Supplementary information 1

Direct sequencing of EGFR exon 20 revealed the presence of T790M secondary mutations in PC9BR clones dependent on EGFR signaling.

A

B
A

The relative copy number of the *c-met* gene in PC9 BIBW resistant clones. Con. indicates parental PC9 cells, the *c-met* copy number of which was set to 1. Numbers indicate PC9 BIBW resistant clone numbers and the bars show the relative *c-met* copy number.

B

PTEN protein expression level was detected by PTEN Western blotting analysis.
Supplementary information 3

<table>
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Case description> A 44-year-old Hispanic female never-smoker was diagnosed with stage IIIA adenocarcinoma in January, 2007. The patient underwent three cycles of neoadjuvant cisplatin/gemcitabine therapy and subsequently underwent a right upper sleeve lobectomy and mediastinal lymphadnectomy on 5/30/2007. Pathology revealed a 3cm well-differentiated adenocarcinoma with perineural invasion. EGFR sequencing of the resected tumor sample revealed the L747_P753 del 17 mutation in exon 19.

This patient subsequently received two more cycles of adjuvant cisplatin/gemcitabine chemotherapy, which were completed by July, 2007 and then she received adjuvant mediastinal radiation of 50.4 Gy from September, 2007 to October, 2007. She was followed up with regular CT scans. On August 2008, two metastatic nodules were noted in the left lung (Supp.Fig. 4A).

The patient was enrolled in the Boehringer-Ingelheim 1200.22 phase II study of BIBW2992 as a 1st or 2nd line treatment of activating EGFR mutation-positive NSCLC patients. The patient started on BIBW2992 at 50 mg po qD but the dose had to be lowered to BIBW2992 at 40 mg due to nailbed toxicities. The patient had a confirmed partial response to BIBW2992 with disappearance of one pulmonary nodule and a marked decrease in size of the other pulmonary nodule (Supp. Fig. 4B).

However, in September, 2009, one of the two metastatic pulmonary nodules that had disappeared reappeared. This was confirmed at a subsequent scan in February, 2010 and the patient was taken off the protocol treatment due to disease progression as assessed by RECIST, even though the other pulmonary nodule remained under control (Supp. Fig. 4C). The resistant pulmonary nodule was resected by a wedge resection in March, 2010. Pathology revealed a 1.5cm poorly-differentiated adenocarcinoma with <5% necrosis. Repeat EGFR sequencing revealed the original L747_P753 del 17 mutation in exon 19 plus the presence of the T790M mutation.

Supplementary Fig. 4A

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