SUPPLEMENTARY MATERIAL

Supplementary Table 1. Biomarker Distribution in 255 Randomized Patients*

Biomarker	Positive Cases
EGFR mutation (exons 18-21)	33 (15%)
EGFR FISH increased copy number	
- Gene amplification	34 (16%)
- High polysomy	60 (28%)
KRAS mutation (codons 12, 13 and 61)	42 (20%)
BRAF mutation (exons 11 and 15)	5 (2%)
VEGF IHC (>100, range 0-300)	191 (89%)
VEGFR-2 IHC (>100, range 0-300)	85 (40%)
RXR - α (nucleus) IHC (>30, range 0-100)	173 (80%)
- α (cytoplasm) IHC (>200, range 0-300)	3 (1%)
- β (cytoplasm) IHC (>200, range 0-300)	12 (6%)
- β (membrane) IHC (>200, range 0-300)	0 (0%)
- γ (cytoplasm) IHC (>200, range 0-300)	23 (11%)
Cyclin D1 IHC (>10%, range 0-100)	114 (54%)
Cyclin D1 FISH amp	27 (13%)
All marker negative	2 (0.9%)
Inadequate tissue for marker analysis: (partial marker information/no marker information)	2/40 (0.9%/19%)

- **NOTE: The percentage of positive cases was calculated based on available data. The denominator may vary from marker to marker. As shown in the last row, 2 patients and 40 patients had only partial marker or no marker information due to inadequate tissue for marker analysis.**

FISH = Fluoresence in situ hybridization; IHC = Immunohistochemistry

Supplementary Table 2. Selected Toxicities(>10% in any one treatment, based on 254 pts)

	Treatment																	
	Total (N=254)				Erlotinib (N=59)				Vandetanib (N=54)				Erlotinib + Bexarotene (N=37)				Sorafenib (N=104)			
EVENT	all grade N (%)		grade 3, 4, or 5 N (%)		all grade N (%)		grade 3, 4, or 5 N (%)		all grade N (%)		grade 3, 4, or 5 N (%)		all grade N (%)		grade 3, 4, or 5 N (%)		all grade N (%)		grade 3, 4, or 5 N (%)	
Rash	123	(48.4%)	12	(4.7%)	43	(72.9%)	2	(3.4%)	17	(31.5%)	1	(1.9%)	21	(56.8%)	2	(5.4%)	42	(40.4%)	7	(6.7%)
Diarrhea	106	(41.7%)	6	(2.4%)	25	(42.4%)	2	(3.4%)	24	(44.4%)	0	(0.0%)	17	(45.9%)	1	(2.7%)	40	(38.5%)	3	(2.9%)
Pain	96	(37.8%)	17	(6.7%)	24	(40.7%)	2	(3.4%)	17	(31.5%)	3	(5.6%)	16	(43.2%)	2	(5.4%)	39	(37.5%)	10	(9.6%)
Fatigue	94	(37.0%)	19	(7.5%)	20	(33.9%)	1	(1.7%)	19	(35.2%)	3	(5.6%)	11	(29.7%)	3	(8.1%)	44	(42.3%)	12	(11.5%)
Anorexia	74	(29.1%)	6	(2.4%)	16	(27.1%)	2	(3.4%)	16	(29.6%)	2	(3.7%)	9	(24.3%)	0	(0.0%)	33	(31.7%)	2	(1.9%)
Hand/Foot Syndrome	65	(25.6%)	17	(6.7%)	.	.	.	.	.	.	.	.	3	(8.1%)	0	(0.0%)	62	(59.6%)	17	(16.3%)
Weight loss	64	(25.2%)	1	(0.4%)	10	(16.9%)	0	(0.0%)	5	(9.3%)	0	(0.0%)	9	(24.3%)	1	(2.7%)	40	(38.5%)	0	(0.0%)
Dyspnea	58	(22.8%)	17	(6.7%)	14	(23.7%)	3	(5.1%)	8	(14.8%)	3	(5.6%)	9	(24.3%)	2	(5.4%)	27	(26.0%)	9	(8.7%)
Anemia	57	(22.4%)	6	(2.4%)	5	(8.5%)	1	(1.7%)	14	(25.9%)	1	(1.9%)	10	(27.0%)	1	(2.7%)	28	(26.9%)	3	(2.9%)
Nausea	49	(19.3%)	6	(2.4%)	10	(16.9%)	1	(1.7%)	7	(13.0%)	1	(1.9%)	7	(18.9%)	1	(2.7%)	25	(24.0%)	3	(2.9%)
Hypertension	38	(15.0%)	15	(5.9%)	.	.	.	.	18	(33.3%)	7	(13.0%)	.	.	.	.	20	(19.2%)	8	(7.7%)
Hypertriglycer	31	(12.2%)	11	(4.3%)	2	(3.4%)	0	(0.0%)	.	.	.	.	28	(75.7%)	11	(29.7%)	1	(1.0%)	0	(0.0%)
Dry skin	30	(11.8%)	1	(0.4%)	13	(22.0%)	0	(0.0%)	3	(5.6%)	0	(0.0%)	4	(10.8%)	1	(2.7%)	10	(9.6%)	0	(0.0%)
Vomiting	29	(11.4%)	1	(0.4%)	6	(10.2%)	0	(0.0%)	5	(9.3%)	0	(0.0%)	5	(13.5%)	0	(0.0%)	13	(12.5%)	1	(1.0%)
Leukopenia/neutropenia	12	(4.7%)	3	(1.2%)	1	(1.7%)	1	(1.7%)	3	(5.6%)	0	(0.0%)	6	(16.2%)	2	(5.4%)	2	(1.9%)	0	(0.0%)

Supplementary Table 3. Summary of Compliance (in %)

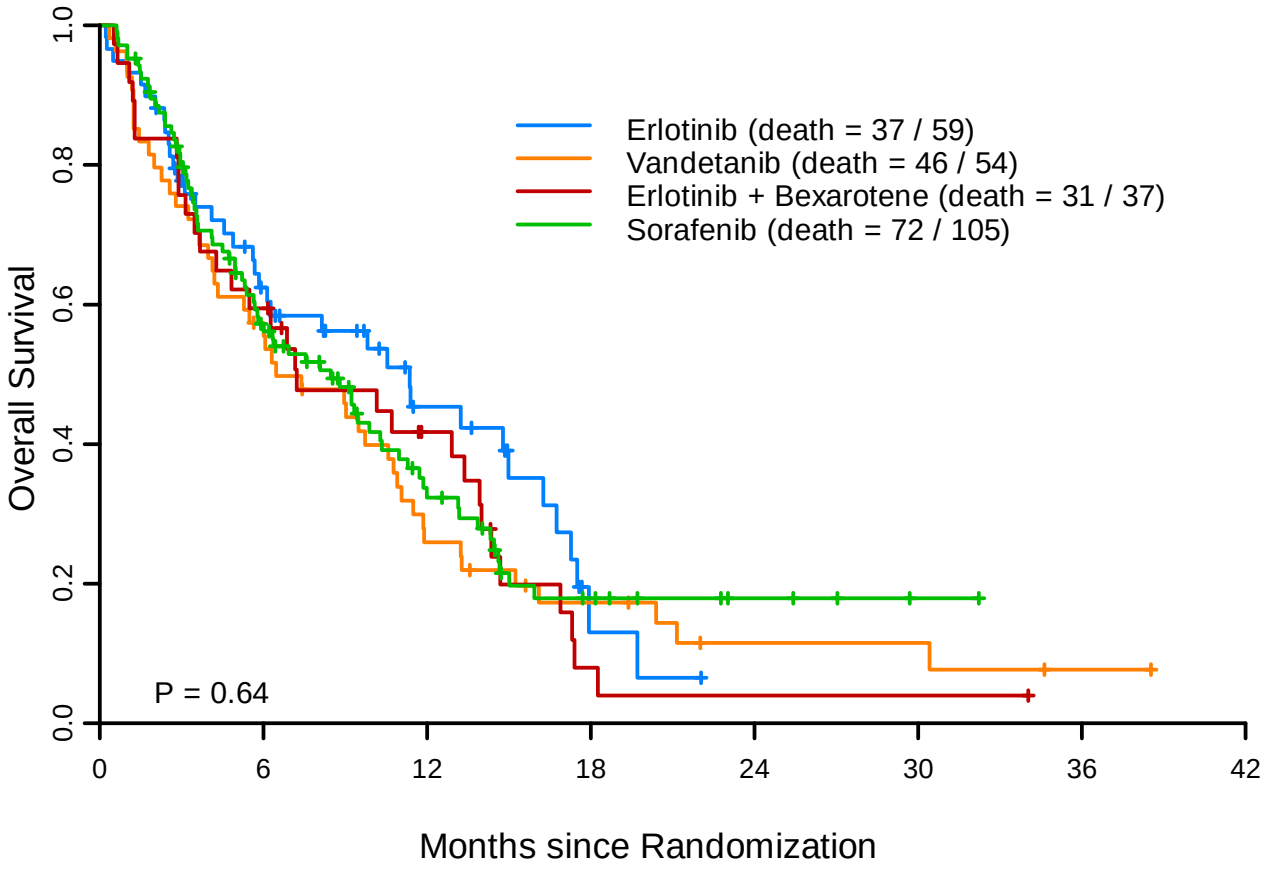
Treatment	N	Minimum	Median	Maximum	Mean	Std Dev
Erlotinib	58	80	100	100	98	4
Vandetanib	54	88	100	100	99	2
Erlotinib (in E+B)	37	66	100	100	98	6
Bexarotene (in E+B)	37	52	100	100	95	10
Sorafenib	104	61	99	100	97	5

***** The compliance% for each patient is calculated by
compliance% = (total number of tablets actually taken)/(targeted total

E: Erlotinib
B: Bexarotene

Supplementary Figure 1. Efficacy

A. Overall Survival by Treatment



Supplementary Appendix

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 (CT) 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 only 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. Using image-guidance to evaluate the needle's trajectory, an 18-or 19-gauge guide needle (Cook, Bloomington, In) 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 to 3 core biopsy samples were obtained with a 20-gauge biopsy needle (Quick-core; Cook, Bloomington, In). The samples were handed over to the appropriate research personnel for handling and processing.

After the biopsy procedure, the 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 after the biopsy procedure. In the absence of a pneumothorax, this was followed by a chest radiograph 3 hours after the biopsy. If the initial chest radiograph showed a pneumothorax, however, 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.

BATTLE Executive Committee Members

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Supplementary Information: Eligibility Criteria

The following inclusion criteria must be met for entry into the study:

1. The patient has a diagnosis of pathologically confirmed NSCLC by tumor biopsy and/or fine-needle aspiration.

2. The patient has a diagnosis of either stage IIIB, stage IV, or advanced, incurable NSCLC, and failed at least one front-line metastatic NSCLC chemotherapy regimen. (Patients who have failed adjuvant or locally advanced therapy within 6 months are also eligible to participate in study).

3. The patient has uni-dimensionally measurable NSCLC.

4. Karnofsky performance status ≥ 60 or ECOG performance status 0-2

5. The patient has biopsy accessible tumor.

6. The patient has adequate hematologic function as defined by an absolute neutrophil count (ANC) ≥ 1,500/mm3, platelet count ≥ 100,000/mm3, WBC ≥ 3,000/ mm3, and hemoglobin ≥ 9 g/dL.

7. The patient has adequate hepatic function as defined by a total bilirubin level ≤ 1.5 X the upper limit of normal, and alkaline phosphatase, AST or ALT ≤ 2.5 X the upper limit of normal.

8. The patient has adequate renal function as defined by a serum creatinine level ≤ 1.5 mg/dL or a calculated creatinine clearance of ≥ 60cc/minute.

9. The patient has PT < 1.5 x upper limit of normal

10. If patient has brain metastasis, they must have been stable (treated or asymptomatic) for at least 4 weeks after radiation if treated with radiation and not have used steroids for at least 1 week. Re-imaging performed after 2 weeks, upon completion of radiation therapy.

11. The patient is ≥ 18 years of age.

12. The patient has signed informed consent.

13. The patient is eligible if disease free from a previously treated malignancy, other than a previous NSCLC, for greater than two years. Patients with a history of prior basal cell carcinoma of the skin or pre-invasive carcinoma of the cervix are exempt from exclusion.

14. Women of childbearing potential must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) prior to study entry and for the duration of study participation. Childbearing potential will be defined as women who have had menses within the past 12 months, who have not had tubal ligation or bilateral oophorectomy. Should a woman become pregnant or suspect that she is pregnant while participating in this study, she should inform her treating physician immediately. The patient, if a man, agrees to use effective contraception or abstinence.

15. Subject must be considered legally capable of providing his or her own consent for participation in this study.

Exclusion Criteria

The following are grounds for exclusion from this study:

1. The patient has received prior investigational therapy, chemotherapy, surgery, or radiotherapy within 4 weeks of initiating study drug

2. The patient has undergone prior thoracic or abdominal surgery within 28 days of study entry, excluding prior diagnostic biopsy.

3. The patient has received radiation therapy to the measurable tumor within 6 months. Patients are allowed to have local irradiation for the management of tumor-related symptoms (bones, brain). However, if a patient has active new disease growing in the previously irradiated site, the patient will be eligible to participate in the study.

4. The patient has a significant medical history or unstable medical condition (unstable systemic disease: congestive heart failure (New York Heart Association Functional Classification class II or worse), recent myocardial infarction within 3 months, unstable angina, active infection (i.e. currently treated with antibiotics), uncontrolled hypertension). Patients with controlled diabetes will be allowed. Patient must be able to undergo procedure for tissue acquisition.

5. The patient has uncontrolled seizure disorder, active neurologic disease, or neuropathy ≥ grade 2. Patients with meningeal or CNS involvement by tumor are eligible for the study if the above exclusion criteria are not met.

6. The patient is pregnant (confirmed by serum β-HCG if applicable) or is breastfeeding.

7. Any condition that is unstable or could jeopardize the safety of the patient and its compliance in the study, in the investigator's judgment.

8. The patient is actively taking herbal remedies or over-the-counter biologics (e.g., shark cartilage, high dose antioxidants).

9. Patients will be allowed to have prior biologic (i.e. VEGF, EGFR, etc.) therapy. However, the patient will be excluded from a given study if he/she has received the same therapy as the clinical trial (i.e. If a patient has been previously treated with bevacizumab, they are allowed to enroll in any of the 4 studies. If a patient has been previously treated with erlotinib, they are excluded from the clinical trials with erlotinib). In addition, if a patient has been previously treated with gefitinib (Iressa), they are excluded from the clinical trials with erlotinib.

Table A-1. Sequences of primers used for DNA polymerase chain reaction amplification for mutation analysis of *EGFR*, *KRAS* and *BRAF*

Gene/Exon	Primers Sequence 5' to 3'	Annealing Temperature in °C
	Forward: CAT GTC TGG CAC TGC TTT CC	
EGFR/18	Reverse: TAT ACA GCT TGC AAG GAC TCT G	63
	Forward: CCA GAT CAC TGG GCA GCA TGT GGC ACC	
EGFR/19	Reverse: AGC AGG GTC TAG AGC AGA GCA GCT GCC	63
	Forward: CTC CTT CTG GCC ACC ATG CG	
EGFR/20	Reverse: AGC GCA GAC CGC ATG TGA GG	63
	Forward: GAC GTG GAG AGG CTC AGA GC	
EGFR/21	Reverse: AGC ATC CTC CCC TGC ATG TG	63
	Forward: TTC ATT ACG ATA CAC GTC TGC	
KRAS/1	Reverse: GTC CTG CAC CAG TAA TAT GC	52
	Forward: CTG TAA TAA TCC AGA CTG TG	
KRAS/2	Reverse: TCC CCA GTC CTC ATG TAC TG	50
	Forward: TCC CTC TCA GGC ATA AGG TAA	
BRAF/11	Reverse: CGA ACA GTG AAT ATT TCC TTT GAT	60
	Forward: TCA TAA TGC TTG CTC TGA TAG GA	
BRAF/15	Reverse: GGC CAA AAA TTT AAT CAG TGG A	60

1. Cappuzzo F, Hirsch FR, Rossi E, et al. Epidermal growth factor receptor gene and protein and gefitinib sensitivity in non-small-cell lung cancer. J Natl Cancer Inst 2005;97:643-55.

2. Uzawa N, Sonoda I, Myo K, Takahashi K, Miyamoto R, Amagasa T. Fluorescence in situ hybridization for detecting genomic alterations of cyclin D1 and p16 in oral squamous cell carcinomas. Cancer 2007;110:2230-9.

Supplementary Information: Biomarker Methodology

To examine eleven molecular biomarkers (Table 2) using the FFPE CNB tissue specimens, thirteen 5μm 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. Fluorescent in situ hybridization (FISH) analysis (*EGFR* and *CCND1*; n=2 sections); 4. Immunohistochemistry (IHC) analysis (VEGF, VEGFR-2, Cyclin-D1, RXRα, RXRβ and RXRγ n=6 sections). All specimens were assigned an ID number, linked with the clinical trial ID number, for subsequent processing in the laboratory. The certification of the presence of adequate tumor tissue in the FFPE tissue specimens by histology examination was performed within 24-48 hours, and the analysis of the eleven 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 5μm-thick HE-stained, FFPE histology sections. DNA was extracted using 25 μl of Pico Pure TM DNA Extraction solution (Arcturus, Mountain View, CA) containing proteinase K and incubated at 65°C for 24 hours. Subsequently, proteinase K inactivation was performed by heating samples at 95°C for ten minutes.

Mutation Analyses. Mutation of *EGFR* (exons 18 to 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 polymerase chain reaction (PCR) amplified using primers shown in Supplementary Table 1. Each PCR amplification was performed in 30 μl volume containing 2 μl DNA (approximately 100 ng of genomic DNA), 0.3 μm forward and reverse primers, 15 μl HotStarTaq (1.5 units of DNA polymerase) Master Mix (Qiagen, Valencia, CA) for 40 cycles at

94°C for 30 seconds, for 30 seconds at the primers pairs' annealing temperature (Supplementary Table 1), and 72°C for 45 seconds, followed by 7 min extension at 72°C. All PCR products were directly sequenced using Applied Biosystems PRISM dye terminator cycle sequencing method (Perkin-Elmer Corp., Foster City, CA). 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**. We examined copy number analysis of both genes using fluorescent in situ hybridization (FISH). For *EGFR*, we analyzed the gene copy number per cell using the LSI EGFR SpectrumOrange/CEP7 SpectrumGreen Probe (Abbott Molecular, Des Plaines, IL), as previously described [1]. For *CCND1*, we used the Vysis LSI *CCND1* (SO)/CEP11 DNA probe set (Abbott Molecular, Des Plaines, IL).

For both FISH analysis, histology sections were incubated at 56°C overnight and deparaffinized by washing in CitriSolv (Fisher Scientific, Pittsburgh, PA). After incubation in denature solution containing 70% formamide and 2× saline sodium citrate buffer (SSC), 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 15–25 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%). We applied the probe set onto the selected area, per the manufacturer's instructions, on the basis of the tumor foci seen on each slide. We then covered the hybridization area with a glass coverslip and sealed the coverslip with rubber cement. The slides were incubated at 80°C for 10 minutes for codenaturation of chromosomal and probe DNA and then placed in a humidified chamber at 37°C for 20–24 hours to allow hybridization to occur. Post-hybridization 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, Burlingame, CA) was applied for chromatin counterstaining.

For both genes, fluorescence signals were scored in at least 50 non-overlapping interphase nuclei per tumor, and the section of the area was guided by image captured in the H&E and eosin stained section. The number of copies of *EGFR* and chromosome 7 probes were assessed independently using a fluorescent microscope (Cytovision^{R} platform, Genetix, Boston, MA). The number of copies of *CCDN1*and chromosome 11 probes were assessed independently using a fluorescent microscope (Cytovision^{R} platform, Genetix, Boston, MA).

For *EGFR*, cases were classified into six 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 the 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 non-increased *EGFR* copy number, as previously published [1].

For *CCDN1*, 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 [2].

Immunohistochemistry Analysis. We examined using immunohistochemistry the protein expression of VEGF, VEGFR-2, RXRα, RXRβ, RXRγ and Cyclin-D1. For VEGF, VEGFR-22, 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. For these analyses we used commercially available antibodies, as follows: VEGF, rabbit polyclonal antibody (Santa Cruz Biotechnology, Inc, Santa Cruz, CA), dilution 1:200; VEGFR-2, mouse monoclonal antibody (Santa Cruz Biotechnology, Inc, Santa Cruz, CA), dilution 1:200; RXRα, rabbit polyclonal antibody (Santa Cruz Biotechnology, Inc, Santa Cruz, CA), dilution 1:300; RXRβ, rabbit polyclonal antibody (Upstate, Lake Placis, NY), dilution 1:100;and RXRγ rabbit polyclonal antibody (Santa Cruz Biotechnology, Inc, Santa Cruz, CA), dilution 1:200; and, Cyclin-D1, rabbit monoclonal antibody (clone SP4; Thermo Scientific, Fremont, CA), dilution 1:100.

All immunostainings were performed using automated stainers (DakoCytomation, Carpinteria, CA). Five-μm-thick sections were deparaffinized, rehydrated, and washed with phosphate buffered saline (PBS) buffer. Antigens were retrieved with 0.01 M citrate buffer (pH 6.0) (DakoCytomation, Carpinteria, CA) for 30 minutes in a steamer. Samples were blocked for endogenous activity in 3% hydrogen peroxide-PBS, avidin-biotin solution (Zymed, San Francisco, CA), 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 anti-rabbit biotinylated immunoglobulins (DakoCytomation). After incubation with the secondary antibody, the sections were incubated with the avidin-biotin-peroxidase complex (DakoCytomation) and developed with 3,3'-

diaminobenzidine. After this, the sections were rinsed in distilled water, counterstained with Mayer's hematoxylin, and mounted for evaluation. As positive controls of the immunohistochemical expression we used surgically resected FFPE NSCLC tumor tissue specimens with known expression of the markers examined. As negative controls, we used the same FFPE tissues and we omitted the primary antibody.

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 expressions were assessed using a semiquantitative analysis of intensity and extension. For cytoplasmic/membrane expression, the percentage of positive tumor cells in the cytoplasm/membrane (0% to 100%) was multiplied by the intensity of staining (0 to 3); therefore, the possible overall score ranged from 0 to 300. Nuclear expression was evaluated for any positive immunostaining was expressed in percentage. Nuclear score >30% was considered positive for RXRα, and nuclear score >10% was considered positive for Cyclin-D1.

Serum Collection

Serum was collected from consenting patients at baseline and after each cycle of treatment. Samples were stored for all patients consented to the optional blood collection, venous blood was collected at the following time points, baseline (pre-treatment), end of cycle 1 and 2, and at every 2 cycles thereafter until off protocol. At each time point, eight (8) mL of venous blood was collected into EDTA-based Vaccutainer® 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 three pre-labeled cryovials and stored at –70°C until analysis.