Combining Targeted Agents: Blocking the Epidermal Growth Factor and Vascular Endothelial Growth Factor Pathways

Alan Sandler¹ and Roy Herbst²

Abstract

Bevacizumab is a recombinant, humanized monoclonal antibody against vascular endothelial growth factor. Erlotinib HCl is a reversible, highly selective epidermal growth factor receptor tyrosine kinase inhibitor. Additionally, both agents have shown benefit in patients with previously treated non–small cell lung cancer (NSCLC). Preclinical data in xenograft models produced greater growth inhibition with the combination than with either agent alone. A phase I/II study in two centers examined combined erlotinib and bevacizumab treatment in patients with nonsquamous stage IIIB/IV NSCLC with one or more prior chemotherapies. In phase I, 150 mg/d erlotinib orally plus 15 mg/kg bevacizumab i.v. every 21 days was established as the phase II dose. A total of 40 patients were enrolled and treated in this study (34 patients at phase II dose); 21 were female, 30 had adenocarcinoma histology, 9 were never smokers, and 22 had two or more prior regimens. The most common adverse events were mild to moderate rash, diarrhea, and proteinuria. Preliminary data showed no pharmacokinetic interaction between erlotinib and bevacizumab. Eight patients (20.0%) had partial responses and 26 (65.0%) had stable disease as their best response. The median overall survival for the 34 patients treated at the phase II dose was 12.6 months, with progression-free survival of 6.2 months. Encouraging antitumor activity and safety of erlotinib plus bevacizumab support further development of this combination for patients with advanced NSCLC. A randomized phase II trial has been completed, and a phase III trial is ongoing.

Over the last decade, biological agents have been developed to target molecules involved in tumor growth and progression. Potential molecular targets include cell surface receptors and their ligands. Two important and relatively well-studied targets are the human epidermal growth factor receptor (HER1 or EGFR) and the vascular endothelial growth factor (VEGF), which is a major regulator of angiogenesis (1).

Rationale for Combination of Erlotinib and Bevacizumab

EGFR and VEGF share common downstream signaling pathways. They exert effects both directly and indirectly on tumor cells, and combining drugs that target these molecules may confer additional clinical benefit. EGFR is involved in angiogenesis; it has been detected in the endothelial cells of tumor vasculature preclinically (2). Coexpression of EGFR and transforming growth factor-α has been correlated with increased microvessel density in invasive breast cancer (3). VEGF is also down-regulated by EGFR inhibition, and a recent study suggested that blockade of VEGF may also inhibit EGFR autocrine signaling (4–6). Therefore, it is rational to suggest that dual blockade of these molecular targets may produce additive and even synergistic cytostatic effects.

Several preclinical studies have investigated the antitumor activity of combined anti-EGFR and anti-VEGF agents and have noted at least additivity, if not synergy (7–9). These encouraging data have led to the initiation of several clinical studies evaluating the combination of erlotinib, an EGFR tyrosine kinase inhibitor, with bevacizumab, an anti-VEGF antibody, in a range of tumor types, including phase II trials in renal cell carcinoma and metastatic breast cancer, a phase I trial in patients with head and neck squamous cell carcinoma, and, lastly, a phase I/II trial in nonsquamous cell non–small cell lung cancer (NSCLC; refs. 10–13).

Erlotinib in NSCLC

There are considerable clinical data supporting the use of erlotinib and bevacizumab individually. The clinical benefit of erlotinib in unselected patients with advanced, recurrent NSCLC was recently confirmed in the phase III trial BR.21 (14). Patients treated with erlotinib had a median survival of 6.7 months compared with 4.7 months for those receiving best supportive care (P = 0.001).

Bevacizumab in Combination with Chemotherapy: Advanced NSCLC

Bevacizumab is a recombinant humanized monoclonal antibody to the VEGF and has shown a survival advantage
when combined with chemotherapy in two phase III studies in colorectal cancer (15, 16). Bevacizumab has also been studied in combination with chemotherapy in NSCLC. In a randomized phase II study of patients with chemotherapy-naive, advanced NSCLC, 99 patients with stage IIIIB, stage IV, or recurrent NSCLC were randomly assigned to receive either paclitaxel/carboplatin alone or paclitaxel/carboplatin plus bevacizumab (either at 7.5 mg/kg or 15 mg/kg every 3 weeks; ref. 17). The response rate was higher with high-dose bevacizumab than with control (investigator assessment; 32% versus 19%). Time to disease progression was also higher for the high-dose bevacizumab arm (investigator assessment; median, 225 versus 129 days for the control).

One unexpected toxicity seen in the phase II NSCLC trial was an unacceptably high incidence of life-threatening pulmonary hemorrhage in patients with squamous cell histology. Therefore, the randomized phase III Eastern Cooperative Oncology Group trial was conducted in patients with metastatic nonsquamous NSCLC. This randomized, multicenter trial examined the effects of the addition of bevacizumab to paclitaxel/carboplatin on overall survival in patients with previously untreated advanced nonsquamous NSCLC (18). Eligibility criteria included Eastern Cooperative Oncology Group performance status of 0 or 1 and adequate hematologic, renal, and hepatic function. Patients with brain metastases and squamous cell histology were excluded. Patients were randomized to receive paclitaxel (200 mg/m²) plus carboplatin (area under the curve = 6) on day 1 every 3 weeks or minus bevacizumab (15 mg/kg) on day 1 every 3 weeks. Patients on the investigational arm continued single-agent bevacizumab after six cycles of chemotherapy until progressive disease or intolerable toxicity. The accrual was completed in April 2004 with 444 patients assigned to chemotherapy alone and 434 to chemotherapy plus bevacizumab. The results presented were based on a second planned interim analysis that was conducted with 469 of 650 (72.2%) deaths needed for full analysis (18). The response rate (10% versus 27%; P < 0.0001, Fisher’s exact test), progression-free survival (4.5 versus 6.4 months; P < 0.0001, two-sided test), and median survival (10.2 versus 12.5 months; P = 0.0075, two-sided Wald test) all favored the bevacizumab arm.

### Erlotinib in Combination with Bevacizumab: Advanced NSCLC

This phase I/II trial examined the safety and efficacy of combining erlotinib and bevacizumab therapy in patients with advanced or metastatic NSCLC. No serious adverse effects or dose-limiting toxicities were reported in any of the cohorts in phase I. In accordance with the study protocol, the dose defined as the phase II dose for this study and the recommended dose for future studies was erlotinib at its previously defined maximum tolerated dose as a single agent (150 mg/d) plus bevacizumab at the highest tolerated dose investigated in this indication (15 mg/kg). The pharmacokinetics of these agents in combination was also assessed, and there was no evidence of a pharmacokinetic interaction between these agents (13).

A total of 40 patients were enrolled in the study. The combination of 150 mg/d erlotinib and 15 mg/kg bevacizumab every 3 weeks was well tolerated; mild to moderate skin rash, proteinuria, and diarrhea were the most common adverse events, and no dose-limiting adverse effects were reported. No arterial thromboembolic events or severe hemoptysis was observed. Objective responses were seen in 20% of patients and 65% had stable disease. Only 15% of the patients had disease progression at the time of initial assessment of response. Even more exciting was the demonstration of a median time to progression of 7 months and a median survival of 12.6 months, both comparing favorably to untreated historical controls who are expected to have a median time to progression of only 2 to 3 months and a median survival of ~4 months. However, results of this phase II study require confirmation in larger phase III studies.

Recently, a somatic mutation in the EGFR tyrosine kinase domain was identified that may correlate with tumor response to EGFR tyrosine kinase inhibitors (19, 20). Although its prognostic value has yet to be fully established, diagnostic tissue from a subset of patients enrolled in this trial was tested for the presence of this mutation.

At the time of reporting, nine patients had sufficient paraffin-embedded tissue available for EGFR tyrosine kinase domain mutational analysis (exons 19, 20, 21, and 23). The patient characteristics and mutational status are shown in Table 1. These nine patients included three who achieved a partial response, three with stable disease as their best response, and three with progressive disease. Mutations were detected in one of the three patients with a partial response and in one of the three patients with stable disease. It is interesting that one of the patients with stable disease and no mutations [wild-type (WT), exons 19-21] was a female nonsmoker with bronchioloalveolar carcinoma, a demographic subset that has been associated with response to EGFR tyrosine kinase inhibitors. Although it is possible that patients reported as WT could have mutations in unexamined exons, these results suggest the possibility that the combination of erlotinib with bevacizumab may provide benefit to patients with WT EGFR and is justification for further study of this combination.

The results of this phase I/II study show that this combination is well tolerated and active in NSCLC. Further investigation into the efficacy and tolerability of combined erlotinib and bevacizumab is ongoing in randomized trials.

### Targeted Agents with Multiple Pathways

AEE 788 (Novartis, Basel, Switzerland) and ZD6474 (AstraZeneca, Wilmington, DE) are two examples of orally available inhibitors of two key pathways in tumor growth: VEGF receptor-dependent tumor angiogenesis and EGFR-dependent tumor cell proliferation and survival. ZD6474 is currently being investigated in three randomized, double-blind phase II trials in NSCLC, in which the efficacy of ZD6474 is being compared with that of the EGFR tyrosine kinase inhibitor gefitinib in combination with the cytotoxic agent docetaxel versus docetaxel alone or in combination with the carboplatin/paclitaxel regimen or gefitinib. In this trial, both response rate, 7% versus 0%, and time to progression, 11.9 versus 8.1 weeks, favored the ZD6474 arm with time to progression reaching statistical significance (P = 0.011;
trials have used these criteria. They have screened something like 300 patients to enroll about 10.

They should be some thought of changing the eligibility criteria. Because accrual is so poor, there is accruing incredibly badly. I think that we need to know what to do about patients with squamous cancers, patients with brain metastases that have been irradiated, and people who are on anticoagulants. Because accrual is so poor, there should be some thought of changing the eligibility criteria. They have screened something like 300 patients to enroll about 10.

I find it somewhat hard to believe that it is totally related to just the exclusion criteria because previous trials have used these criteria.

In the ZD6474 trials, patients with squamous cell have been included as well as patients with brain metastases who have been treated and are stable off steroids. We have not run into any problems thus far. The numbers are quite small, but there is at least the suggestion that there is a benefit in squamous cell just as there is in nonsquamous disease.

You can argue from the early randomized phase II study with chemotherapy plus or minus bevacizumab that the squamous cell tumors potentially did better, since some of them had the most brisk responses, but the issue of bleeding was a serious concern.

Dr. Thomas Lynch: Heymach, you have spent a lot of time on angiogenesis in the laboratory. As we know, there are several potential mechanisms for how bevacizumab may be working in this setting with erlotinib. Do you think that this drug is just simply a better way of delivering either chemotherapy or erlotinib to tumors? Or do you think that drugs like erlotinib actually have antiangiogenic properties on their own, and we may be seeing synergy from the antiangiogenesis standpoint?

Dr. Heymach: I'll show some preclinical data tomorrow addressing this question both with bevacizumab and erlotinib and with ZD6474. Regarding the delivery question and the concept that normalization of the vasculature is a way to improve delivery, the data that have emerged preclinically suggest that if that happens it is within a narrow window at the very beginning of treatment. But you do see synergy in terms of killing endothelial cells by blocking VEGF and then hitting endothelial cells with chemotherapy. With regard to the combination with erlotinib, we know that the EGFR contributes to the control of expression of various angiogenic factors. So in culture when you inhibit EGFR, expression of VEGF drops, basic FGF, IL-8, and a host of other angiogenic factors. So the combination is turning down the angiogenic drive of the tumor as well as blocking the most important angiogenic factor, at least in some tumors.

Dr. Jeffrey Settleman: There may be a naive assumption overall about whether the VEGF antibody is only working on the vasculature. It could actually be doing something in the tumor cell. I wonder how much effort has there been to see in a cell culture setting whether a VEGF inhibitor has an additive effect when you put it together with gefitinib or erlotinib.

Table 1. Preliminary results of EGFR mutations analysis

<table>
<thead>
<tr>
<th>Response</th>
<th>Response duration (wk)</th>
<th>Sex</th>
<th>Race</th>
<th>Histology</th>
<th>Smoking history</th>
<th>Mutation status exon 19</th>
<th>Mutation status exon 20</th>
<th>Mutation status exon 21</th>
<th>Mutation status exon 23</th>
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<tr>
<td>PR</td>
<td>55</td>
<td>M</td>
<td>White</td>
<td>NSCLC-PDf</td>
<td>Prior</td>
<td>WT (3)</td>
<td>WT (3)</td>
<td>WT (3)</td>
<td>WT (1)</td>
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<tr>
<td>PR</td>
<td>28</td>
<td>F</td>
<td>White</td>
<td>Adenocarcinoma</td>
<td>Never</td>
<td>WT (3)</td>
<td>P772_H773insNS; H773Y (3)</td>
<td>WT (3)</td>
<td>WT (3)</td>
</tr>
<tr>
<td>PR</td>
<td>19</td>
<td>M</td>
<td>White</td>
<td>Adenocarcinoma</td>
<td>Prior</td>
<td>WT (1)</td>
<td>0</td>
<td>WT (1)</td>
<td>0</td>
</tr>
<tr>
<td>SD</td>
<td>25</td>
<td>F</td>
<td>White</td>
<td>Adenocarcinoma-PDf</td>
<td>Prior</td>
<td>WT (1)</td>
<td>WT (1)</td>
<td>WT (1)</td>
<td>0</td>
</tr>
<tr>
<td>SD</td>
<td>34</td>
<td>F</td>
<td>White</td>
<td>BAC</td>
<td>Never</td>
<td>WT (2)</td>
<td>WT (1)</td>
<td>WT (3)</td>
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<tr>
<td>SD</td>
<td>14</td>
<td>F</td>
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<td>Adenocarcinoma</td>
<td>Never</td>
<td>E746-A750del (2)</td>
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<td>F</td>
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<td>Prior</td>
<td>WT (3)</td>
<td>WT (1)</td>
<td>WT (2)</td>
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<tr>
<td>PD</td>
<td>NA</td>
<td>F</td>
<td>White</td>
<td>Adenocarcinoma</td>
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<td>WT (1)</td>
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<tr>
<td>PD</td>
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<td>Black</td>
<td>NSCLC</td>
<td>Prior</td>
<td>WT (1)</td>
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NOTE: Numbers in parentheses indicate the number of independent rounds of PCR/sequencing done for each exon.

Abbreviations: PR, partial response; PDf, poorly differentiated; SD, stable disease; BAC, bronchioloalveolar carcinoma; PD, progressive disease; NA, not applicable.
Dr. Heymach: Regarding the clinical situation, there are data now on colorectal cancer to show that some do express VEGF receptor on the tumor cells themselves. The fact that there is a greater additive benefit of the combination in colorectal cancer may be because tumor cell VEGF receptor is being affected to some extent. We have screened all the lung cancer cell lines that we use in xenografts for VEGF receptor, and about a fourth or a fifth of them do express levels that we can detect by Western blot and by flow cytometry. So the levels are lower than with endothelial cell expression, but it is not an insignificant portion that do express VEGF receptors. Certainly, the data suggest there is at least some antitumor effect occurring, but it is probably not a big one.

Dr. Lynch: A question for the clinicians: What proportion of lung cancer patients that you see would you feel comfortable treating with bevacizumab?

Dr. Rogerio Lilenbaum: In a general lung cancer population, I would say 30% to 40%. Now, we have a Phase II study of a different chemotherapy debulking with bevacizumab and we have never had such a hard time to accrue patients to a stage IV first-line trial such as this one, primarily because of the exclusion criteria.

Dr. Lynch: We also have had an incredibly hard time accruing to our adjuvant trial of carboplatin/paclitaxel/bevacizumab. Excluding the concurrent use of anticoagulants and the cardiovascular disease has been the biggest block on accrual.

Dr. Sandler: We have spent 7 to 8 hours now talking about EGFR inhibitors and breaking it down into 1%, 3%, 4% of the population that might get the best benefit. So here we have a drug that works in maybe 35% of patients and it gets criticized. But erlotinib and gefitinib—fabulous! Five percent of the drug that works in maybe 35% of patients and it gets criticized. So here we have a EGFR inhibitors and breaking it down into 1%, 3%, 4% of the population that might get the best benefit. So here we have a drug that works in maybe 35% of patients and it gets criticized. But erlotinib and gefitinib—fabulous! Five percent of the population!

Dr. Panos Fidias: I think the truly eligible population is probably more than 50%; it ends up being less than that, primarily because we are worried about it. We start worrying about initial blood pressure in the office, which we’ve never looked at before—it’s 160 over something and we worry about it! Somebody had a treated brain metastasis of 9 mm picked up on an MRI and we start to worry about it. These are the kinds of considerations that reduce the number of eligible patients.

Dr. Sarada Gurubhagavatula: I agree. I think about bevacizumab for many patients, but for whatever reason—brain metastases or squamous cell or even central tumors—there is always something that seems to come up.

Dr. David Johnson: The other issue is the high cost of this bevacizumab/chemotherapy regimen, particularly if the patient doesn’t have adequate insurance coverage. There are a lot of factors that are conscious and perhaps subconscious that affect our decision making about using this particular drug. Having said that, it is the first drug in 20 years that has had an impact on the survival of patients with stage IV disease. So we need to find safer ways of using it. Remember, paclitaxel caused arrhythmias in everybody when you put an EKG on them. When you took the EKG off, it suddenly became a nonissue.

Dr. Fidias: You can go back to the deaths in the ECOG 4599 study to see the characteristics of patients who had hemoptysis, those presumably carefully selected patients with adenocarcinomas?

Dr. Sandler: We are doing that. Basically, the overall pulmonary hemorrhage rate was 4.5%; it was 1.9% grade 3+ for the bevacizumab arm. An independent radiologic facility is looking at the CT scans for all of those patients. We are going to analyze whether size or location mattered, since we did not use those factors as an exclusion criteria. Also, there was no central pathology review at ECOG for this study, so we are going to reexamine histology to see if some patients with squamous cell did go on trial.


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