HHS Public Access

Author manuscript

JCO Precis Oncol. Author manuscript; available in PMC 2019 April 25.

Published in final edited form as:

JCO Precis Oncol. 2017 ; 1: . doi:10.1200/PO.17.00123.

Corresponding author: Lecia V. Sequist, MD, Massachusetts General Hospital, PO Box 212, 55 Fruit St, Boston, MA 02114; lvsequist@partners.org.

AUTHOR CONTRIBUTIONS

Conception and design: Meghan J. Mooradian, Zofia Piotrowska, Lecia V. Sequist

Provisionofstudymaterialorpatients: Konstantinos Arnaoutakis

Collection and assembly of data: Meghan J. Mooradian, Zofia Piotrowska, Benjamin J. Drapkin, Dora Dias-Santagata, Konstantinos Arnaoutakis, Anthony J. Iafrate, Mari Mino-Kenudson, Lecia V. Sequist

Data analysis and interpretation: Meghan J.Mooradian, Zofia Piotrowska, Benjamin J. Drapkin, Nicolas Marcoux, Rebecca J. Nagy, Richard Lanman, Anthony J. Iafrate, Anna F. Farago, Mari Mino-Kenudson, Aaron N. Hata, Lecia V. Sequist

Manuscript writing: All authors

Final approval of manuscript: All authors

Accountable for all aspects of the work: All authors

Meghan J. Mooradian

No relationship to disclose

Zofia Piotrowska

Consulting or Advisory Role: Boehringer Ingelheim, AstraZeneca, ARIAD Pharmaceuticals, Takeda Pharmaceuticals, superDimension

Research Funding: Guardant Health (Inst)

Benjamin J. Drapkin

No relationship to disclose

Dora Dias-Santagata

No relationship to disclose

Nicolas Marcoux

Honoraria: Bristol-Myers Squibb

Konstantinos Arnaoutakis

Honoraria: Foundation Medicine, Precision Advisors, ARIAD Pharmaceuticals

Consulting or Advisory Role: Foundation Medicine, Precision Advisors, ARIAD Pharmaceuticals

Rebecca J. Nagy

Employment: Guardant Health

Stock and Other Ownership Interests: Guardant Health

Richard Lanman

Employment: Guardant Health, Veracyte

Leadership: Guardant Health

Stock and Other Ownership Interests: Guardant Health, Veracyte

Research Funding: Guardant Health

Anthony J. Iafrate

Stock and Other Ownership Interests: Archer Biosciences

Consulting or Advisory Role: Debiopharm Group, Constellation Pharmaceuticals, Chugai Pharmaceutical, Roche

Research Funding: Blueprint Medicines

Patents, Royalties, Other Intellectual Property: ArcherDx exclusive license to AMP technology

Anna F. Farago

Honoraria: Foundation Medicine

Consulting or Advisory Role: PharmaMar, Merrimack, Takeda Pharmaceuticals, AbbVie, Intervention Insights

Research Funding: PharmaMar (Inst), AbbVie (Inst), AstraZeneca (Inst), Bristol-Myers Squibb (Inst), Merck (Inst), Loxo (Inst), Ignyta (Inst)

Travel, Accommodations, Expenses: PharmaMar, AbbVie, Stemcentrx

Mari Mino-Kenudson

Consulting or Advisory Role: Merrimack, H3 Biomedicine, ACD Pharmaceuticals, Roche, Genentech

Aaron N. Hata

Research Funding: Amgen, Novartis, Relay Therapeutics

Lecia V. Sequist

Honoraria: AstraZeneca

Consulting or Advisory Role: AstraZeneca, Genentech, Roche, Bristol-Myers Squibb, Pfizer

Research Funding: Boehringer Ingelheim (Inst), Clovis Oncology (Inst), Genentech (Inst), Merrimack (Inst), Novartis (Inst), AstraZeneca (Inst), Johnson & Johnson (Inst), Pfizer (Inst)

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The following represents disclosure information provided by authors of this manuscript. All relationships are considered compensated. Relationships are self-held unless noted. I = Immediate Family Member, Inst = My Institution. Relationships may not relate to the subject matter of this manuscript. For more information about ASCO's conflict of interest policy, please refer to www.asco.org/rwc or po.ascopubs.org/site/ifc.

Clonal Evolution and the Role of Serial Liquid Biopsies in a Case of Small-Cell Lung Cancer–Transformed *EGFR* Mutant Non–Small-Cell Lung Cancer

Meghan J. Mooradian[#], **Zofia Piotrowska**[#], **Benjamin J. Drapkin**, **Dora Dias-Santagata**, and **Nicolas Marcoux**
Massachusetts General Hospital, Boston, MA

Konstantinos Arnaoutakis
University of Arkansas for Medical Sciences, Little Rock, AR;

Rebecca J. Nagy and **Richard Lanman**
Guardant Health, Redwood City, CA.

Anthony J. Iafrate, **Anna F. Farago**, **Mari Mino-Kenudson**, **Aaron N. Hata**, and **Lecia V. Sequist**
Massachusetts General Hospital, Boston, MA

[#] These authors contributed equally to this work.

INTRODUCTION

Epidermal growth factor receptor (*EGFR*) mutation–positive lung cancers respond dramatically to EGFR tyrosine kinase inhibitors (TKIs),[1–3] and repeat biopsies at acquired resistance can illuminate the critical molecular resistance mechanisms.[4,5] Historically, resistance mutations were conceptualized as binomial variables (eg, cancers were either positive or negative for a given mutation); however, growing appreciation of intra- and intertumoral heterogeneity has shifted the paradigm toward resistant clones as dynamic populations, which shift in prevalence on the basis of the selective pressure of sequential therapies.[6] This case report illustrates how serial molecular monitoring may provide unique insight into clonal evolution.

CASE REPORT

A 63-year-old man with back pain and a minimal smoking history presented for medical attention in April 2015. Lumbar spine magnetic resonance imaging (MRI) demonstrated multiple bone lesions, and computed tomography scans revealed a 4-cm right-sided hilar lung mass, regional thoracic lymphadenopathy, multiple hepatic metastases, a left-sided adrenal metastasis, and several osseous lesions(Fig 1A). Brain MRI visualized three asymptomatic brain metastases. Biopsies of the subcarinal lymph node and the left-side adrenal lesion were performed, which confirmed adenocarcinoma of lung origin (Figs 2A and 2B).

The patient was treated with radiation therapy to the painful vertebral metastasis and stereotactic radiosurgery to two brain lesions. Molecular testing of the subcarinal node

through a next-generation sequencing (NGS) panel that covers > 200 genes (FoundationOne; Foundation Medicine, Cambridge, MA) revealed an *EGFR* exon 19 deletion (del19) mutation, a *TP53*V173L mutation, an *EGFR* amplification, and an *RB1* loss of exons 18 and 19. Oral erlotinib 150 mg daily was initiated in May 2015, and uniform disease response was evident on restaging scans in July 2015 (Fig 1B). However, in October 2015, repeat imaging showed significant growth in a single liver lesion and a new 2.6-cm lesion in the spleen, with continued response elsewhere, including the brain (Fig 1C).

Repeat biopsy of the enlarging liver lesion in November 2015 revealed nests of highly atypical cells with finely dispersed chromatin, inconspicuous nucleoli, and abundant mitoses (Figs 2C to 2E). Immunohistochemical stains were positive for synaptophysin and chromogranin and negative for thyroid transcription factor 1 and napsin A. The protein encoded by the *MKI67* gene labeling index was 80%. The overall features were consistent with small-cell lung cancer (SCLC) transformation.[4] NGS that consisted of targeted hotspot evaluation in 39 genes (SNaPshot NGS; Massachusetts General Hospital, Boston, MA) confirmed the presence of the original *EGFR* del19 and *TP53* mutations and showed additional mutations in *PIK3CA* (G545L), *PIK3CA* (G726L), *ERBB3* (G337A), and *FBXW7* (L8P). Biallelic loss of *RB1* was detected (Figs 3A and 3B). A patient-derived xenograft generated from this biopsy specimen lacked RB protein expression but retained minimal *EGFR* expression and demonstrated activation of the mitogen-activated protein kinase and AKT pathways (Fig 3C), likely as a result of the presence of an activating *PIK3CA* mutation.

The patient was treated with carboplatin and etoposide chemotherapy and ongoing erlotinib. Scans after four cycles of chemotherapy showed a mixed response with slight regression in the previously biopsied (SCLC-transformed) liver metastasis, stable brain metastases, and multiple new distinct sites of hepatic progression (Fig 1D). Plasma-based cell-free circulating tumor DNA (ctDNA) genotyping (Guardant360; Guardant Health, Redwood City, CA) revealed the following genes (and mutant allele frequencies [MAFs]; Fig 4): *EGFR* del19 (11.1%), *TP53* V173L (11.2%), *CCND2* S271S(5%),*EGFR*T790M (3.5%), *PIK3CA* E726K (2.7%), *PIK3CA* E545K (2.6%), and *NRAS* V188V (1.4%). Of note, the Guardant360 assay can detect *RB1* inactivating mutations but not allelic losses.

We interpreted the emergence of an *EGFR* T790M–positive clone as the most likely resistance mechanism within the growing liver nodules. The patient discontinued chemotherapy, and the T790M-specific TKI osimertinib[7] was administered in March 2016. Restaging in June 2016 (performed with MRI because of renal dysfunction) revealed that the hepatic lesions that had most recently progressed on chemotherapy (presumed T790M positive) had stabilized, but the liver lesion biopsied in 2015 (SCLC histology at that time) had again enlarged with no other sites of systemic or intracranial progression (Fig 1E). We hypothesized that the SCLC-transformed clone was driving radiographic progression. A repeat plasma Guardant360 test in June 2016 confirmed that *EGFR* T790M was now undetectable, but increases were found in the MAFs of *PIK3CA* E726K (50.9%), *PIK3CA* E545K (54.3%), *TP53* V173L (54.8%), and *EGFR* del19 (45.5%; Fig 4). In addition, new (compared with prior plasma testing) moderate-level amplification was noted in *ERBB2* (HER2; 3.3-fold amplified in plasma), *PIK3CA* (3.3-fold), *c-MYC* (2.7-fold), and *FGFR1*

(2.6-fold). The patient was subsequently treated with docetaxel and then nivolumab without response. He died in November 2016. An autopsy was not pursued.

Discussion

EGFR mutation–positive lung adenocarcinomas have been observed to transform to an SCLC phenotype as a resistance mechanism to frontline EGFR inhibitors in 5% of patients.[4,5,8] This particular case of SCLC transformation illustrates the complexities of clonal evolution in acquired resistance and, importantly, demonstrates how serial genotyping through plasma and tissue may help us to follow the various clones clinically and to prioritize therapeutics.

We hypothesize that all tumor cells in this patient carried common founder mutations in *EGFR* del19 and *TP53* V173L (Fig 5). However, one resistant clone with SCLC morphology emerged clinically in October 2015, and genotyping confirmed an additional private *PIK3CA* E545L mutation, which often is seen in SCLC-transformed *EGFR* mutant clones.[4] During chemotherapy, we believe that the SCLC clone diminished, whereas another clone that harbored an acquired *EGFR* T790M mutation and perhaps two other distinct *PI K3CA* mutations (E545K and E726K) emerged as observed in plasma ctDNA in March 2016. Although a tissue biopsy specimen could not be obtained at that time, on the basis of our prior observation that SCLC and adenocarcinoma populations can oscillate in response to specific treatment[4] and that T790M is rarely seen in SCLC-transformed tissue biopsy specimens, we hypothesize that the T790M-positive clone detected in plasma maintained an adenocarcinoma phenotype. The T790M clone was no longer detectable by June 2016 after treatment with osimertinib, but one or more other clones became dominant with increasing MAFs, and subsequently, the disease became refractory to therapy (Fig 4). This elevation in ctDNA and subsequent clinical decline mirror data that demonstrated the correlation of increased MAFs and decreased overall survival.[9–11]

In addition, the patient had a tumor with baseline *RB1* mutation that was expected to lead to loss of function and is believed to play an essential role in the histologic transformation to SCLC among *EGFR* mutant cancers. We previously demonstrated that *RB1* is uniersally lost in SCLC-transformed cancers, although not sufficiently for transformation.[12] Recent work has demonstrated that baseline *RB1* loss among *EGFR* mutant tumors is a strong predictor for subsequent SCLC transformation.[13] As the clinical use of NGS panels increases and baseline inactivating *RB1* mutations are more frequently detected, more data to understand the implications will be required, including a better understanding of the critical steps that lead to the lineage shift, so that we can develop more-effective treatment strategies.[14]

Finally, this case report illustrates the potential utility of longitudinal molecular profiling during targeted therapy. At present, two mutation-specific Food and Drug Administration–approved plasma tests may be used to select EGFR TKIs.[15,16] Clinical practice is rapidly evolving, but on the basis of current evidence, it is reasonable to offer plasma genotyping upon progression with frontline EGFR TKIs to evaluate for T790M. If a T790M clone is detected, initiation of osimertinib is standard; however, if plasma testing is negative for T790M, reflexive tissue biopsy should be performed because approximately 30% of ctDNA

test results will be false negative.[17] The exact role of liquid biopsies for serial monitoring requires more rigorous evaluation, but a key appeal is that tumor biopsy findings may underestimate the full spectrum of resistant clones present at the time of progression.[6,18,19] In practice, we commonly see the type of mixed radiographic response as observed in this patient with regression of some sitesbut continued growth in others. Heterogeneity among distinct cancer subclones may explain such disparate responses, and longitudinal plasma testing might be a valuable adjunct to tissue biopsies to understand the dynamic evolution of various clones.

For example, although tissue biopsy may offer critical information about the histology and molecular alterations of a specific progressing lesion, it may lack information about other sites of disease. Conversely, ctDNA genotyping could paint a more-complete picture of the competing resistance clones within a patient, although precise determination of which molecular alterations coexist within one clone or in one anatomic site are not currently possible. Indeed, other studies have demonstrated that multiple resistance mechanisms can be detected within plasma,[18] and we and others have observed that longitudinal ctDNA analyses can track the rise and fall of distinct subclones.[6,20]

In summary, this case report illustrates that the relative magnitude of resistant subclones can fluctuate in response to therapy, that liquid biopsies hold great potential to detect and monitor distinct genetic subpopulations within a patient, and that the presence of a baseline *RB1* mutation in an *EGFR* mutant cancer and subsequent SCLC transformation raises important questions about monitoring such patients. For those with *EGFR* mutant lung cancers, both tissue and liquid biopsy specimens can yield critical information to elucidate dominant clones that drive cancer growth at various time points. As our appreciation of the complexities of resistance and cancer heterogeneity grows, longitudinal plasma testing likely will play an increasing clinical role.

REFERENCES

1. Sequist LV, Yang JC, Yamamoto N, et al.: Phase III study of afatinib or cisplatin plus pemetrexed in patients with metastatic lung adenocarcinoma with EGFR mutations. J Clin Oncol 31:3327–3334, 2013 [PubMed: 23816960]
2. Mok TS, Wu YL, Thongprasert S, et al.: Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma. N Engl J Med 361:947–957, 2009 [PubMed: 19692680]
3. Rosell R, Carcereny E, Gervais R, et al.: Erlotinib versus standard chemotherapy as first-line treatment for European patients with advanced EGFR mutation-positive non-small-cell lung cancer (EURTAC): A multicentre, open-label, randomised phase 3 trial. Lancet Oncol 13:239–246, 2012 [PubMed: 22285168]
4. Sequist LV, Waltman BA, Dias-Santagata D, et al.: Genotypic and histological evolution of lung cancers acquiring resistance to EGFR inhibitors. Sci Transl Med 3:75ra26, 2011
5. Yu HA, Arcila ME, Rekhtman N, et al.: Analysis of tumor specimens at the time of acquired resistance to EGFR-TKI therapy in 155 patients with EGFR-mutant lung cancers. Clin Cancer Res 19:2240–2247, 2013 [PubMed: 23470965]
6. Piotrowska Z, Niederst MJ, Karlovich CA, et al.: Heterogeneity underlies the emergence of EGFRT790 wild-type clones following treatment of T790M-positive cancers with a third-generation EGFR inhibitor. Cancer Discov 5:713–722, 2015 [PubMed: 25934077]
7. Jänne PA, Yang JC, Kim DW, et al.: AZD9291 in EGFR inhibitor-resistant non-small-cell lung cancer. N Engl J Med 372:1689–1699, 2015 [PubMed: 25923549]

8. Camidge DR, Pao W, Sequist LV: Acquired resistance to TKIs in solid tumours: Learning from lung cancer. Nat Rev Clin Oncol 11:473–481, 2014 [PubMed: 24981256]
9. Bettegowda C, Sausen M, Leary RJ, et al.: Detection of circulating tumor DNA in early- and late-stage human malignancies. Sci Transl Med 6:224ra24, 2014
10. Schwaederlé MC, Patel SP, Husain H, et al.: Utility of genomic assessment of blood-derived circulating tumor DNA (ctDNA) in patients with advanced lung adenocarcinoma. Clin Cancer Res 23:5101–5111, 2017 [PubMed: 28539465]
11. El Messaoudi S, Mouliere F, Du Manoir S, et al.: Circulating DNA as a strong multimarker prognostic tool for metastatic colorectal cancer patient management care. Clin Cancer Res 22:3067–3077, 2016 [PubMed: 26847055]
12. Niederst MJ, Sequist LV, Poirier JT, et al.: RB loss in resistant EGFR mutant lung adenocarcinomas that transform to small-cell lung cancer. Nat Commun 6:6377, 2015 [PubMed: 25758528]
13. Lee JK LJ, Lee J, Kim S, et al.: Clonal history and genetic predictors of transformation into small-cell carcinomas from lung adenocarcinomas. J Clin Oncol 35:3065–3074, 2017 [PubMed: 28498782]
14. Farago AF, Piotrowska Z, Sequist LV. Unlocking the mystery of small-cell lung cancer transformation in *EGFR* mutant adenocarcinoma. J Clin Oncol 35:2987–2988, 2017 [PubMed: 28692381]
15. Food and Drug Administration: FDA approves first blood test to detect gene mutation associated with non-small cell lung cancer, 2016 https://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm504488.htm
16. Kwapisz D: The first liquid biopsy test approved. Is it a new era of mutation testing for non-small cell lung cancer? Ann Transl Med 5:46, 2017 [PubMed: 28251125]
17. Oxnard GR, Thress KS, Alden RS, et al.: Association between plasma genotyping and outcomes of treatment with osimertinib (AZD9291) in advanced non–small-cell lung cancer. J Clin Oncol 34:3375–3382, 2016 [PubMed: 27354477]
18. Chabon JJ, Simmons AD, Lovejoy AF, et al.: Circulating tumour DNA profiling reveals heterogeneity of EGFR inhibitor resistance mechanisms in lung cancer patients. Nat Commun 7:11815, 2016 [PubMed: 27283993]
19. Suda K, Murakami I, Sakai K, et al.: Small cell lung cancer transformation and T790M mutation: Complimentary roles in acquired resistance to kinase inhibitors in lung cancer. Sci Rep 5:14447, 2015 [PubMed: 26400668]
20. Russo M, Siravegna G, Blaszkowsky LS, et al.: Tumor heterogeneity and lesion-specific response to targeted therapy in colorectal cancer. Cancer Discov 6:147–153, 2016 [PubMed: 26644315]

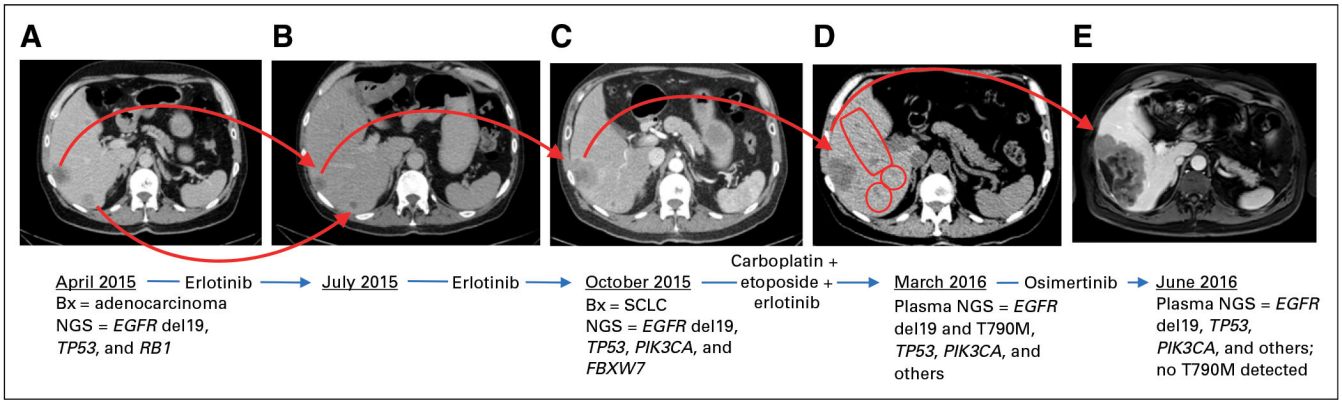

Fig 1.

Clinical course and representative radiographic images. (A to E) Selected radiographic images of the liver illustrate involvement with cancer. Treatments and key biopsy (Bx) results (tissue or liquid) are indicated underneath each image in chronologic order. NGS, next-generation sequencing; SCLC, small-cell lung cancer.

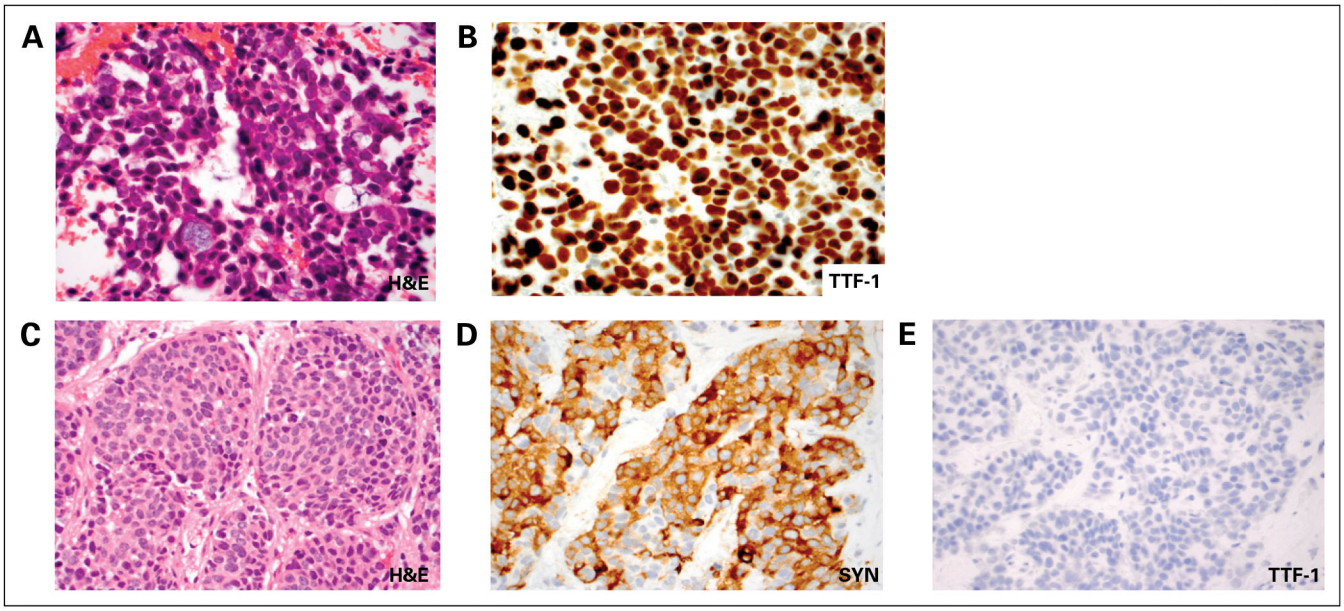

Fig 2.

Histopathologic findings. (A) Hematoxylin and eosin (H&E) stain and (B) thyroid transcription factor 1 (TTF-1) immunostain of fine-needle aspiration of a subcarinal lymph node at diagnosis show adenocarcinoma with diffuse TTF-1 expression consistent with a lung primary. (C) H&E stain and (D) synaptophysin (SYN) and (E) TTF-1immunostains of a liver core biopsy at the time of acquired resistance to erlotinib demonstrate small-cell lung cancer with solid nests of highly atypical epithelial cells with finely dispersed chromatin, inconspicuous nucleoli, and brisk mitotic activity. The tumor cells are positive for SYN and negative for TTF-1.

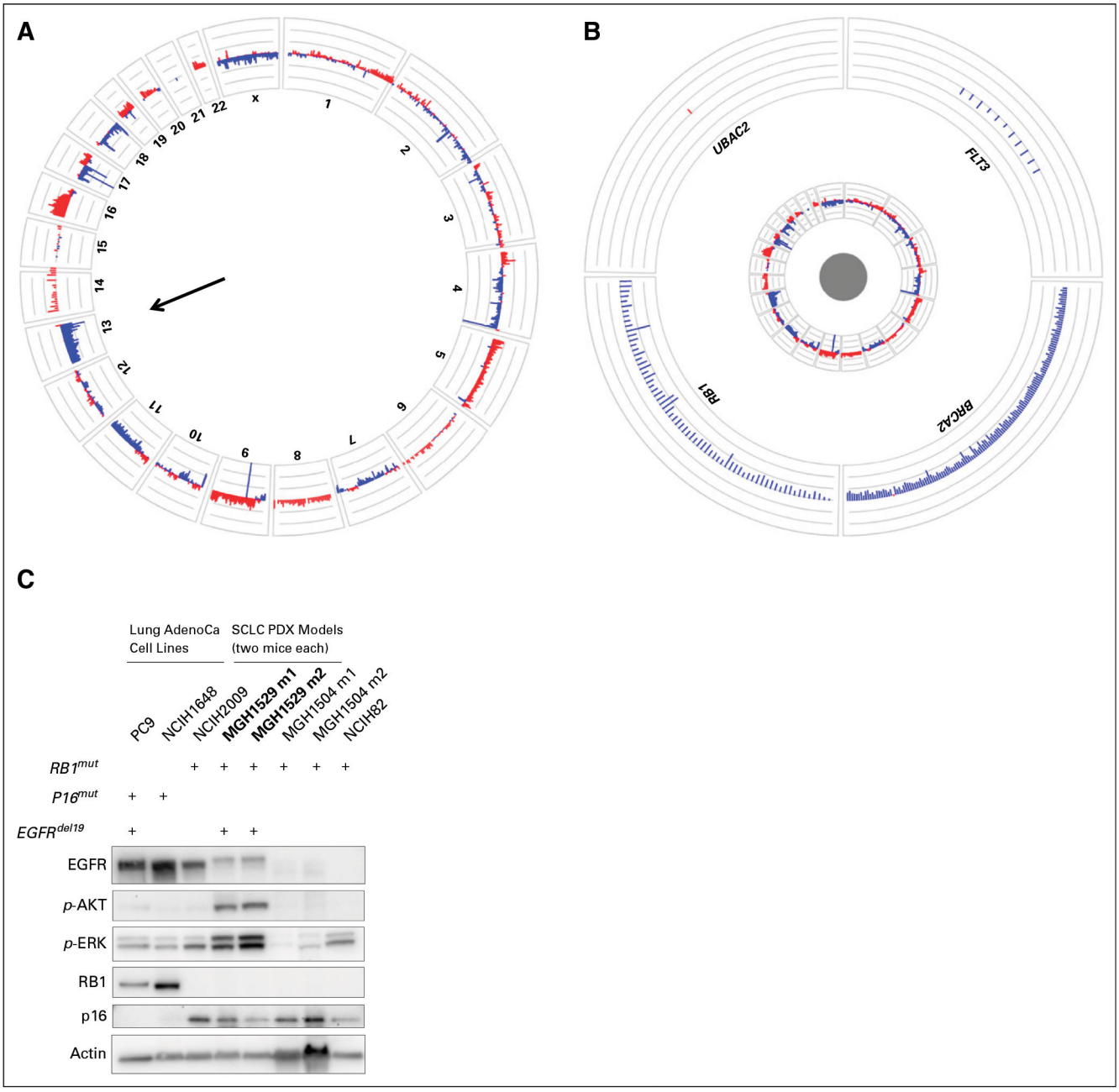

Fig 3.

RB1 loss within the small-cell lung cancer (SCLC)–transformed tumor. Circos plots provide illustrative overviews of the next-generation sequencing (NGS) analyses of the liver biopsy specimens with SCLC transformation. (A) Summary of all evaluable probes across all chromosomes (red, genomic gains; blue, genomic losses) shows diffuse losses across chromosome 13, including the *RB1* gene locus. (B) A magnified view of four specific genes on chromosome 13 shows that all examined loci of *RB1* are lost, with only blue signals and complete absence of red signals. (C) Tissue obtained from the November 2015 liver biopsy (which shows SCLC) was used to generate a patient-derived xenograft (PDX) in an NSG mouse and subsequently passaged through additional NSG mice. The PDX tumor

demonstrated SCLC histologic features consistent with the patient biopsy sample (not shown). A Western blot demonstrates relative protein levels of EGFR, *p*-AKT (S473), *p*-ERK, RB, p16, and actin (loading control) among PDX tumors (MGH1529; two tumors shown) with control lung adenocarcinoma (AdenoCa) and SCLC samples for comparison. Genetic characteristics of the various cell lines (*RB1*, *p16*, and *EGFR* exon 19 deletion [del19] mutations [mut]) are shown above the Western blot. Of note, the PDX tumor retained mild EGFR expression, although not as strongly as the AdenoCa controls, but had complete loss of RB1 expression similar to the de novo SCLC lines.

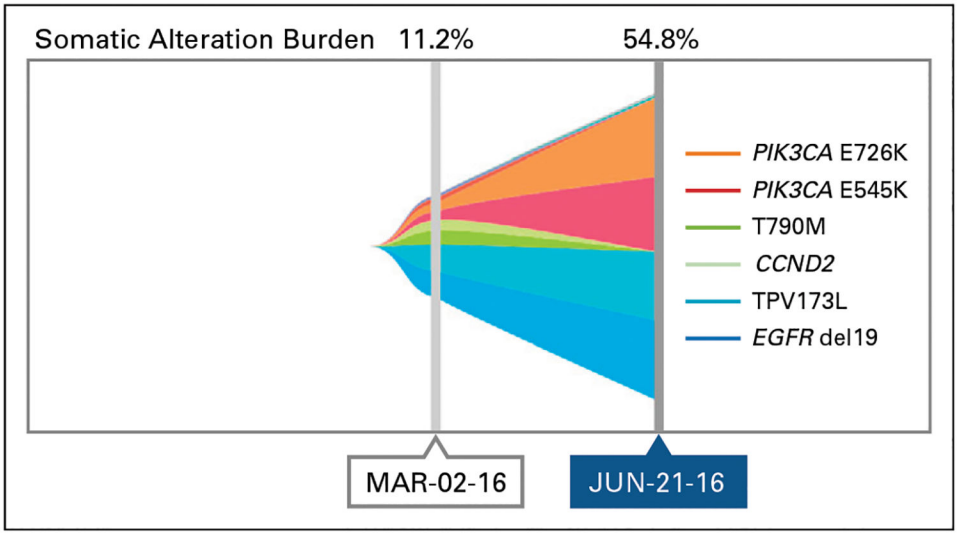

Fig 4.

Plasma genotyping. Graphic representation of the relative mutant allele frequencies detected in plasma circulating tumor DNA over time.

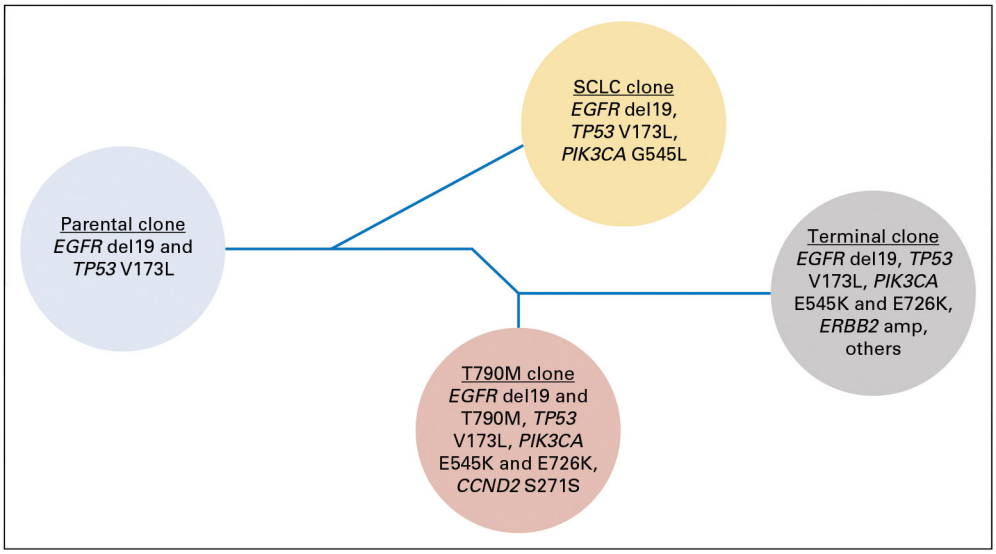

Fig 5.

Clonal evolution schematic. Dendrogram of a hypothetical phylogenetic evolution of subclones in this patient. Each circle represents a hypothesized clone and its key genetic features. The timing of the branch points is illustrative and not meant to convey exact data. Not all documented molecular changes are included in the illustration. SCLC, small-cell lung cancer.