Oncogenic Fusions May Be Frequently Present at Resistance of EGFR Tyrosine Kinase Inhibitors in Patients With NSCLC: A Brief Report

Diego Enrico, MD,a Ludovic Lacroix, PharmD, PhD,b,c,d,e Jeanne Chen, MD,b,c Etienne Rouleau, PharmD, PhD,e Jean-Yves Scoazec, MD, PhD,c,d,e Yohann Loriot, MD, PhD,a,b,c, Lambros Tselikas, MD,f Cécile Jovelet, PharmD, PhD,g David Planchard, MD, PhD,a Anas Gazzah, MD,h Laura Mezquita, MD, PhD,a Maud Ngo-Camus, MSc,h Stefan Michiels, PhD,e Christophe Massard, MD, PhD,b,c,h Gonzalo Recondo, MD, PhD,b,c Francesco Facchinetti, MD,b,c Jordi Remon, MD,j Jean-Charles Soria, MD, PhD,b,c Fabrice André, MD, PhD,a,b,c Gilles Vassal, MD, PhD,k Luc Friboulet, PhD,b,c,a Benjamin Besse, MD, PhD,a,c

aDepartment of Medical Oncology, Gustave Roussy Cancer Campus, Villejuif, France
bINSERM U981, Gustave Roussy Cancer Campus, Villejuif, France
cUnivrsité Paris-Saclay, Paris, France
dExperimental and Translational Pathology Platform (PETRA), Genomic Platform-Molecular Biopathology Unit (BMO) and Biological Resource Center, AMMICA, INSERM US23/CNRS UMS3655, Gustave Roussy Cancer Campus, Villejuif, France
*eDepartment of Medical Biology and Pathology, Gustave Roussy Cancer Campus, Villejuif, France
fDepartment of Interventional Radiology, Gustave Roussy Cancer Campus, Villejuif, France

*Corresponding author.
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Address for correspondence: Luc Friboulet, PhD, Gustave Roussy Cancer Campus, Université Paris-Saclay, 114 Rue Edouard Vaillant, Villejuif 94805, France. E-mail: luc.friboulet@gustaveroussy.fr

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ABSTRACT

Introduction: Despite initial benefit, virtually all patients suffering from EGFR-mutant NSCLC experience acquired resistance to tyrosine kinase inhibitors (TKIs), driven by multiple mechanisms. Recent reports have identified oncogenic kinase fusions as off-target resistance mechanisms; however, these alterations have been rarely investigated at EGFR TKIs progression.

Methods: Patients with EGFR-mutated metastatic NSCLC (N = 62) with tissue and plasma biopsies at EGFR TKI progression between January 2015 and June 2019, at a French hospital and optionally before progression, were identified from the prospective MATCH-R study (NCT02517892). Postprogression biopsy samples were analyzed for gene fusions using targeted gene panel sequencing, whole-exome sequencing, RNA sequencing, and comparative genomic hybridization array.

Results: Six gene fusions were detected in tumor progression biopsies under an EGFR TKI from 62 consecutive patients (9.7%) with EGFR-mutated advanced NSCLC. Among 31 patients progressing to first- or second-generation EGFR TKIs, one (3%) had an Eukaryotic translation initiation factor 4 gamma 2–GRB2 associated binding protein 1 (EIF4G2-GAB1) fusion. Among 31 patients progressing to the third-generation osimertinib, five (16%) presented oncogene fusions of fibroblast growth factor receptor 3–transforming acidic coiled-coil containing protein 3 (FGFR3-TACC3) (n = 2), kinesin family member 5B–Ret proto-oncogene (KIF5B-RET) (n = 1), striatin–anaplastic lymphoma kinase (STRN-ALK) (n = 1), and zinc finger DHHC-Type palmitoyltransferase 20–Thr790Met (ZDHHC20-BRAF) (n = 1) transcripts. Out of two patients that received osimertinib at first-line, one acquired an FGFR3-TACC3 fusion at progression. In all patients, fusions co-occurred with the original activating EGFR mutation; however, among four patients with an acquired T790M mutation, three (75%) lost the T790M mutation.

Conclusions: Oncogenic fusions at the time of EGFR TKI resistance were identified at a relatively high frequency, mainly after the third-generation TKI osimertinib. Patients progressing to EGFR TKIs may have a new opportunity for targeted therapy when oncogenic fusions are identified.
longitudinally during treatment and at progression for circulating tumor DNA (ctDNA) sequencing. Targeted gene panel sequencing was performed with an Ion Torrent PGM (ThermoFisher Scientific) sequencer using a customized panel (Mosc3 or 4) covering 75 to 82 critical oncogenes or tumor suppressor genes developed with Ion AmpliSeq custom design. Whole-exome sequencing, RNA sequencing (RNA-seq), and Affymetrix CytoScan HD comparative genomic hybridization array were performed as previously reported (see Supplementary Methods). CtdNA samples were analyzed by next-generation sequencing (50-gene panel) (Supplementary Methods). All molecular oncogenic alterations were respectively classified in either definitive (or potential) resistance or concomitant genetic alterations according to OncokB and Cancer Genome Interpreter. Patients were analyzed according to first- or second-generation TKIs (erlotinib, gefitinib, or afatinib) and the third-generation TKI osimertinib. The Kaplan-Meier method was used to estimate progression-free survival 2 (time from initiation of subsequent line therapy after osimertinib progression to the first documented disease progression or death) and overall survival in the post-osimertinib cohort (Supplementary methods).

Results
Between January 2015 and June 2019, 62 consecutive patients with EGFR-mutated advanced NSCLC underwent genotyping of tumor tissue and ctDNA samples collected at the time of EGFR TKIs progression and were analyzed according to TKI-generation (Supplementary Fig. 1). A total of 60 patients (97%) had adenocarcinomas, 37 (60%) were nonsmokers, and the mean age was 58 years (± SD 10.7). An exclusive thoracic progression was more frequent at osimertinib recurrence, and extrathoracic progression patterns were more frequent after first- or second-generation EGFR TKI (p = 0.03) (Table 1).

Table 1. Patient Clinical Characteristics

| Characteristics | First- or Second-Generation EGFR TKI Cohort, n (%) | Third-Generation EGFR TKI Cohort, n (%) | p value |
|-----------------|--------------------------------------------------|----------------------------------------|---------|
| Total           | 31                                               | 31                                     |         |
| Median age (range), y | 60 (37–89)                                         | 58 (40–72)                            | 0.40    |
| Sex, n (%)      |                                                  |                                        |         |
| Male            | 7 (23)                                           | 8 (26)                                 | 0.77    |
| Female          | 24 (77)                                          | 23 (74)                                |         |
| Smoking history, n (%) |                                             |                                        |         |
| Never           | 21 (68)                                          | 16 (52)                                | 0.24    |
| Current and former | 9 (29)                                           | 13 (42)                                |         |
| NS              | 1 (3)                                            | 2 (6)                                  |         |
| Baseline driver alteration |                                    |                                        |         |
| Exon 19, deletion | 20 (65)                                          | 24 (77)                                | 0.40    |
| Exon 21, L858R  | 10 (32)                                          | 7 (23)                                 |         |
| Exon 18, G719A  | 1 (3)                                            | 0                                      |         |
| First- or second-generation EGFR TKI before resistance biopsy | |                                        |         |
| Erlotinib or Gefitinib | 26 (84)                                         | 20 (65)                                | 0.17    |
| Afatinib        | 5 (16)                                           | 9 (29)                                 |         |
| Third-generation EGFR TKI before resistance biopsy | |                                        |         |
| Osimertinib     | 0                                                | 31 (100)                               |         |
| Response to TKI |                                                  |                                        |         |
| CR/PR           | 24 (77)                                          | 22 (71)                                | 0.71    |
| SD/PD           | 7 (23)                                           | 8 (26)                                 |         |
| NS              | 0                                                | 1 (3)                                  |         |
| Progression pattern at TKI resistance | |                                        |         |
| Solitary        | 19 (61)                                          | 24 (77)                                | 0.23    |
| Multiple        | 11 (36)                                          | 7 (23)                                 |         |
| NS              | 1 (3)                                            | 0                                      |         |
| Site of progression |                                               |                                        |         |
| Thoracic        | 10 (32)                                          | 19 (61)                                | 0.03    |
| Extrathoracic   | 20 (65)                                          | 12 (39)                                |         |
| NS              | 1 (3)                                            | 0                                      |         |

Missing data were excluded from the statistical analysis.
NS, not specified; TKI, tyrosine kinase inhibitor; PD, progressive disease; CR, complete response, PR, partial response.
In six patients (9.7%), fusions were detected by RNA-seq analyses on tissue samples (Table 2). In the post–first- or second-generation EGFR TKIs cohort (n = 31), one case (3%) had a transcript fusion involving Eukaryotic translation initiation factor 4 gamma 2 (EIF4G2) and GRB2-associated binding protein 1 (GAB1) after gefitinib treatment. In the post-osimertinib cohort (n = 31, two and 29 receiving the drug in the first and subsequent lines, respectively), the resistance alteration landscapes at progression biopsy are described in Figure 1. Five patients (16%) presented oncogenic fusions including fibroblast growth factor receptor 3–transforming acidic coiled-coil containing protein 3 (FGFR3-TACC3) (n = 2), kinesin family member 5B–Ret proto-oncogene (KIF5B-RET) (n = 1), striatin–anaplastic lymphoma kinase (STRN-ALK) (n = 1), and zinc finger DHHC-type palmtoyltransferase 20 (ZDHHC20-BRAF) (n = 1) (Table 2).

One of the FGFR3-TACC3 fusions was acquired after osimertinib first-line treatment, and the remaining in the subsequent lines of treatment. In terms of EGFR mutations identified at the time of fusion occurrence, all tumors retained the original activating EGFR mutation, but three of four patients (75%) lost the acquired Thr790Met (T790M) mutation. Median progression-free survival in patients that presented fusions at osimertinib progression was longer than patients with other known...
resistance alteration, but not statistically significant (6 months [95% confidence interval (CI): 0.7–16.8] versus 3 months [95% CI: 2.5–3.5], respectively; hazard ratio, 3.31 [95% CI: 0.7–16.7]; \(p = 0.09\)) (Supplementary Fig. 2). No difference was observed either in the same analysis on overall survival (\(p = 0.95\)) (Supplementary Fig. 3).

Only two tumors, in particular, had other well-established resistance alterations to EGFR TKI (receptor tyrosine-protein kinase erbB-2 \(\text{ERBB2}\) amplification and T790M mutation) concomitantly with the fusions. When the putative resistance mechanisms differed from fusions, 37% (23 of 62) had more than one concomitant resistance alterations in the progression biopsy.

**Discussion**

We found a higher rate of fusions at resistance after a third-generation EGFR TKI (five of 31, 16%) compared with first- or second-generation TKIs (one of 31, 3%). Recent reports have described fusions as off-target resistance mechanisms to EGFR TKIs, but at lower frequencies. Among 3873 patients with EGFR-positive NSCLC, Xu et al.\(^8\) found 16 fusions (0.4%) at progression to EGFR TKI, including \(\text{RET}\) (\(n = 6\), \(\text{ALK}\) (\(n = 5\)), neurotrophic receptor tyrosine kinase 1 \((\text{NTRK1})\) (\(n = 4\)), \(\text{ROS1}\) (\(n = 1\)), and \(\text{FGFR3}\) (\(n = 1\)).\(^8\) Analyzing ctDNA and tumor tissue samples at osimertinib progression from three lung cancer studies, fusions were found in 3% (one of 91) to 7% (three of 41) of cases.\(^9\)–\(^11\) Interestingly, all our fusions were detected on tissue but not on ctDNA analysis, which may explain our higher incidence, because most previous studies have used targeted gene panels sequencing DNA extracted from blood rather than tissue biopsies.

\(\text{FGFR3-TACC3}\) rearrangement has been identified as a driver alteration in several solid tumors and could lead to EGFR TKI resistance by promoting sustained activation of the ERK pathway.\(^12\) Fusions involving \(\text{RET}\) were also described at EGFR TKI resistance and were successfully reversed by a combination of EGFR and RET TKIs.\(^13\)

The \(\text{ALK}\) partner \(\text{STRN}\), found in our study, has rarely been established in the EGFR TKI resistance setting. Our patients did not respond to the \(\text{ALK}\) inhibitor crizotinib but achieved a stable disease of 6 months when both EGFR and ALK were inhibited with brigatinib. A published case report offers conflicting results about the effectiveness of \(\text{ALK}\) inhibitors on \(\text{STRN-ALK}\) translocated tumors.\(^14\)

Although \(\text{BRAF}\) alterations in NSCLC are represented mainly by mutations (2%–4%), fusions display another mechanism of \(\text{BRAF}\) activation, which has also been suggested as a resistance mechanism to EGFR TKIs at a low frequency (~2%).\(^3\) The \(\text{ZDHHC20}\) fusion partner has not been previously reported. It is anticipated to be an oncogenic fusion because the loss of the N-terminal inhibitory domain permits the constitutive dimerization of RAF proteins with consequent activation of the downstream pathways.

The \(\text{EIF4G2-GAB1}\) rearrangement found at gefitinib progression is also a novel reported fusion. GAB1 activity is relevant for some cellular functions such as the regulation of proliferation, migration, and survival by associating with TKI receptors such as met proto- oncogene (hepatocyte growth factor receptor) \(\text{cMET}\). Aberrant GAB1 activity has been associated with resistance mechanisms in \(\text{BRAF}\)-mutant melanomas owing to altered feedback regulation of \(\text{BRAF}\) signaling.\(^15\) Despite
the well-established oncogenic property of this fusion, its role in EGFR resistance needs preclinical validations.

Interestingly, at osimertinib resistance, tumors with fusion emergence retained the original activating EGFR mutation, but most of them (75%) lost the resistant T790M mutation. In agreement with these findings, Xu et al. reported a 50% T790M loss from 10 patients who presented fusions after osimertinib treatment. Furthermore, Oxnard et al. found similar results in three tumors with fusions and without T790M mutations at osimertinib progression (cell division cycle 6 [CDC6]-RET, FGFR3-TACC3, and extended synaptotagmin 2 [ESYT2]-BRAF). Together, these findings suggest that tumors drive the resistance and growth through these oncogenic fusions over the EGFR-dependent pathway.

Limitations
Our study has limitations. The sample size is limited and comes from a single center. Nevertheless, it is the first study using systematic RNA-seq at resistance to EGFR TKI. In addition, the lack of whole-exome sequencing–RNA-seq analyses in the TKI-naive biopsies does not allow us to define these oncogenic fusions as confirmed acquired resistance to EGFR TKIs. However, it should be noted that oncogenic fusions have been reported at a very low frequency at diagnosis.

Conclusions
In our cohort, oncogenic fusions identified at the time of EGFR TKI resistance were more frequent than expected, in particular after treatment with a third-generation TKI. A significant proportion of these fusions can be targeted so their identification could influence treatment selection and overall survival of patients failing EGFR TKIs.

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Supplementary Data
Note: To access the supplementary material accompanying this article, visit the online version of the Journal of Thoracic Oncology Clinical and Research Reports at www.jtocrr.org and at https://doi.org/10.1016/j.jtocrr.2020.100023.

References
1. Recondo G, Facchinetti F, Olaussen KA, Besse B, Friboulet L. Making the first move in EGFR-driven or ALK-driven NSCLC: first-generation or next-generation TKI? Nat Rev Clin Oncol. 2018;15:694-708.
2. Gkolfinopoulos S, Mountzios G. Beyond EGFR and ALK: targeting rare mutations in advanced non-small cell lung cancer. Ann Transl Med. 2018;6:142-142.
3. Klempner SJ, Bazhenova LA, Braiteh FS, et al. Emergence of RET rearrangement co-existing with activated EGFR mutation in EGFR-mutated NSCLC patients who had progressed on first- or second-generation EGFR TKI. Lung Cancer. 2015;89:357-359.
4. Vojnic M, Kubota D, Kurzatkowski C, et al. Acquired BRAF Rearrangements Induce Secondary Resistance to EGFR therapy in EGFR-Mutated Lung Cancers. J Thorac Oncol. 2019;14:802-815.
5. Massard C, Michiels S, Ferté C, et al. High-throughput genomics and clinical outcome in hard-to-treat advanced cancers: results of the MOSCATO 01 trial. Cancer Discov. 2017;7:586-595.
6. Chakravarty D, Gao J, Phillips SM, et al. OncoKB: a precision oncology knowledge base. JCO Precision Oncology. 2017;1:1-16.
7. Tamborero D, Rubio-Perez C, Deu-Pons J, et al. Cancer Genome Interpreter annotates the biological and clinical relevance of tumor alterations. Genome Med. 2018;10:25.
8. Xu H, Shen J, Xiang J, et al. Characterization of acquired receptor tyrosine-kinase fusions as mechanisms of resistance to EGFR tyrosine-kinase inhibitors. Cancer Manag Res. 2019;11:6343-6351.
9. Papadimitrakopoulou VA, Wu Y, Han J, et al. Analysis of resistance mechanisms to osimertinib in patients with EGFR T790M advanced NSCLC from the AURA3 study. Paper presented at: ESMO 2018 Congress. October 19, 2018.
10. Oxnard GR, Hu Y, Mileham KF, et al. Assessment of resistance mechanisms and clinical implications in patients with EGFR T790M-positive lung cancer and acquired resistance to osimertinib. JAMA Oncol. 2018;4:1527-1534.
11. Ramalingam SS, Cheng Y, Zhou C, et al. Mechanisms of acquired resistance to first-line osimertinib: preliminary data from the phase III FLAURA study. Ann Oncol. 2018;29(suppl 8):vii840.
12. Daly C, Castanaro C, Zhang W, et al. FGFR3-TACC3 fusion proteins act as naturally occurring drivers of tumor resistance by functionally substituting for EGFR/ERK signaling. Oncogene. 2017;36:471-481.
13. Piotrowska Z, Isozaki H, Lennerz JK, et al. Landscape of acquired resistance to osimertinib in EGFR-mutant NSCLC and clinical validation of combined EGFR and RET inhibition with osimertinib and BLU-667 for acquired RET fusion. Cancer Discov. 2018;8:1529-1539.
14. Nakanishi Y, Masuda S, Iida Y, Takahashi N, Hashimoto S. Case report of non-small cell lung cancer with STRN-ALK translocation: a nonresponder to alectinib. J Thorac. 2017;12:e202-e204.
15. Caenepeel S, Cooke K, Wadsworth S, et al. MAPK pathway inhibition induces MET and GAB1 levels, priming BRAF mutant melanoma for rescue by hepatocyte growth factor. Oncotarget. 2017;8:17795-17809.