I. SUPPLEMENTARY FIGURES

Supplemental Figure 1. Sanger sequencing identifies two glioblastoma cell lines with EGFR mutation. Shown are results of Sanger sequencing forward and reverse reactions performed on genomic DNA from SF268 and SKMG3 glioblastoma cell lines. Mutations in codon 289 of EGFR are indicated with arrows.

Supplemental Figure 2. SKMG3 glioma cells were acutely transduced with control, EGFR-targeted, or HER2-targeted shRNAs. The extent of EGFR and HER2 knockdown was evaluated by immunoblot (left). Cell death was assessed by trypan blue staining (right).

Supplemental Figure 3. Immortalized human astrocytes were stably transduced with the indicated alleles of EGFR and treated with increasing doses of erlotinib or lapatinib as indicated. Cells were lysed and whole cell lysates analyzed by immunoblot with the indicated antibodies.

Supplemental Figure 4. Human non-small cell lung carcinoma (A and B) and glioblastoma (C) cell lines were treated with the indicated doses of lapatinib and erlotinib. Following treatment, cells were lysed and lysates subjected to immunoblot with the indicated antibodies to document the effects of each drug on target inactivation and signal transduction.
**Supplemental Figure 5.** Whole cell lysates from NR6 cells stably transduced with an EGFR-A289D cDNA were subjected to an ATP competition-binding assay (see Figure 4A) where ATP binding was competed with the indicated doses of erlotinib and lapatinib. Avidin pulldowns following the labeling step were subjected to western blot analysis with the indicated antibodies.

**Supplemental Figure 6.** SKMG3 whole cell lysates were subjected to the ATP competition-binding assay (see Figure 4A) where ATP binding was competed with the indicated doses of CI-1033 and HKI-272. Avidin pulldowns following the labeling step were subjected to western blot analysis with the indicated antibodies.

**Supplemental Figure 7.** SKMG3 (A) and KNS-81-FD (B) glioblastoma cells were treated with the indicated doses of lapatinib. Cell death was assessed 5 days after treatment by trypan blue exclusion. Note that significant cell death starts to occur with lapatinib concentrations greater than 1.5µM.

**Supplemental Figure 8.** SKMG3 cells were acutely transduced with a dilution series of a lentiviral EGFR-targeted shRNA (dilution factor is indicated in parenthesis) or with a control shRNA (empty vector). A fraction of the cells from each infection was used for immunoblot analysis with the indicated antibodies (right). Lysates were probed with an anti-Src antibody to control for loading. The remaining cells were re-seeded and allowed to grow for 5 days post-infection at the end of which cell death was assessed by trypan blue staining (left).
**Supplemental Figure 9.** Effects of erlotinib and lapatinib on the viability and anchorage-independent growth of patient-derived glioma sphere lines. (A and B) Two EGFR-amplified glioma sphere lines (GS676 nad GS600) were grown in the presence or absence of the indicated doses of erlotinib or lapatinib. The effects of each drug on cell death were assessed by the trypan-blue method after 5 days of treatment. (C) Representative image of a colony formation assay of GS676 cells treated with the indicated doses of lapatinib.

**Supplemental Figure 10.** Histological examination of GS676 xenografts. GS676 tumors (see Figure 6D) were stained with H&E (top panel) and an antibodies against cleaved Caspase3 (bottom panel) to assess apoptotic cell death.
II. SUPPLEMENTARY TABLES

**Supplemental Table 1.** Patient Characteristics for NABTC 04-01 clinical trial.
Summary for NABTC 04-01 clinical trial.

**Supplemental Table 2.** Demographic, clinical outcome, and molecular profiling results for all NABTC 04-01 patients.

**Supplemental Table 3.** Adverse Events on NABTC 04-01 Trial.

**Supplemental Table 4.** Lapatinib concentrations in all examined tumor and plasma samples.

**Supplemental Table 5.** Median Lapatinib tumor and plasma concentrations in context of other demographic and tumor profiling results.

**Supplemental Table 6.** Control tumor samples for EGFR/pEGFR profiling.

**Supplemental Table 7.** EGFR profiling results for all NABTC 04-01 with sufficient frozen tumor tissue for molecular analysis.

**Supplemental Table 8.** NABTC 04-01 patient outcomes, tumor concentrations, plasma concentrations, EGFR immunoblot and EGFR mutation data. Rows 2-7 define patients whose lapatinib tumor levels were $\geq 1500$ nM.
III. SUPPLEMENTARY CLINICAL TRIAL INFORMATION

1. Protocol. This Biomarker Phase II trial was registered with http://www.Clinical Trials.gov (#NCT00107003) (see also http://clinicaltrials.gov/ct2/show/ NCT00107003? term=glioblastoma+lapatinib&rank=1). The clinical trial protocol (NABTC 04-01) conduct and regulatory activities were run through the central office of NABTC and approved by the Institutional Review Boards of each of the NABTC institutions taking part in the study (UCSF, UCLA, University of Wisconsin, University of Pittsburgh, MD Anderson Cancer Center, Dana Farber Cancer Institute, Duke University, Memorial Sloan Kettering Cancer Center, Neuro-Oncology Branch of the NCI).

2. Objectives. The primary goals of this biomarker phase II trial were as follows: (1) to determine the 6-month progression-free survival rate; (2) to determine if lapatinib inhibits the phosphorylation of its cellular targets and the downstream signaling pathways in glioblastoma tissue; and (3) to determine tumor concentrations of lapatinib.

3. Enrollment. Enrollment was restricted to non-pregnant, contraceptive practicing patients with a histological diagnosis of glioblastoma (GBM), including secondary GBM, radiographic evidence for disease recurrence after radiation therapy, no previous signal transduction inhibitor therapy, off all enzyme inducing anticonvulsants or other agents know it interfere with metabolism of lapatinib, and availability of archived formalin fixed paraffin embedded (FFPE) tumor sample from a prior surgery. Other enrollment criteria included age > 18 y, Karnofsky performance score (KPS) ≥ 60, life expectancy ≥ 8 wk, adequate bone
marrow function (white blood cell [WBC] ≥ 3,000/μl, absolute neutrophil count [ANC] ≥ 1,500/μl, platelets ≥ 100,000/μl, hemoglobin ≥ 10 gm/dl), adequate liver and renal function (serum glutamic oxaloacetic transaminase [SGOT] and bilirubin < 2.5× upper limits of normal, creatinine < 1.5 mg/dl) cardiac ejection fraction within the range of normal. Patients must have recovered from the toxic effects of prior therapy and at least 28 days from any investigational agent, 28 days from prior cytotoxic therapy, 28 days from radiation, 14 days from vincristine, 42 days from nitrosoureas, 21 days from procarbazine administration, and 7 days from FDA approved non-cytotoxic agents. Any patient with prior use of interstitial brachytherapy or stereotactic radiosurgery must have confirmation of true progressive disease rather than radiation necrosis based upon either PET or Thallium scanning, MR spectroscopy or surgical documentation of disease. Patients with up to two prior relapses were eligible. All patients enrolled in the clinical trial gave written informed consent to participate in these evaluations.

Forty-four patients, who also met all other eligibility criteria, were enrolled at the time of tumor recurrence and received neoadjuvant oral daily lapatinib (750mg bid) for 7 to 10 days prior to salvage surgical resection. After recovery from surgery, patients resumed twice daily lapatinib treatment at the neoadjuvant dose until clinical and/or radiographic evidence for tumor progression was found. Please see schema in Figure 5A. Patient characteristics are found on Supplementary Table 1.
The following table defines the number of patients used to achieve the objectives above.

<table>
<thead>
<tr>
<th>Primary Objective</th>
<th>Patients Enrolled</th>
<th>Evaluable for Primary Endpoint</th>
</tr>
</thead>
<tbody>
<tr>
<td>PFS6</td>
<td>44</td>
<td>33 (Suppl. Table 2)</td>
</tr>
<tr>
<td>Impact on EGFR signaling</td>
<td>44</td>
<td>18 (Suppl. Table 6)</td>
</tr>
<tr>
<td>Lapatinib Tumor Concentr.</td>
<td>44</td>
<td>27 (Suppl. Table 5)</td>
</tr>
</tbody>
</table>

Supplementary Table 2 provides details on each patient for each of these three objectives.

4. Adverse Events. Adverse events were graded using NCI Common Terminology Criteria for Adverse Events (CTCAE) version 3.0. Adverse events were recorded from registration to 30 days post last dose of study drug. Adverse events attributed (possible, probable and definite) to lapatinib are provided in Supplementary Table 3. There are 14 grade 3 or greater events. Most are expected either from lapatinib (diarrhea, rash, nausea, vomiting, fatigue, and leucopenia) or are common adverse events seen in glioblastoma patients (lymphopenia and headache).

5. Clinical Outcomes.

- Supplementary Table 2, columns A through J, provides the detailed patient-by-patient characteristics, evaluability and clinic outcomes including time to tumor progression (TTP) and time to survival (TTS). TTP was defined as the time from post surgical initiation of lapatinib to tumor progression. TTS was defined as time from post surgical initiation of lapatinib to death.
- Eleven patients did not resume therapy post operatively.
- Of those who were evaluable for TTP and went off treatment for reasons other than progression or death, 4 died within 30 days of last dose date and date of death was
used for calculation of PFS. One additional patient was considered censored at 5 weeks although the patient died a little over 1 month later. One patient had no last dose reason given, but that patient died while on treatment at 65 weeks.

- Only 1 patient had PFS greater than 26 weeks for a success rate of 3%. That patient died at 65 weeks. 5 patients were censored. None with more than 7 weeks of follow up. The KM estimates were: median PFS was 12 weeks (95% c.i. 8-16). The KM estimate of PFS6 was also 3%.

6. Lapatinib concentrations. Results are described in the main manuscript and in Figure 5B. Individual patient information is listed in Supplementary Tables 4 (all aliquots) and Supplementary Table 5 (median values).

7. EGFR inhibition. Results are described in the main manuscript and in Figure 5C. Individual patient information is listed in Supplementary Table 6. Sample information for the control cohort of recurrent GBM tumor samples is summarized in Supplementary Table 7.

8. Supplementary Methods.

8a. Sample Collection. A pre-dose PK blood sample (10mL, Na EDTA) and a PG blood sample (10mL, Na Heparin) were obtained followed by 7 days of twice daily lapatinib ending the evening prior to surgery. A PK blood and tumor tissue sample (minimum 50 mg, snap frozen) were obtained at the same time of resection. Post centrifugation of the blood samples, the plasma for PK and RBC pellet for PG and the tissue sample were frozen (≤ - 200C) until analysis.
8b. LC/MS/MS Analysis. Lapatinib concentrations were determined by a validated liquid chromatography-tandem mass spectroscopy method with an electrospray interface in the positive ion mode (Rapid Common Mass Spectrum 18:285, 2004). The lower limit of in plasma was 5 ng/mL and 0.08 ng/mL in brain tumor tissue extract. Lapatinib concentrations at the time of resection were the observed values.

8c. Pharmacogenomics Analysis. Genomic DNA was extracted using standard procedures. Genomics DNA and separate primersequences were amplified by PCR and genotyped by pyrosequencing. The following polymorphisms were genotype: ABCB1 [(G2677TA), (C3435T), (C1236T)], SLCO1A2 [(A516C), (T38C)], CYP3A4*1B (A-392G) CYP3A5*3A (A6986G), ABCC1 (G4002A) and EGF (A61G). Nineteen patients were genotyped, thirteen of which had their tumor tissue analyzed for lapatinib. The allelic frequencies (%) were: ABCB1 [(G2677TA – 68%); (C3435T – 68%): (C1236T – 79%); ABCC1 (G4002A – 37%); SLCO1A2 [(A516C – 11%), (T38C – 21%)]; CYP3A4*1B (A – 392G – 5%); CYP 3A5 (A6986G – 100%); EGF (A61G – 0%). There was no statistically significant relationship between any of the genotypes and tumor concentrations. However, we did observe a trend with the SLCO1A2 (T38C) genotype and higher lapatinib tumor concentrations. One additional observation (n=1) was that genotypes for the genomic DNA and tumor tissue DNA were identical.