The BATTLE Trial: Personalizing Therapy for Lung Cancer
ABSTRACT

The Biomarker-integrated Approaches of Targeted Therapy for Lung Cancer Elimination (BATTLE) trial represents the first completed prospective, biopsy-mandated, biomarker-based, adaptively randomized study in 255 pretreated lung cancer patients. Following an initial equal randomization period, chemorefractory non–small cell lung cancer (NSCLC) patients were adaptively randomized to erlotinib, vandetanib, erlotinib plus bevaxotene, or sorafenib, based on relevant molecular biomarkers analyzed in fresh core needle biopsy specimens. Overall results include a 46% 8-week disease control rate (primary end point), confirm prespecified hypotheses, and show an impressive benefit from sorafenib among mutant-KRAS patients. BATTLE establishes the feasibility of a new paradigm for a personalized approach to lung cancer clinical trials. (ClinicalTrials.gov numbers: NCT00409968, NCT00411671, NCT00411632, NCT00410059, and NCT00410189.)

SIGNIFICANCE: The BATTLE study is the first completed prospective, adaptively randomized study in heavily pretreated NSCLC patients that mandated tumor profiling with “real-time” biopsies, taking a substantial step toward realizing personalized lung cancer therapy by integrating real-time molecular laboratory findings in delineating specific patient populations for individualized treatment.

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INTRODUCTION

The leading cause of cancer-related mortality, lung cancer accounts for more U.S. deaths each year than do breast, colon, prostate, liver, and kidney cancers and melanoma combined (1). Systemic chemotherapy is the mainstay for metastatic lung cancer. Although approved therapies in this setting include a few biologic agents, subjective physician preference based on clinical characteristics such as age, gender, or performance status largely drives treatment decisions (2–4).

Tumor biomarker evaluations have recently emerged as an important factor in planning treatment for non–small cell lung cancer (NSCLC) after improved outcomes with the epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKI) erlotinib and gefitinib in patients with NSCLC harboring EGFR mutations (5–8). Notwithstanding this success, biologic agents have not been effective in many randomized trials in NSCLC. There is a paucity of effective predictive markers of drug sensitivity or resistance, due in large part to difficulties in prospectively obtaining baseline tumor tissue in patients with metastatic NSCLC. In patients with pretreated NSCLC, tumor biomarker evaluation is frequently based on the tissue obtained at diagnosis and may not reflect the current state of biomarkers after treatment with chemotherapy.

In the novel phase II Biomarker-integrated Approaches of Targeted Therapy for Lung Cancer Elimination (BATTLE) program of personalized medicine (ClinicalTrials.gov numbers: NCT00409968, NCT00411671, NCT00411632, NCT00410059, and NCT00410189) reported in this article, we prospectively biopsied tumors and, based on tumor markers, used adaptive randomization to assign NSCLC patients to the treatment with greatest potential benefit based on cumulative data (Fig. 1). The signaling pathways and targeted

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Note: Supplementary data for this article are available at Cancer Discovery Online (http://www.aacrjournals.org).

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agents were selected on the basis of the highest scientific and clinical interest at the time (2005) and included EGFR (erlotinib), KRAS/BRAF (sorafenib), retinoid-EGFR signaling (bexarotene and erlotinib), and vascular endothelial growth factor receptor [VEGFR; vandetanib (refs. 9–12)]. All of these compounds were being tested in the phase II or III setting and thus were appropriate for treatment of patients with advanced NSCLC. Testing the feasibility of performing core biopsy procedures in pretreated disease and utilizing real-time biomarker analyses for treatment were major challenges in BATTLE and, if successful, were proposed as major steps toward personalizing therapy for patients with NSCLC.

## RESULTS

### Patient Characteristics

A total of 341 patients were enrolled in the BATTLE study between November 30, 2006, and October 28, 2009, with equally random assignments for the first 97 patients and adaptive randomization for the remaining 158. The numbers of randomized patients per treatment arm were 59 (erlotinib), 54 (vandetanib), 37 (erlotinib plus bexarotene), and 105 (sorafenib). Seventeen patients were randomly assigned twice, and 1 patient 3 times.

Eighty-six patients could not be randomly assigned because of intercurrent illnesses (n = 29) or worsening overall condition (n = 22), conditions preventing a biopsy (n = 17), or choice of an alternative treatment (n = 18; Fig. 2).

Notable patient characteristics included 83 patients (33%) with prior brain metastases, 116 (45%) with prior treatment with an EGFR TKI, and a median of 2 prior chemotherapies (Table 1). Our patient population was reflective of a heavily pretreated NSCLC population, with 44% (102 patients) having progression as their best response to prior therapy. Supplementary Table S1 lists the distribution of individual biomarkers. The prevalence of mutations in our study population included 15% EGFR and 20% KRAS. Forty-two patients had inadequate tissue for biomarker analysis, and 2 patients were negative for all study biomarkers.

### Efficacy

The overall 8-week disease control rate (DCR) in 244 patients eligible for this analysis was 46% (Table 2); median progression-free survival (PFS) was 1.9 months [95% confidence interval (CI), 1.8–2.4]; median overall survival (OS) was 8.8 months (95% CI, 6.3–10.6); and 1-year survival was 35% (Supplementary Fig. S1). The median patient follow-up was 10.3 months. There were no complete responses and only 7% partial responses. Supplementary Table S2 lists the median duration of response.

<table>
<thead>
<tr>
<th>Agent</th>
<th>n eval</th>
<th>EGFR:</th>
<th>KRAS/BRAF:</th>
<th>VEGF:</th>
<th>RXR/CycD1:</th>
<th>None:</th>
</tr>
</thead>
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<tr>
<td>Erlotinib</td>
<td>25</td>
<td>8</td>
<td>2</td>
<td>10</td>
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<td>4</td>
</tr>
<tr>
<td>Vandetanib</td>
<td>23</td>
<td>16</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Erlotinib +</td>
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<td>11</td>
<td>2</td>
<td>3</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Bexarotene</td>
<td>21</td>
<td>10</td>
<td>2</td>
<td>3</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Sorafenib</td>
<td>28</td>
<td>10</td>
<td>2</td>
<td>3</td>
<td>1</td>
<td>4</td>
</tr>
</tbody>
</table>

Figure 2. CONSORT diagram of the BATTLE study.
### Table 1. Patient characteristics by treatment

<table>
<thead>
<tr>
<th>Age (mean, 62; range, 26 – 84)</th>
<th>All (n = 113)</th>
<th>Erlotinib (n = 59)</th>
<th>Vandetanib (n = 54)</th>
<th>Erlotinib + bexarotene (n = 37)</th>
<th>Sorafenib (n = 105)</th>
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<tbody>
<tr>
<td>≤50</td>
<td>41 (16%)</td>
<td>5 (8%)</td>
<td>11 (20%)</td>
<td>6 (16%)</td>
<td>19 (18%)</td>
</tr>
<tr>
<td>51–60</td>
<td>73 (29%)</td>
<td>23 (39%)</td>
<td>15 (28%)</td>
<td>11 (30%)</td>
<td>24 (23%)</td>
</tr>
<tr>
<td>61–70</td>
<td>86 (34%)</td>
<td>19 (32%)</td>
<td>19 (35%)</td>
<td>11 (30%)</td>
<td>37 (35%)</td>
</tr>
<tr>
<td>&gt;70</td>
<td>55 (22%)</td>
<td>12 (20%)</td>
<td>9 (17%)</td>
<td>9 (24%)</td>
<td>25 (24%)</td>
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<tr>
<td>Gender</td>
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<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Female</td>
<td>118 (46%)</td>
<td>26 (44%)</td>
<td>29 (54%)</td>
<td>12 (32%)</td>
<td>51 (49%)</td>
</tr>
<tr>
<td>Male</td>
<td>137 (54%)</td>
<td>33 (56%)</td>
<td>25 (46%)</td>
<td>25 (68%)</td>
<td>54 (51%)</td>
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<td>Ethnicity</td>
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<tr>
<td>Caucasian</td>
<td>209 (82%)</td>
<td>51 (86%)</td>
<td>41 (76%)</td>
<td>31 (84%)</td>
<td>86 (82%)</td>
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<td>Hispanic</td>
<td>16 (6%)</td>
<td>2 (3%)</td>
<td>7 (13%)</td>
<td>0 (0%)</td>
<td>7 (7%)</td>
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<td>African American</td>
<td>16 (6%)</td>
<td>3 (5%)</td>
<td>2 (4%)</td>
<td>4 (11%)</td>
<td>7 (7%)</td>
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<tr>
<td>Asian</td>
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<td>3 (5%)</td>
<td>4 (7%)</td>
<td>2 (5%)</td>
<td>5 (5%)</td>
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<td>Smoker</td>
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<td>Current</td>
<td>23 (9%)</td>
<td>9 (15%)</td>
<td>5 (9%)</td>
<td>3 (8%)</td>
<td>6 (6%)</td>
</tr>
<tr>
<td>Former</td>
<td>177 (69%)</td>
<td>41 (69%)</td>
<td>31 (57%)</td>
<td>32 (86%)</td>
<td>73 (70%)</td>
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<tr>
<td>Never</td>
<td>55 (22%)</td>
<td>9 (15%)</td>
<td>18 (33%)</td>
<td>2 (5%)</td>
<td>26 (25%)</td>
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<td>Histology</td>
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<td>Adenocarcinoma</td>
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<td>31 (53%)</td>
<td>35 (65%)</td>
<td>23 (62%)</td>
<td>71 (68%)</td>
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<tr>
<td>Squamous</td>
<td>46 (18%)</td>
<td>16 (27%)</td>
<td>7 (13%)</td>
<td>6 (16%)</td>
<td>20 (19%)</td>
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<tr>
<td>Others</td>
<td>49 (19%)</td>
<td>12 (20%)</td>
<td>12 (22%)</td>
<td>8 (22%)</td>
<td>14 (13%)</td>
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<td>Prior erlotinib therapy</td>
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<td></td>
<td></td>
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<td>No</td>
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<td>59 (100%)</td>
<td>9 (17%)</td>
<td>37 (100%)</td>
<td>34 (32%)</td>
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<td>Yes</td>
<td>116 (45%)</td>
<td>0 (0%)</td>
<td>45 (83%)</td>
<td>0 (0%)</td>
<td>71 (68%)</td>
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<td>0</td>
<td>22 (9%)</td>
<td>5 (8%)</td>
<td>9 (17%)</td>
<td>2 (5%)</td>
<td>6 (6%)</td>
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<tr>
<td>1</td>
<td>197 (77%)</td>
<td>44 (75%)</td>
<td>36 (67%)</td>
<td>30 (81%)</td>
<td>87 (83%)</td>
</tr>
<tr>
<td>2</td>
<td>36 (14%)</td>
<td>10 (17%)</td>
<td>9 (17%)</td>
<td>5 (14%)</td>
<td>12 (11%)</td>
</tr>
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<td>Prior cytotoxic chemotherapy (median, 2; range, 1 – 6)</td>
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<tr>
<td>1</td>
<td>95 (37%)</td>
<td>25 (42%)</td>
<td>21 (39%)</td>
<td>18 (49%)</td>
<td>31 (30%)</td>
</tr>
<tr>
<td>2</td>
<td>84 (33%)</td>
<td>20 (34%)</td>
<td>17 (31%)</td>
<td>12 (32%)</td>
<td>35 (33%)</td>
</tr>
<tr>
<td>3</td>
<td>40 (16%)</td>
<td>9 (15%)</td>
<td>7 (13%)</td>
<td>4 (11%)</td>
<td>20 (19%)</td>
</tr>
<tr>
<td>4</td>
<td>24 (9%)</td>
<td>4 (7%)</td>
<td>6 (11%)</td>
<td>2 (5%)</td>
<td>12 (11%)</td>
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<tr>
<td>5</td>
<td>9 (4%)</td>
<td>1 (2%)</td>
<td>2 (4%)</td>
<td>1 (3%)</td>
<td>5 (5%)</td>
</tr>
<tr>
<td>6</td>
<td>3 (1%)</td>
<td>0 (0%)</td>
<td>1 (2%)</td>
<td>0 (0%)</td>
<td>2 (2%)</td>
</tr>
</tbody>
</table>

*Cells showing effective treatments within specific marker groups defined as the probability of DCR given data is 80% or greater. Only 1 patient in the RXR/Cyclin D1 marker group received erlotinib + bexarotene.

### Table 2. Eight-week disease control status by treatment and marker groups

<table>
<thead>
<tr>
<th>Marker group</th>
<th>Treatment</th>
<th>Erlotinib</th>
<th>Vandetanib</th>
<th>Erlotinib + bexarotene</th>
<th>Sorafenib</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>EGFR</td>
<td></td>
<td>6/17 (35%)</td>
<td>11/27 (41%)</td>
<td>11/20 (55%)</td>
<td></td>
<td>37/87 (43%)</td>
</tr>
<tr>
<td>KRAS/BRAF</td>
<td>1/7 (14%)</td>
<td>0/3 (0%)</td>
<td>1/3 (33%)</td>
<td>11/14 (79%)</td>
<td></td>
<td>13/27 (48%)</td>
</tr>
<tr>
<td>VEGF/VEGFR-2</td>
<td>10/25 (40%)</td>
<td>6/16 (38%)</td>
<td>0/3 (0%)</td>
<td>25/39 (64%)</td>
<td></td>
<td>41/83 (49%)</td>
</tr>
<tr>
<td>RXR/Cyclin D1</td>
<td></td>
<td>0/1 (0%)</td>
<td>0/0 (NA)</td>
<td>1/1 (100%)</td>
<td></td>
<td>1/4 (25%)</td>
</tr>
<tr>
<td>None</td>
<td>3/8 (38%)</td>
<td></td>
<td></td>
<td>5/9 (56%)</td>
<td></td>
<td>19/41 (46%)</td>
</tr>
<tr>
<td>Total</td>
<td>20/58 (34%)</td>
<td>17/52 (33%)</td>
<td></td>
<td>18/36 (50%)</td>
<td></td>
<td>57/98 (58%)</td>
</tr>
</tbody>
</table>

"Cells showing effective treatments within specific marker groups defined as the probability of DCR given data is 80% or greater. Only 1 patient in the RXR/Cyclin D1 marker group received erlotinib + bexarotene."
9 partial responses in these heavily pretreated patients. In an 8-week landmark analysis, the median survival of patients with 8-week disease control (DC) was 9.6 months (95% CI, 7.4–12.5), compared with 7.5 months (95% CI, 4.2–9.2) for patients without 8-week DC (Fig. 3A; \( P = 0.018 \)). The overall 8-week DCRs were 34% (erlotinib), 33% (vandetanib), 50% (erlotinib plus bexarotene), and 58% (sorafenib). Effective treatment-marker-group pairings, defined as having a 0.8 posterior probability of exceeding a DCR of 30%, were as follows: erlotinib in the VEGF/VEGFR-2 (VEGFR-2) group; vandetanib in the EGFR group; erlotinib plus bexarotene in the EGFR, retinoid X receptor (RXR)/Cyclin D1, and no-marker groups; and sorafenib in the KRAS/BRAF, VEGF/VEGFR-2, and no-marker groups (Table 2).

In addition to analysis of prespecified marker groups, we also studied effects of individual markers on treatment efficacy. In confirmation of our prespecified scientific hypotheses, individual markers that predicted a better 8-week DC of treatment [versus the marker’s opposite status (absence or presence)] were EGFR mutations for erlotinib (\( P = 0.04 \)), high VEGFR-2 expression for vandetanib (\( P = 0.05 \)), and high Cyclin D1 expression for erlotinib plus bexarotene (\( P = 0.01 \)). Exploratory predictive marker analysis results were as follows: a better 8-week DC with EGFR amplification for erlotinib plus bexarotene (\( P = 0.006 \)); a worse 8-week DC with EGFR mutation (\( P = 0.01 \)) or high EGFR polysomy (\( P = 0.05 \)) for sorafenib; and, compared with the combined other treatments, sorafenib had a higher DCR (64% versus 33%) in EGFR–wild-type patients (\( P < 0.001 \)) and a non-statistically significant trend toward better DCR (61% versus 32%) in mutant-KRAS patients (\( P = 0.11 \); Fig. 3B). In addition, in the KRAS/BRAF marker group, sorafenib had a 79% DCR compared to a 14% DCR with erlotinib (\( P = 0.016 \)).

**Toxicity**

All 4 treatments were well tolerated, each having toxicity consistent with prior reports. Treatment-related grade 3–4 toxicity was 6.5% (Supplementary Table S2). Average compliance in each arm was >95%. Sorafenib produced the most toxicity, which caused discontinuation of treatment in 19% and dose reductions in 21% of sorafenib-treated patients (Supplementary Table S3). Lung biopsy was well tolerated by the 139 patients who underwent the procedure, with pneumothorax in 11.5%, and only 1 grade 3 event, which required overnight hospitalization.

**DISCUSSION**

The phase II randomized BATTLE trial made important clinical discoveries and demonstrated the feasibility of its novel design for advancing personalized treatment of NSCLC. BATTLE is the first completed prospective, biopsymanded, biomarker-based, adaptively randomized clinical study in patients with pretreated, advanced lung cancer. The trial data validated prespecified scientific hypotheses.
regarding predictive biomarkers for targeted agents and identified potential new predictive markers. The BATTLE study is important in demonstrating several key points: 1) establishing the feasibility of performing biopsies and real-time biomarker analyses in previously treated lung cancer patients; 2) identifying interactions between the treatments and markers (e.g., DCR of 79% with sorafenib but only 14% with erlotinib in the KRAS/BRAF marker group) for guiding adaptive randomization; and 3) confirming the prespecified hypotheses of treatment efficacy in the presence of individual markers related to the treatments’ mechanism of action.

EGFR mutations have been adopted as a predictive biomarker for directing NSCLC patient treatment with EGFR TKIs but are present in only 10% to 15% of the lung cancer population. Results from the vast majority of chemotherapy-based clinical trials in NSCLC, which continue to treat NSCLC as a homogeneous disease, have been disheartening, and personalized trials targeting molecular NSCLC characteristics of individual patients may be a viable option for improving treatment outcomes.

We showed that 8-week DC status is a good surrogate for OS in previously treated patients, as also reported by the Southwest Oncology Group during BATTLE (13). This clinically relevant, short-term, surrogate end point facilitated the rapid integration of outcome data into adaptive randomization, confirming its utility for personalizing treatment assignments. In addition, the short-term nature of the 8-week DC end point was not considered to be affected by patients who had received multiple prior treatments before BATTLE. Our overall response rate of 4% is reflective of a heavily pretreated NSCLC population and consistent with other published studies in this population (5, 9, 14).

Results of the BATTLE study support the potential of various biomarkers to predict the sensitivity or resistance of patients to targeted agents. Sorafenib was active against tumors with mutated or wild-type KRAS, but had a worse DCR (compared with other study agents) in patients with EGFR mutations. As expected (5–7, 15–17), erlotinib was beneficial in patients with mutated-EGFR tumors. Erlotinib plus bexarotene improved DC in patients with a higher expression of Cyclin D1, suggesting a potential role for bexarotene in lung cancer treatment (11); similar to sorafenib, the combination also improved DC in the KRAS-mutant patient population. Future randomized, controlled studies are needed to further confirm the predictive value of these biomarkers. These findings (e.g., association of increased expression of Cyclin D1 with benefit from treatment with bexarotene and erlotinib, and sorafenib’s activity in patients with both KRAS-mutant and KRAS-wild-type tumors) have fueled enthusiasm to further test these compounds in future clinical trials.

Biomarker profiles may differ between early-stage and advanced lung tumors. In current practice, biomarker profiles are determined from the original diagnostic tissue and may not reflect the current tumor biomarker status after receiving treatments, thus hampering decision making for personalized treatment. The present study performed real-time biopsies for assessing the current status of tumor biomarkers in patients, thus validating the feasibility of this paradigm-shifting approach.

The BATTLE approach could be expanded to develop personalized cytotoxic therapy. ERCC1 or RRM1 protein overexpression can help direct cytotoxic therapy, but these markers are not widely used in the clinical setting; other cytotoxic-therapy markers need further elucidation (18, 19). We mandated at least 2 core needle biopsies (CNB) in BATTLE and collected additional tissue and blood for discovering new biomarkers, including gene signatures, which may help further define patient populations sensitive to specific cytotoxic and biologic treatments.

Our study has some important limitations. First, and probably most important, our biomarker groups were less predictive than were individual biomarkers, which diluted the impact of strong predictors in determining treatment probabilities. For example, EGFR mutations were far more predictive than was the overall EGFR marker group. The unfortunate decision to group the EGFR markers also impacted the other marker groups and their interactions with other treatments, resulting in a suboptimal overall DCR as described. Second, several of the prespecified markers (e.g., RXR) had little, if any, predictive value in optimizing treatment selections. This limitation will be addressed in future studies by not grouping or prespecifying biomarkers prior to initiating these biopsy-mandated trials. In addition, adaptive randomization, which assigns more patients to the more effective treatments within each biomarker group, only works well with a large differential efficacy among the treatments (as evident in the KRAS/BRAF group), but its role is limited without such a difference (e.g., in the other marker groups). Allowing prior use of erlotinib was another limitation and biased treatment assignments; in fact, the percentage of patients previously treated with erlotinib steadily increased during trial enrollment. Overall, 45% of our patients were excluded from the 2 erlotinib-containing arms because of prior EGFR TKI treatment. As erlotinib is a standard of care therapy in NSCLC second-line, maintenance, and front-line settings, the number of patients receiving this targeted agent will likely continue to increase.

The BATTLE approach requires a highly integrated team of multidisciplinary investigators and should be implemented at specialized centers in carefully designed clinical trials. However, once a validated biomarker predicting benefit of treatment is identified, conducting this type of study in both academic and community environments will help promote the use of biomarkers to select patients for optimal treatment assignments.

While proving that the BATTLE-type platform is feasible, we have also learned several important lessons from our initial experience that have and will impact the design and conduct of future BATTLE studies focused on pretreated NSCLC populations. A forthcoming study (BATTLE-2) of targeted agents in pretreated patients with advanced NSCLC will further refine our experience with this approach. In BATTLE-2, we prespecify an extremely limited set of markers and will use the first half of the study population (approximately 200 patients) to conduct prospective testing of biomarkers/signatures. Upon completing this analysis, the “best” (most predictive) markers and signatures will be used to guide patient assignments to the most favorable matched treatments in the second half of the study (approximately 200 patients). Patients enrolled would be screened for EGFR mutations and ALK...
translocations (20). If positive, they would not be eligible for enrollment in this study but would be referred to other ongoing trials testing agents targeting those mutations. We believe this is an ethical design and would allow patients to be exposed to additional novel therapies for lung cancer treatment.

BATTLE is the first completed prospective, adaptively randomized study in heavily pretreated NSCLC patients that mandated tumor profiling with real-time CNBs, demonstrating the feasibility of this approach and creating a new paradigm for translational research. This trial took a substantial step toward realizing personalized lung cancer therapy by integrating real-time molecular laboratory findings in delineating specific patient populations for individualized treatment. BATTLE accumulated increasing probabilities of a positive treatment outcome and showed the potential of its mandatory-biopsy design for developing specific predictive biomarkers and associated treatments for subsequent definitive clinical testing. This approach will be important for future evaluations of new molecular targets and predictive biomarkers (21–23). The successful completion of BATTLE reported in this article will potentially facilitate the implementation of future trials of personalized treatments in lung and other cancers with even more efficient designs, as a forerunner in the quest for discovery of novel cancer treatments.

**METHODS**

**Patient Population**

We recruited patients with pretreated NSCLC at the University of Texas MD Anderson Cancer Center (MDACC) who agreed to a baseline tumor biopsy procedure. Eligibility also included age ≥18 years and adequate performance status (ECOG grade 0–2). Prior treatment with erlotinib was allowed, but such patients were excluded from the erlotinib-containing study arms, and stable (for at least 4 weeks) or treated brain metastases were permitted. Patients with multiple lines of prior therapy were eligible if they had adequate performance status. Radiographic imaging of tumors was reviewed to determine suitability for biopsy. All participants provided written informed consent. Other eligibility criteria appear in the Supplementary Data.

**Study Design**

BATTLE was a randomized phase II, single-center, open-label study in patients with advanced NSCLC refractory to prior chemotherapy (Fig. 1). Following molecular tumor-biomarker assessments, patients were randomly assigned to oral treatment with erlotinib therapy (Fig. 1). Following molecular tumor-biomarker assessments, patients were randomly assigned to oral treatment with erlotinib was allowed, but such patients were excluded from the erlotinib-containing study arms, and stable (for at least 4 weeks) or treated brain metastases were permitted. Patients with multiple lines of prior therapy were eligible if they had adequate performance status. Radiographic imaging of tumors was reviewed to determine suitability for biopsy. All participants provided written informed consent. Other eligibility criteria appear in the Supplementary Data.

**Biopsy Procedure**

Written informed consent was obtained from each patient before each biopsy. Coagulopathies were corrected prior to biopsy. All biopsies were performed under computed tomographic or sonographic guidance by a board-certified interventional radiologist with the patient in the prone, supine, or lateral decubitus position, depending on the location of the lesion. During the biopsy, patients received either local anesthesia or monitored, moderate sedation. Patients’ skin was aseptically prepared and draped, and 1% lidocaine was administered subcutaneously for local anesthesia. A coaxial biopsy technique was used for all patients. With image guidance to evaluate the needle's trajectory, an 18- or 19-gauge needle (Cook) was inserted through the skin and advanced to a position close to the target lesion. After imaging confirmation of the needle tip's position, 2 or 3 core biopsy samples were obtained with a 20-gauge biopsy needle (Quick-core; Cook). The samples were handed over to the appropriate research personnel for handling and processing.

After the biopsy procedure, patients were monitored by the nursing staff in the radiology department's recovery area. In patients
who underwent a lung or mediastinal biopsy, an upright inspiratory posteroanterior chest radiograph was obtained within 30 minutes of the biopsy procedure. In the absence of a pneumothorax, the procedure included a second chest radiograph 3 hours after the biopsy. If the initial chest radiograph showed a pneumothorax, a follow-up radiograph was obtained after 1 hour. Chest tubes were inserted if the pneumothorax size was >30%, the pneumothorax increased in size, or patients experienced pain, shortness of breath, or a decrease in oxygen saturation.

**Biomarker Methodology**

To evaluate 11 molecular biomarkers (Supplementary Table S1) using the formalin-fixed paraffin-embedded (FFPE) CNB tissue specimens, 13 5-μm histology sections were obtained, as follows: 1) H&E histology analysis (n = 1 section); 2) DNA extraction for mutation analyses (EGFR, KRAS, and BRAF; n = 1 or 2 sections); 3) FISH analysis (EGFR and CCND1; n = 2 sections); and 4) immunohistochemistry (IHC) analysis (VEGF, VEGFR-2, Cyclin D1, RXRγ, RXRβ, and RXRα; n = 6 sections). All specimens were assigned an identification number linked to the clinical trial identification number for subsequent processing in the laboratory. Certification of the presence of adequate tumor tissue in the FFPE tissue specimens by histologic examination was performed within 24 to 48 hours, and analysis of the 11 molecular biomarkers was performed, completed, and reported, in most cases, within 14 days.

**Microdissection and DNA extraction** Malignant tumor cells were manually microdissected from 4 sequential 5-μm-thick H&E-stained FFPE histology sections. DNA was extracted using 25 μL of Pico Pure TM DNA Extraction solution (Arcturus) containing proteinase K and incubated at 65°C for 24 hours. Subsequently, proteinase K inactivation was performed by heating samples at 95°C for 10 minutes.

**Mutation analysis** Mutations of EGFR (exons 18–21), KRAS (exons 1, codons 12 and 13; and exon 2, codon 61), and BRAF (exons 11 and 15) were studied using DNA extracted from microdissected FFPE tumor cells. The DNA sequences were PCR amplified using primers shown in Supplementary Table S1. Each PCR amplification was performed in 30 μL of volume containing 2 μL of DNA (approximately 100 ng of genomic DNA), 0.3 μM forward and reverse primers, and 15 μL of HotStarTaq (1.5 units of DNA polymerase) Master Mix (Qiagen) for 40 cycles at 94°C for 30 seconds, at the primer pairs’ annealing temperature (Supplementary Table S1), and at 72°C for 45 seconds, followed by 7 minutes of extension at 72°C. All PCR products were directly sequenced using Applied Biosystems PRISM dye terminator cycle sequencing method (Perkin-Elmer). All sequence variants were confirmed by independent PCR amplifications from at least 2 independent DNA extractions, and sequenced in both directions.

**EGFR and CCND1 copy number analysis** Copy number of both genes was analyzed using FISH. For EGFR, gene copy number per cell was analyzed using the LSI EGFR SpectrumOrange/CEP7 SpectrumGreen Probe (Abbott Molecular), as previously described (16). For CCND1, the Vysis LSI CCND1 (SO)/CEP11 DNA probe set (Abbott Molecular) was used. For both FISH analyses, histology sections were incubated at 56°C overnight and deparaffinized by washing in CitriSolv (Fisher Scientific). After incubation in denaturing solution containing 70% formamide and 2× SSC buffer, pH 7.0, at 73°C for 5 minutes, the histology sections were digested with proteinase K (0.25 mg/mL in 2× SSC) at 37°C for 15 to 25 minutes, rinsed in 2× SSC (pH 7.0) at room temperature for 5 minutes, and dehydrated using ethanol in a series of increasing concentrations (70%, 85%, 100%). The probe set was applied onto the selected area, per the manufacturer’s instructions, on the basis of the tumor foci seen on each slide. The hybridization area was covered with a glass coverslip and then sealed with rubber cement. The slides were incubated at 80°C for 10 minutes for co-denaturation of chromosomal and probe DNA and then placed in a humidified chamber at 37°C for 20 to 24 hours to allow hybridization to occur. Posthybridization washes were performed in 1.5-M urea and 0.1× SSC (pH, 7.0–7.5) at 45°C for 30 minutes and in 2× SSC for 2 minutes at room temperature. After the samples were dehydrated in a series of increasing ethanol concentrations, 4’,6’-diamidino-2-phenylindole (0.15 mg/mL) in Vectashield Mounting Medium (Vector Laboratories) was applied for chromatin counterstaining.

For both genes, fluorescence signals were scored in at least 50 nonoverlapping interphase nuclei per tumor, and the section of the area was guided by images of the H&E-stained section. The number of copies of EGFR and chromosome 7 probes was assessed independently using a fluorescence microscope (CytoVision platform, Genetix). The number of copies of CCND1 and chromosome 11 probes was assessed independently using a fluorescence microscope (CytoVision platform, Genetix). For EGFR, cases were classified into 6 FISH strata according to the frequency of cells with the EGFR gene copy number and referred to the chromosome 7 centromere, as follows: 1) disomy (≤3 copies in <10% of cells); 2) low trisomy (3 copies in 10%–40% of cells, 4 copies in <10% of cells); 3) high trisomy (3 copies in >40% of cells, 4 copies in <10% of cells); 4) low polysomy (≥4 copies in 10%–40% of cells); 5) high polysomy (≥4 copies in 40% of cells); and 6) gene amplification (ratio of EGFR gene to chromosome ≥2, presence of tight EGFR gene clusters and 15 copies of EGFR per cell in 10% of the analyzed cells). The high polysomy and gene amplification categories were considered to be high EGFR copy number, and the other categories were considered to be nonincreased EGFR copy number, as previously published (24, 26). For CCND1, cases were considered to have gene copy number gain when the average ratio of CCND1 copy number to chromosome 11 centromere copy number was >1, or when clusters of CCND1 signals were observed in >20% of nuclei, as previously published (27).

**IHC analysis** Protein expression of VEGF, VEGFR-2, RXRα, RXRβ, RXRγ, and Cyclin D1 was determined by IHC. For VEGF, VEGFR-2, RXRα, RXRβ, and RXRγ proteins, combined expression of cytoplasmic and membrane staining was assessed, and for RXRα and Cyclin D1 proteins, expression of nuclear staining was examined. Commercially available antibodies were used, as follows: VEGF, rabbit polyclonal antibody (Santa Cruz Biotechnology, Inc.), dilution 1:200; VEGFR-2, mouse monoclonal antibody (Santa Cruz Biotechnology, Inc.), dilution 1:200; RXRα, rabbit polyclonal antibody (Santa Cruz Biotechnology, Inc.), dilution 1:300; RXRβ, rabbit polyclonal antibody (Upstate), dilution 1:100; RXRγ rabbit polyclonal antibody (Santa Cruz Biotechnology, Inc.), dilution 1:200; and Cyclin D1, rabbit monoclonal antibody (clone SP4; Thermo Scientific), dilution 1:100.

All immunostaining was performed using automated stainers (DakoCytomation). Sections 5-μm thick were deparaffinized, rehydrated, and washed with PBS. Antigens were retrieved with 0.01-M citrate buffer (pH 6.0). DakoCytomation) for 30 minutes in a steamer. Samples were blocked for endogenous activity in 3% hydrogen peroxide-PBS, avidin-biotin solution (Zymed), and serum-free protein block (DakoCytomation) before incubation at room temperature with the primary antibody for 60 minutes for VEGF, RXRα, and RXRγ, and 65 minutes for VEGFR-2, RXRβ, and Cyclin D1. The sections were then washed in Tris-buffered saline (pH 7.4) and incubated with goat antirabbit biotinylated immunoglobulin (DakoCytomation). After incubation with the secondary antibody, the sections were incubated with the avidin-biotin-peroxidase complex (DakoCytomation) and developed with 3, 3’-diaminobenzidine. The sections were then rinsed in distilled water, counterstained with Mayer’s hematoxylin,
and mounted for evaluation. Surgically resected FFPE NSCLC tumor tissue specimens with known expression of the markers were used as positive controls. The same FFPE tissues processed without the primary antibody were used as negative controls.

**Biomarker scoring** For VEGF, VEGFR-2, RXRα, RXRβ, and RXRγ proteins, combined expression of cytoplasmic and membrane staining was assessed, and for RXRα and Cyclin D1 proteins, expression of nuclear staining was examined. All expression was assessed using semiquantitative analysis of intensity and extension. For cytoplasmic/membrane expression, the percentage of positive tumor cells in the cytoplasm/membrane (0%–100%) was multiplied by the intensity of staining (0–3); therefore, the possible overall score ranged from 0 to 300. Cytoplasmic and membrane expression scores >100 were considered positive for VEGF and VEGFR-2, and scores >200 were considered positive for RXRβ and RXRγ. Nuclear expression was evaluated for any positive immunostaining, which was expressed in percentage. A nuclear score >30% was considered positive for RXRα, and a nuclear score >10% was considered positive for Cyclin D1.

**Serum Collection** Samples were collected from consenting patients at baseline and after each cycle of treatment. Venous blood was collected at the following time points: baseline (pretreatment), end of cycles 1 and 2, and every 2 cycles thereafter until the patient went off protocol. At each time point, 8 mL of venous blood was collected into an EDTA-based Vacutainer and plasma was separated via centrifugation, 1500 RPM for 15 minutes at 4°C within 30 minutes of collection. The resultant plasma was aliquoted into 3 prelabeled cryovials and stored at –70°C until analysis.

**Statistical Analysis** The accrual goal was 250 randomized patients to achieve a sample size of 200 evaluable patients with complete marker profiles, which would allow an 80% power, with a 20% type I error rate, to identify effective treatments within each marker group. A high type I error rate prevented missing any potentially effective treatments that could be confirmed in larger, future studies (28).

The primary end point was the 8-week DCR [complete or partial response or stable disease via Response Evaluation Criteria in Solid Tumors (RECIST) (29)], which we compared with the historical 30% DCR estimate in similar patients (14). Treatment efficacy (a positive finding) was defined as >0.80 probability of achieving >30% DCR.

The statistical design was based on adaptive randomization under a Bayesian hierarchical model that would increasingly assign patients into treatments with the greatest potential for efficacy based on individual biomarker profiles (28). We planned to randomly assign at least the initial 80 patients equally to the 4 treatments, to allow at least 1 patient in each marker group to complete treatment, thus providing sufficient data to estimate the prior probability of DC for subsequent patients. Subsequent randomization “switched” to an adaptive algorithm, which incorporated the data of each patient evaluated at the 8-week time point (treatment, biomarker group, and 8-week DCR) into recalculations of the posterior probability of efficacy for treatments in relation to biomarker groups. This scheme adapts randomization probabilities for each of the 4 treatments from an equal chance, to a Bayesian adaptive algorithm, which incorporated the prior probability and DC data into a “posterior” probability of the DCR for a given treatment; the resulting posterior probability was continually updated per accumulating data on the associations between the DC and biomarkers of patients.

**Clinical Assessments** Patients were evaluated clinically at the end of each treatment cycle (defined as lasting 4 weeks), and underwent imaging studies every 2 cycles, or every 8 weeks. Patients who progressed could reenter the clinical trial and be reassigned randomly to treatment if still eligible and agreeable to a new biopsy. A radiologist independently assessed DC, which was defined as a complete or partial response or stable disease according to the RECIST (29) at the end of 8 weeks (start of treatment to end of second treatment cycle). PFS was assessed from the date of randomization to the earliest sign of disease progression or death from any cause. OS was assessed from the date of randomization until death from any cause. Tumor response was assessed every 8 weeks until disease progression. Toxicity was assessed in accordance with the National Cancer Institute Common Terminology Criteria for Adverse Events, version 3.0.

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