Spatial Tumor Heterogeneity in Lung Cancer with Acquired Epidermal Growth Factor Receptor-Tyrosine Kinase Inhibitor Resistance

Targeting High-Level MET-Amplification and EGFR T790M Mutation Occurring at Different Sites in the Same Patient

Matthias Scheffler, MD, Sabine Merkelbach-Bruse, PhD,† Marc Bos, MD,‡ Jana Fassunke, PhD,†
Masyar Gardizi, MD,§ Sebastian Michels, MD,§ Laura Groneck, MD,‖ Anne M. Schultheis, MD,†
Florian Malchers, PhD,‡ Frauke Leenders, PhD,‡ Carsten Kobe, MD,¶ Katharina König, PhD,†
Lukas C. Heukamp, MB, PhD,# Martin L. Sos, MD,‡ Roman K. Thomas, MD,‡
Reinhard Büttner, MD,† and Jürgen Wolf, MD**

Different mechanisms underlying acquired resistance (AR) to epidermal growth factor receptor (EGFR) inhibitors in *EGFR*-mutated lung cancer are known, among them the *EGFR*[T790M] mutation in approximately 50% and *MET*-amplifications in approximately 5% of these patients.[1] Here, we describe a patient with AR harboring both genetic aberrations at distinct tumor sites resulting in mixed response patterns following biopsy-guided sequential targeted therapy.

CASE REPORT

A 55-year-old male Caucasian patient was diagnosed with lung adenocarcinoma Union for International Cancer Control stage IV (cT4cN1cM1b PUL/PLEU/OSS) in October 2011. *EGFR*[L858R] mutation was diagnosed by dideoxy sequencing.

*Lung Cancer Group Cologne, Department I of Internal Medicine, University Hospital of Cologne, Cologne, Germany; †Center for Integrated Oncology Köln Bonn, Institute of Pathology, University Hospital of Cologne, Cologne, Germany; ‡Department of Translational Genomics, Center for Integrated Oncology Köln Bonn, University of Cologne, Cologne, Germany; §Lung Cancer Group Cologne, Department I of Internal Medicine, Center for Integrated Oncology Köln Bonn, University Hospital of Cologne, Cologne, Germany; ‖Department I of Internal Medicine, Center for Integrated Oncology Köln Bonn, University Hospital of Cologne, Cologne, Germany; ¶Center for Integrated Oncology Köln Bonn, Institute of Nuclear Medicine, University Hospital of Cologne, Cologne, Germany; and #Institute of Hematopathology Hamburg, Hamburg, Germany

After several lines of treatment including erlotinib, cisplatin/pemetrexed, and afatinib/cetuximab, he presented in July 2013 with disease progression. Next-generation sequencing based analysis of the rebiopsy of the primary tumor (left lung upper lobe) revealed *EGFR*[L858R] and two different *TP53* mutations (R110C and C277* [allele frequencies 4% vs. 26%, suggesting biclonality]), but no aberration associated with AR (Table 1; Sanger sequencing was used for validation and showed no discrepancies). Treatment was initiated with docetaxel/gemcitabine, and massive progression was diagnosed in September 2013 (lung, liver, myocard, and soft-tissue left arm; Fig. 1*A*). Rebiopsy of the lesion in the left arm detected *EGFR*[L858R], *TP53*[C277*], and high-level *MET*-amplification (*MET*/centromer7 ratio, 8.3; cutoff for high-level amplification, 2.0)[2] in the absence of *EGFR*[T790M]. Treatment with crizotinib (2×250 mg/d) in combination with erlotinib (100 mg/d) was started. After 1 week of therapy, fluorodeoxyglucose (18F)-positron emission tomography–computed tomography showed a dramatic metabolic response (Fig. 1*B*) in the arm, the liver, and the myocardium, with improvement of the patient's performance state. However, the primary tumor (left lung) did not respond (Fig. 1*B*). In December 2013, the patient presented with increasing dyspnea (Fig. 1*C*). Although complete metabolic response was maintained in liver and soft-tissue metastases, metabolic progression occurred in myocardial metastasis. Combination therapy was maintained. In January 2014, the patient presented with dyspnea and left pleural effusion. Analysis of pleural effusion (Fig. 1*D*) revealed *EGFR*[L858R], *TP53*[R110C], low-level *MET*-amplification, and *EGFR*[T790M]. The same mutations were detected in peripheral blood. Crizotinib was stopped, and the patient was enrolled into a trial evaluating a third generation EGFR-tyrosine kinase inhibitor (TKI) (NCT01802632) in February 2014. After 2 weeks, fluorodeoxyglucose (18F)-positron emission tomography–computed tomography showed control of the primary lung tumor but a strong increase in activity in the liver and myocardium (Fig. 1*E*). The patient was withdrawn from the trial

TABLE 1. Biopsies Performed and Molecular Findings

Date	Localization	Method	Results
October 2011	Lung (primarius)	Sanger	*EGFR* L858R
June 2013	Lung (primarius)	NGS/FISH/Sanger	*EGFR* L858R, *TP53* C277*, *TP53* R110C, and no *MET*amp
October 2013	Muscle right arm	NGS/FISH/Sanger	*EGFR* L858R, *TP53* C277*, and high-level *MET*amp
January 2014	Pleural effusion	NGS/FISH/Sanger	*EGFR* L858R, *TP53* R110C, low-level *MET*amp, and *EGFR* T790M
January 2014	Peripheral blood	CAGE[a]	*EGFR* L858R, *EGFR* T790M, and *TP53* R110C
April 2014	Pleural effusion	NGS/FISH	*EGFR* L858R, *EGFR* T790M, *TP53* R110C, and no *MET*amp

[a] An NGS-based target enrichment assay detecting rearrangements, amplifications, deletions, and point mutations, in more than 300 cancer relevant genes.
NGS, next-generation sequencing; FISH, fluorescence in situ hybridization; Sanger, dideoxy sequencing; CAGE, Cancer genome scanner® (Blackfield AG, Cologne, Germany).
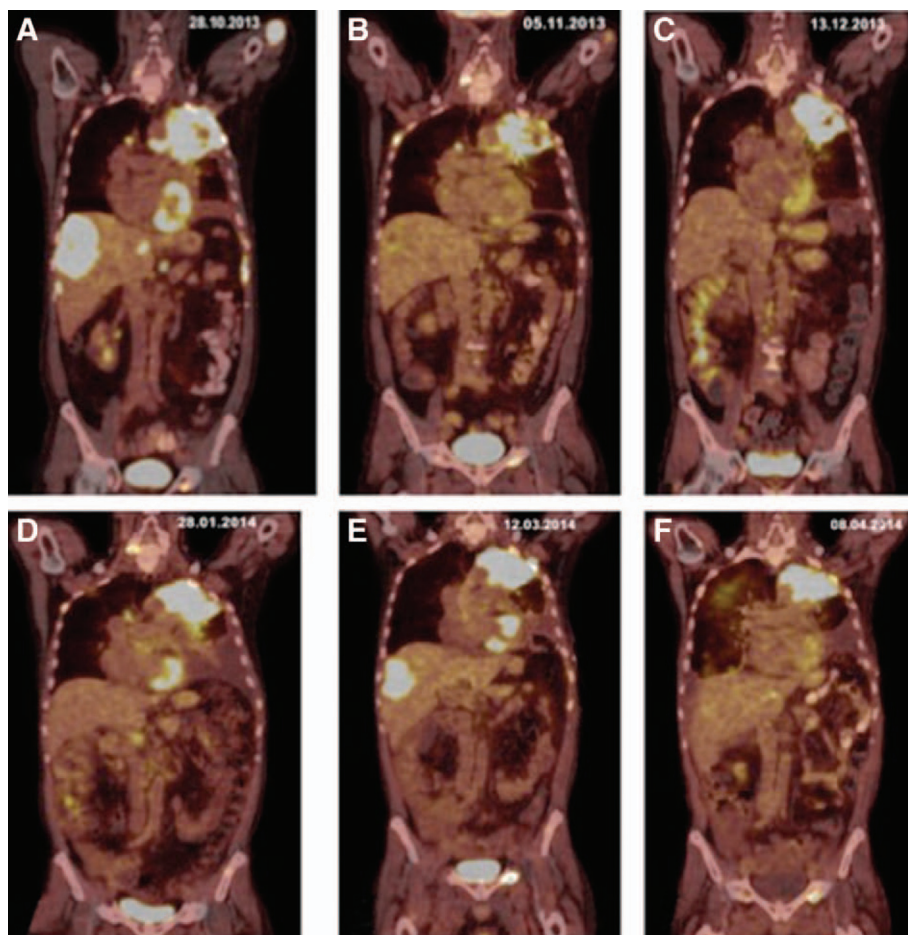

FIGURE 1. Fluorodeoxyglucose (18F)-positron emission tomography–computed tomography results. *A,* October 2013: massive progression after epidermal growth factor receptor (EGFR)-tyrosine kinase inhibitor resistance and chemotherapy (lung, myocardial infiltration, liver, and soft-tissue left arm). Rebiopsy left arm: *EGFR*^{L858R}, high-level *MET*amp, *TP53*^{C277*}, and *TP53*^{R110C}. *B,* November 2013: complete metabolic response of myocard-tissue, liver-tissue, and soft-tissue metastases after 8 days of crizotinib and erlotinib. *C,* December 2013: ongoing metabolic response of myocard-tissue, liver-tissue, and soft-tissue metastases under crizotinib and erlotinib. *D,* January 2014: ongoing metabolic response of liver-tissue and soft-tissue metastases. Metabolic progression of myocard infiltrating tumor and progressive pleural effusion left. Molecular analysis of pleural effusion: *EGFR*^{L858R}, *EGFR*^{T790M}, *TP53*^{R110C}, and low-level *MET*amp. Peripheral blood: *EGFR*^{L858R}, *EGFR*^{T790M}, and *TP53*^{R110C} (*MET* status not assessable). Stop of crizotinib and erlotinib treatment, start of AZD9291. *E,* March 2014: stable metabolic disease in lung tumor, tumor progression in myocard- and liver metastases, tumor control of the soft-tissue metastasis in the arm. Stop of AZD9291, restart with crizotinib. *F,* April 2014: complete metabolic response of liver- and myocardial metastases, ongoing tumor control of the soft-tissue metastasis. Progress of pleural effusion. Patient died after 3 weeks of crizotinib treatment. Molecular analysis of pleural effusion: *EGFR*^{L858R}, *EGFR*^{T790M}, *TP53*^{R110C}, and no *MET*amp.

FIGURE 2. Overview of the findings in the resistance setting after erlotinib treatment (November 2011–July 2012) and afatinib/cetuximab treatment (November 2012–June 2013) with intercurrent radiation/chemotherapy.

as no concomitant antineoplastic treatment was allowed, and crizotinib treatment was restarted. Three weeks later, again a nearly complete metabolic response of the metastases presumably driven by high-level *MET*-amplification was observed (Fig. 1*F*). However, the patient suffered from recurrent pleural effusions and his performance state worsened. He finally died in April 2014.

DISCUSSION

This report describes a patient with acquired EGFR-tyrosine kinase inhibitor resistance harboring two different resistance mechanisms, namely *EGFR*[T790M] and high-level *MET*-amplification, at different tumor sites. Despite rebiopsy-guided effective temporary control of the tumor clone harboring high-level MET amplification with a potent MET-inhibitor[3] tumor activity could not be persistently controlled because of clonal heterogeneity and finally led to fatal progression of the disease (Figs. 2 and 3). These observations not only provide a rationale for early combination therapy in AR but also point to the limitations of our current paradigmatic understanding of driver-mutation directed therapy and underlines recent analyses demonstrating genetic variability in patients with lung cancer.[4,5]

REFERENCES

1. Sequist LV, Waltman BA, Dias-Santagata D, et al. Genotypic and histological evolution of lung cancers acquiring resistance to EGFR inhibitors. *Sci Transl Med* 2011;3:75ra26.
2. Schildhaus H, Schultheis AM, Ruschoff J, et al. MET amplification status in therapy-naive adeno- and squamous cell carcinomas of the lung. *Clin Cancer Res* 2015;21:907–915.
3. Ou SH, Kwak EL, Siwak-Tapp C, et al. Activity of crizotinib (PF02341066), a dual mesenchymal-epithelial transition (MET) and anaplastic lymphoma kinase (ALK) inhibitor, in a non-small cell lung cancer patient with de novo MET amplification. *J Thorac Oncol* 2011;6:942–946.
4. Zhang J, Fujimoto J, Zhang J, et al. Intratumor heterogeneity in localized lung adenocarcinomas delineated by multiregion sequencing. *Science* 2014;346:256–259.
5. de Bruin EC, McGranahan N, Mitter R, et al. Spatial and temporal diversity in genomic instability processes defines lung cancer evolution. *Science* 2014;346:251–256.
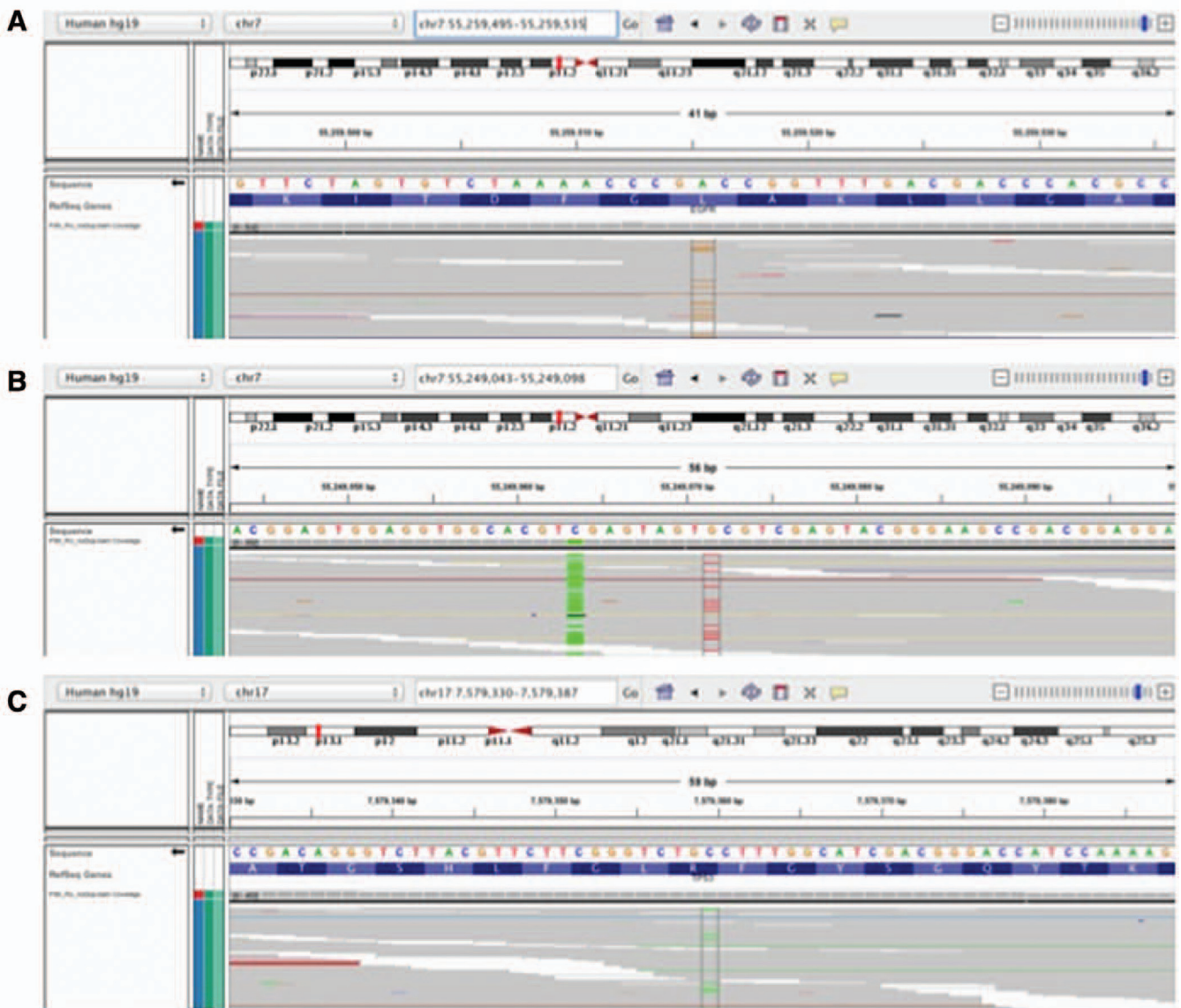
FIGURE 3: Liquid biopsy reveals epidermal growth factor receptor (*EGFR*) and TP53 mutations: results of the peripheral blood analysis (January 2014). *A, EGFR*[L858R] mutation with an allele frequency of 13%. *B, EGFR*[T790M] mutation with an allele frequency of 10%. *C, TP53*[R110C] mutation with an allele frequency of 9%. All identified and illustrated by integrative genome viewer.