

Supplementary Materials for

Genotypic and Histological Evolution of Lung Cancers Acquiring Resistance to EGFR Inhibitors

Lecia V. Sequist,* Belinda A. Waltman, Dora Dias-Santagata, Subba Digumarthy, Alexa B. Turke, Panos Fidias, Kristin Bergethon, Alice T. Shaw, Scott Gettinger, Arjola K. Cosper, Sara Akhavanfard, Rebecca S. Heist, Jennifer Temel, James G. Christensen, John C. Wain, Thomas J. Lynch, Kathy Vernovsky, Eugene J. Mark, Michael Lanuti, Anthony J. Iafrate, Mari Mino-Kenudson, Jeffrey A. Engelman*

*To whom correspondence should be addressed. E-mail: lvsequist@partners.org (L.V.S.); jengelma@partners.org (J.A.E.)

Published 23 March 2011, *Sci. Transl. Med.* **3**, 75ra26 (2011)
DOI: 10.1126/scitranslmed.3002003

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Figure S1. Percent Change in Measurable Disease, by Patient

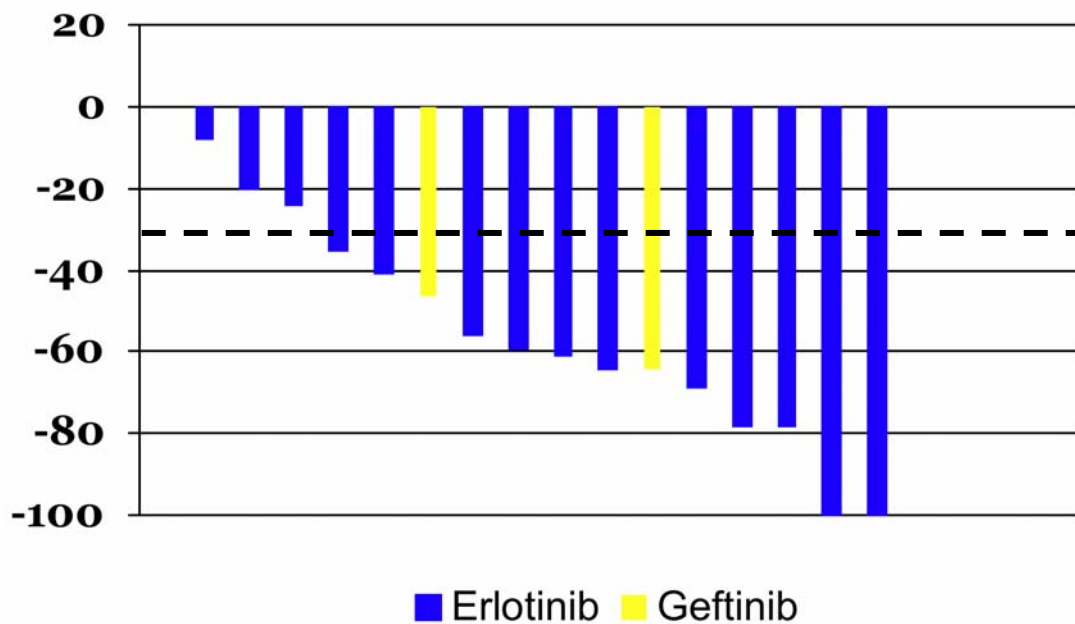


Fig S1. The percent change in size of the measurable tumor from baseline to best response while on the first EGFR TKI therapy received is displayed per individual patient using standard RECIST measurement technique. A dashed line is at negative 30% to represent the RECIST-defined threshold for partial response to therapy.

Figure S2. Amplification of the T790M Allele in Tumors with *EGFR* Amplification

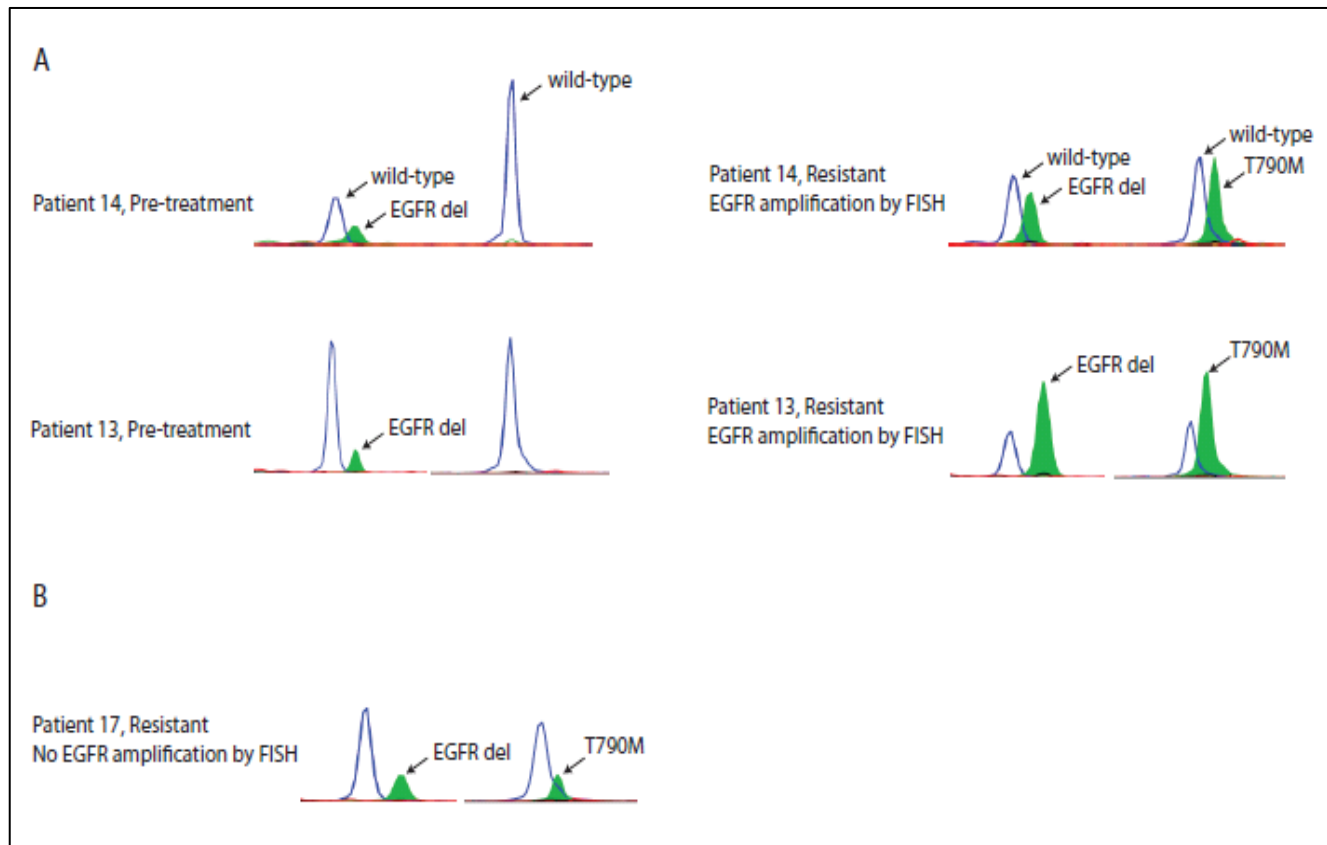


Fig S2. Chromatograms of two patients with acquired amplification of the T790M allele. The chromatograms are magnified to show only the assays of interest. Blue lines depict the wild type allele at each tested locus and green shaded peaks identify the mutations present in the tumor specimens. A) Patient 14 showed an exon 19 deletion mutation but no T790M at baseline (*left*). Upon repeat biopsy (*right*) there was a higher amplitude peak at exon 19 as well as a high amplitude peak at T790M suggesting that the *EGFR* amplification found on FISH analysis led to increased copy number of both the sensitizing and resistance mutations. Patient 13 also demonstrated marked *EGFR* amplification in the resistant specimen (see Fig. 2), and the chromatogram demonstrates amplification of both his original exon 19 deletion mutation and T790M on the repeat biopsy. B) This is in contrast to Patient 17, who acquired T790M but there was no *EGFR* amplification determined by FISH.

Figure S3. The Overall Distribution of the Genetic and Histological Findings in the Resistant Biopsies

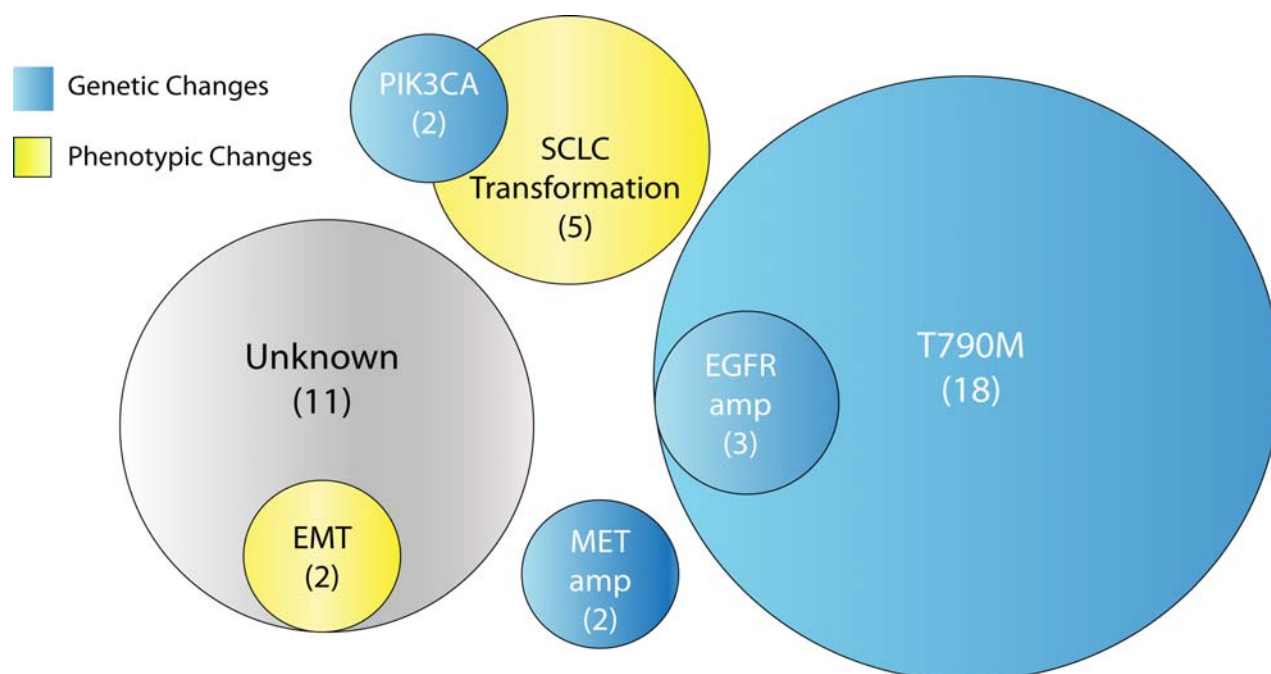


Fig S3. The prevalence and overlap of the genetic and histological changes in the acquired resistant biopsies is shown. Among the 18 patients with acquired T790M mutations, 3 also had acquired *EGFR* amplification. Two patients developed *MET* amplification. Five patients underwent a NSCLC to SCLC phenotypic transformation, and one of those patients also acquired a *PIK3CA* mutation. Another patient developed isolated acquisition of a *PIK3CA* mutation. Within the 11 patients without acquired genetic alterations or change to SCLC, two had evidence of EMT (only 7 of the 11 were assessed for EMT changes).

Table S1: Detailed Results for 37 Paired Specimens with TKI resistance

Pre-Treatment						Drug Resistant					Summary of Changes	Biopsy Data			Therapy Data	
#	A g e	S e x	EGFR mut	Histo	Add'l Muts	Histo	T 7 9 0 M	Add'l Muts	Acq EGFR amp	MET amp		Initial Bx Site	Repeat Bx Site	Type of Repeat Bx	Primary TKI (time on TKI)	TKI status at Repeat Bx
T790M																
1	66	M	L858R	Adeno	ST	NSCLC	+	--	--	--	T790M	Sclav LN	Adrenal	CT-guided Core	Erlo (6 mos)	Off† (2 mos)
2	74	F	E 19 del	Adeno	B-cat	Adeno	+	B-cat	--	--	T790M	Med LN	Liver	US-guided Core	Erlo (12 mos)	On
3	47	F	E 19 del	Adeno	NT	Adeno	+	-- ◇	--	--	T790M	RLL lung	Sclav LN	Excision	Gef (15 mos)	On
4	60	F	E 19 del	Adeno	NT	Adeno	+	-- ◇	--	--	T790M	Sclav LN	RLL lung	EBUS FNA/CB	Erlo (7 mos)	On
5	57	M	L858R	Adeno	NT	Adeno	+	-- ◇	--	--	T790M	LUL lung	LUL lung §	CT-guided FNA/CB	Gef (5+ yrs)	On
6	47	M	E 19 del	Adeno	NT	Adeno	+	--	Ins	Ins	T790M	LLL lung	Liver	CT-guided Core	Erlo (12 mos)	Off† (14 mos)
7	58	F	E 19 del	Adeno	ST	Adeno	+	--	--	--	T790M	RUL lung	Liver	US-guided Core	Erlo (3+ yrs)	On
8	69	M	L858R	Adeno	TP53	Adeno	+	--^	--	--	T790M^	Med LN	LLL lung	CT-guided Core	Erlo (2 yrs)	On
9	58	F	G719C, S768I	Adeno	NT	Adeno	+	--	Ins	Ins	T790M	Liver	Liver §	US-guided Core	Erlo (2+ yrs)	On
10	46	F	E 19 del	Adeno	NT	Adeno	+	TP53	Ins	Ins	T790M	Cerv LN	RUL lung	Bronch FNA/CB	Erlo (3 yrs)	Off (3 mos)
11	53	F	E 19 del	Adeno	NT	Adeno	+	--	Ins	--	T790M	RLL lung	RLL lung §	CT-guided Core	Erlo (16 mos)	On
12	59	F	L858R	Adeno	TP53	Adeno	+	TP53	--	--	T790M	RUL lung	LLL lung	CT-guided Core	Erlo (8 mos)	Off† (5 mos)
T790M + EGFR amp																
13	42	M	E 19 del	Adeno	--	Adeno	+	--	+	--	T790M, EGFR amp	RML lung	RUL Lung	CT-guided FNA/CB	Erlo (5 mos)	On
14	55	M	E 19 del	Adeno	TP53	Adeno	+	TP53 ◇	+	--	T790M, EGFR amp	RLL lung	RUL lung	CT-guided Core	Erlo (10 mos)	On
15	37	F	E 19 del	Adeno	--	Adeno	+	--	+	--	T790M, EGFR amp	LLL lung	Liver	CT-guided Core	Erlo (6 mos)	On
T790M + new, additional mutations																
16	88	F	E 19 del	Adeno	--	Adeno	+	B-cat	Ins	--	T790M, B-cat	Pleural fluid	Cerv LN	Excision	Erlo (2+ yrs)	On
17	85	M	E 19 del	Adeno	--	Adeno	+	B-cat ◇	--	--	T790M, B-cat	Liver	Pleural fluid	US-guided tap/CB	Erlo (22 mos)	On
18	75	F	E 19 del	Adeno	ST	NSCLC	+	APC	--	Ins	T790M, APC•	LUL lung	Liver	CT-guided Core	Erlo (18 mos)	On

MET amplification																
19	61	M	L858R	Adeno-Squam	NT	NSCLC	--	--	--	+	MET amp, loss EGFR amp	RUL lung	RML lung	CT-guided Core	Erlo (15 mos)	On
20	76	M	L858R	Adeno	NT	Adeno	--	--	Poly	+	MET amp	Pleura	Periton. fluid	US-guided tap/CB	Erlo (13 mos)	Off† (5 mos)
Acquired PIK3CA mutation																
21	65	M	E 19 del	Adeno	ST	NSCLC	--	PIK3 CA	--	--	PIK3CA acquisition	Med LN	Jaw mass	Excision	Erlo (21 mos)	On
Histologic Transformation (1 with acquired PIK3CA mutation)																
22	67	F	L858R	Adeno	ST	SCLC	--	--	--	--	SCLC trans	Cerv LN	Lingula lung	CT-guided Core	Erlo (22 mos)	On
23	54	F	E 19 del	Adeno	NT	SCLC	--	--	--	--	SCLC trans	Med LN	Med LN	EBUS Core	Erlo (3+ yrs)	On
24	56	F	L858R	Adeno	ST	SCLC	--	PIK3 CA	Ins	--	SCLC trans, PIK3CA	Pleural fluid	LUL lung	CT-guided Core	Erlo (14 mos)	On
25	40	F	E 19 del	Adeno	ST	SCLC	--	-- ◇	--	--	SCLC trans	Pleura	CW mass	Excision	Erlo (2+ yrs)	Off (2 mos)
26	61	F	L858R	Adeno	ST	SCLC	--	--	Ins	--	SCLC trans	LUL lung	Subc nodule	Direct FNA	Erlo (18 mos)	On
27	66	M	L858R	Adeno	ST	Adeno/ EMT	--	-- ◇	Ins	--	EMT	RUL lung	Med LN	Bronch Core	Erlo (11 mos)	On
28	59	M	Exon 20 ins*	Adeno	--	Adeno/ EMT	--	-- ◇	--	--	EMT	LLL lung	Subc nodule	Excision	Gef (11 mos)	On
29	64	M	L858R	Adeno	B-cat	SarcC	--	-- ◇	Poly	--	SarcC, loss of B-cat	Brain	Cerv LN	Excision	Erlo (11 mos)	Off (2 weeks)
No Histological or Genetic Changes Identified																
30	62	F	L858R	Adeno	NT	Adeno	--	--	Ins	Ins	None	Liver	Pleural fluid	US-guided tap/CB	Erlo (6 mos)	On
31	52	F	E 19 del	Adeno	NT	Adeno	--	--	--	--	None	LUL lung	Omen-tum	CT-guided Core	Gef (17 mos)	On
32	58	F	E 19 del	Adeno	NT	Adeno	--	--	Ins	--	None	Med LN	RUL lung	CT-guided Core	Erlo (14 mos)	On
33	61	F	L858R	Adeno	NT	Adeno	--	TP53	Ins	--	None	Pleural fluid	Med LN	EBUS FNA/CB	Erlo (13 mos)	On
34	85	F	E 19 del	Adeno	--	Adeno	--	-- ◇	--	--	None	RUL Lung	Stomach	Excision	Erlo (6 mos)	On
35	62	M	L858R	NSCLC	NT	NSCLC	--	--	Ins	Ins	None	Lumbar vert.	RUL lung	CT-guided FNA/CB	Gef (3+yrs)	On
36	56	M	L858R	Adeno	--	Adeno	--	-- ◇	--	--	None	LUL lung	LUL lung §	CT-guided Core	Erlo (5 mos)	Off (<2 weeks)
37	51	F	E 19 del	Adeno	NT	Adeno	--	-- ◇	--	--	None	LUL lung	Pleural fluid	US-guided tap/CB	Erlo (8 mos)	On

Table S1 Legend:

EGFR = epidermal growth factor receptor

TKI = tyrosine kinase inhibitor

Mut = mutation

Histo = histology

Acq. = acquired

Amp = amplification

E 19 del = exon 19 deletion

Adeno = adenocarcinoma, **AdenoSquam** = adenosquamous

+ = yes/positive, **--** = no/negative

NSCLC = Non-small cell lung cancer not otherwise specified, **SCLC** = small cell lung cancer

SarcC = sarcomatoid carcinoma

Bx = Biopsy

ST = Some additional mutations tested and negative, but not the full panel

NT = Additional mutations not tested because of limited tissue

B-cat = beta-catenin

Ins = Insufficient tissue

Poly = EGFR polysomy; EGFR amp not tested in initial samples in these cases, so acquisition unknown

EMT = epithelial to mesenchymal transformation

◇ Testing for EMT performed on this patient

^TP53 mutation suspected to be present, but not confirmed

*Exon 20 insertion assay added to SNaPshot for this patient given direct sequencing result from pre-treatment sample

•APC mutation present in resistant specimen, but not confirmed to be present in initial biopsy

Sclav LN = supraclavicular lymph node, **Med LN** = mediastinal lymph node, **Cerv LN** = cervical lymph node

Adrenal = adrenal gland

Subc = subcutaneous

RUL/RLL/RML/LUL/LLL = lobes of the lung designated as right or left, and upper, middle or lower

Periton. fluid = peritoneal fluid

CW = chest wall

Vert = vertebra

§ = repeat biopsy location is the exact same lesion as pre-treatment biopsy location

CT = computed tomogram

US = ultrasound, **EBUS** = endobronchial ultrasound

FNA/CB = Fine Needle Aspiration with cell block made from composite of multiple passes

Tap/CB = fluid drainage was performed with cell block made

Erlo = erlotinib, **Gef** = gefitinib

†Denotes chemotherapy given between end of TKI administration and prior to repeat biopsy

Table S2. Mutations Tested in SNaPshot Version I

Gene	Amino Acid – cDNA Residue	Total Loci Tested
APC	R1114 - 3340C, Q1338 - 4012C, R1450 - 4348C, T1556fs* - 4666_4667insA	4
BRAF	V600 - 1798G, V600 - 1799T	2
CTNNB1(b-Cat)	D32 - 94G, D32 - 95A, S33 - 98C, G34 - 101G, S37 - 109T, S37 - 110C, T41 - 121A, T41 - 122C, S45 - 133T, S45 - 134C	10
EGFR	G719 - 2155G, T790 - 2369C, L858 - 2573T E746_A750 - 2235_2249del, E746_A750 - 2236_2250del	5
FLT3	D835 - 2503G	1
JAK2	V617 - 1849G	1
KIT	D816 - 2447A	1
KRAS	G12 - 34G, G12 - 35G, G13 - 37G, G13 - 38G	4
NOTCH1	L1575 - 4724T, L1601 - 4802T	2
NRAS	G12 - 34G, G12 - 35G, G13 - 37G, G13 - 38G Q61 - 181C, Q61 - 182A, Q61 - 183A	7
PIK3CA	R88 - 263G, E542 - 1624G, E545 - 1633G, Q546 - 1636C Q546 - 1637A, H1047 - 3139C, H1047 - 3140A, G1049 - 3145G	8
PTEN	R130 - 388C, R173 - 517C, R233 - 697C, K267fs* - 800delA	4
TP53	R175 - 524G, G245 - 733G, R248 - 742C, R248 - 743G R273 - 817C, R273 - 818G, R306 - 916C	7

Sizing Assay

EGFR exon 19 deletions

Table S3. Results from First Autopsy Case

Gender, Age	Autopsy site	Histology	Genotype*
Female, 67	Liver, nodule1	SCLC	L858R
	Liver, nodule 2	SCLC	L858R
	Liver, nodule 3	SCLC	L858R
	Lymph node – paratracheal	SCLC	L858R
	Lymph node – intraparenchymal, left upper lobe apex	SCLC	L858R
	Diaphragm	SCLC	L858R
	Brain	Adenocarcinoma	L858R

*The original activating mutation for each patient was also found in all of the primary and metastatic sites. No additional genetic mutations (including T790M and MET amplification) were identified.

Table S4. Results from Second Autopsy Case

Gender, Age	Autopsy site	Histology	Genotype*	Other
Male, 59	Omentum	Adenocarcinoma	Exon 20 insertion	
	Right upper lobe	Adenocarcinoma	Exon 20 insertion	
	Left bronchial lymph node	Adenocarcinoma	Exon 20 insertion	No EMT
	Right lower lobe	Sarcomatoid	Exon 20 insertion	EMT

*The original activating mutation for each patient was also found in all of the primary and metastatic sites. No additional genetic mutations (including T790M and MET amplification) were identified.