, 60 mg/kg/administration, p.o., q3d×6. These results and the effects of selected combination therapies applied to mice bearing GEO tumors are summarized in Table 2.

Combination therapies were evaluated with the GEO carcinoma in an attempt to extend the observation of therapeutic synergy achieved with cetuximab plus oral taxane in the L2987 lung carcinoma. A combination of 60 mg/kg/administration oral taxane plus 1 mg/mouse of cetuximab produced a therapeutically synergistic 1.9 log cell kill, which was statistically different (P < 0.001) than either optimal monotherapy, and with associated body weight loss no worse than seen with BMS-275183 alone. The advantage of this combination over optimal monotherapies is illustrated in Fig. 2.

**DISCUSSION**

Parenteral administration of drugs is not conducive to protracted, repetitive, chronic treatment applications. The recent advent of weekly regimens for the delivery of taxanes clinically (23–27), and the apparent success associated with their deployment in this manner, provides an impetus for the development of oral versions with at least comparable efficacy and no worse a toxicological profile. With the availability of a clinically active oral taxane, many different schedule options become feasible.

The preclinical activity of BMS-275183 was observed with several different schedules, with an intermittent administration regimen being optimal in one carefully examined tumor model (22). The oral taxane also has shown activity and manageable toxicity in a phase I clinical trial with a weekly schedule of
Synergistic combined modality therapy with oral taxane, BMS-275183, plus the anti-EGFR antibody, cetuximab, versus the human GEO colon carcinoma xenograft. Optimal regimens for each therapy evaluated and their associated LCK effects are shown in the legend.

No increase in toxicity was noted when combining BMS-275183 with cetuximab versus L2987. Essentially, full doses, i.e., the biologically optimal dose or MTD of each therapeutic, could be administered in combination without causing unexpected body weight loss or deaths. Against the human L2987 lung carcinoma model, neither the oral taxane nor cetuximab at optimal dose levels was associated with more than modest, borderline antitumor activity when given as a solo therapy. Yet, the combination of these two agents yielded a therapeutically synergistic outcome. Improvements of >1 log cell kill beyond the therapeutic potential of either solo treatment were observed in confirmatory L2987 experiments, resulting even in the cure of several mice. The finding of therapeutic synergy for the oral taxane plus cetuximab was further confirmed in the GEO tumor model. Unlike claims for synergy often described for agents when a combination produces greater activity than either solo agent used at the same dose found in the combination, the oral taxane plus cetuximab therapeutic synergy is one whose combination effects are superior to those produced by the optimal tolerable dose of either component used alone. In future investigations, we will attempt to additionally optimize the combination, for example, by evaluating sequential or intermittent administrations, as well as evaluate the combination against an EGFR-negative tumor line.

Acknowledgments

We thank the following individuals for their assistance in conducting the experiments described herein: Amy Camuso, Stephen Castaneda, Krista Fager, Christine Friefeh, Ivan Inigo, David Kan, and Kelly McGlincey.

References


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