BRIEF REPORT

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

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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.

Keywords: EGFR resistance; Oncogene fusions; NSCLC; EGFR tyrosine kinase inhibitors

Introduction

During the past decade, tyrosine kinase inhibitors (TKIs) have displayed a substantial clinical benefit for patients with *EGFR*-mutant NSCLC.[1] Despite tremendous advances, the long-term effectiveness of these targeted therapies has been limited by the unavoidable development of acquired resistance, leading to clinical disease progression. Several resistance mechanisms have been studied recently, schematically dichotomized between on-target (molecular alterations involving the target itself) and off-target (alterations involving other molecular elements). Gene fusions that activate tyrosine kinase receptors, such as *ALK*, *ROS1*, and *RET*, which occur in 1% to 5% of NSCLC, are usually mutually exclusive with *EGFR* mutations and represent meaningful therapeutic targets.[2] Recent studies have also documented the emergence of oncogenic fusions as an off-target resistance mechanism to EGFR TKIs; however, limited cases have been reported and the estimated frequency remains unclear.[3,4]

Through tumor genotyping of tissue and plasma biopsies, we analyzed the presence of fusions and concurrent genetic alterations at biopsy progression under EGFR TKIs in patients with advanced NSCLC.

Materials and Methods

Patients with EGFR-mutant advanced NSCLC with tissue and plasma biopsies at the time of TKI progression (and optionally before starting targeted therapy) were selected from the prospective MATCH-R study (NCT02517892) (Supplementary methods). Postprogression samples were obtained from formalin-fixed, paraffin-embedded pathology blocks or fresh biopsies, if available. Blood samples were collected

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.

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.[5] Whole-exome sequencing, RNA sequencing (RNA-seq), and Affymetrix CytoScan HD comparative genomic hybridization array were performed as previously reported (see Supplementary Methods).[5] 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 GenomeInterpreter.[6,7] 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 2. Characteristics and Genomic Alterations in Pre- and Post-EGFR TKIs Samples in Patients With a Fusion at EGFR TKI Progression

Case	Age/Sex	EGFR TKI/Line Before Resistance Biopsy	Genomic Alterations Pre-EGFR TKI (TGPS on Tissue)	Genomic Alterations on Tissue at EGFR TKI Progression (TGPS, CGH Array, and WES-RNAseq on Tissue)	
				Fusion	Other Alterations
MR 04	62/F	Gefitinib/2	EGFR: L858R EGFR exon 18, I706Y CTNNB1: S33C NOTCH4: G1821E	EIF4G2-GAB1[a]	EGFR: L858R[a,b] EGFR: I706T[a,b] MET: R988C[c] EGFR: T790M[c] NOTCH4: G1821E[b] CTNNB1: G34V[a] FGFR3: S804A[c] BRD4: R1329Q[a] GATA2: D184A[a] WT1: S255A[a] TSC1: T1020P[a] PDPK1 amp[d]
MR 211	57/F	Osimertinib/4	EGFR exon 19 del EGFR: T790M TP53 loss[d] CDK4 amp[d] HMGA2 amp[d] MDM2 amp[d]	FGFR3-TACC3[a]	EGFR exon 19 del[a,b] CDK4 amp[a,d] HMGA2 amp[d] MDM2 amp[a,d] TP53 loss[d] MAP3K7: p.Asp211His[a] GATA3: p.Thr156Pro[a]
MR 393	64/F	Osimertinib/1	EGFR: L858R	FGFR3-TACC3[a]	EGFR exon 21, L858R[a] PIK3R1 mut[a] MYCN: Pro345Thr[a] FANCA: p.Pro1220Arg[a]
MR 48	51/F	Osimertinib/8	EGFR exon 19 del EGFR: T790M TP53: C238F	KIF5B-RET[a]	EGFR exon 19 del[a,b] TP53: C238F[a,b]
MR 240	65/F	Osimertinib/2	EGFR exon 19 del EGFR: T790M[c] CDKN2A mut[a,c] FGFR2: S252S[c] TP53 mut[c]	STRN-ALK[a,d]	EGFR exon 19 del[a,b] EGFR: T790M[a,b] TP53: p.Lys132f[a,b] PTEN: F278L[b] TERT amp[d]
MR 01	54/M	Osimertinib/3	EGFR exon 19 del EGFR: T790M TP53 mut	DHHC20-BRAF[a]	EGFR exon 19 del[a,b] TP53: Cys135Serfs[a,b] ERBB2 amp[a,d] CDK12 amp[a]

[a]Genomic alterations found in WES-RNAseq analyses.
[b]Genomic alterations found in TGPS analyses.
[c]Genomic alterations found only in the ctDNA analyses.
[d]Genomic alterations found in cytoscan HD CGH array.
CGH, comparative genomic hybridization; ctDNA circulating tumor DNA; F, female; M, male; RNA-seq, RNA sequencing; TGPS, targeted gene panel sequencing; TKI, tyrosine kinase inhibitor; WES, whole-exome sequencing; Amp, amplification; MR, patient number.

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 palmitoyltransferase 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 2 in patients that presented fusions at osimertinib progression was longer than patients with other known

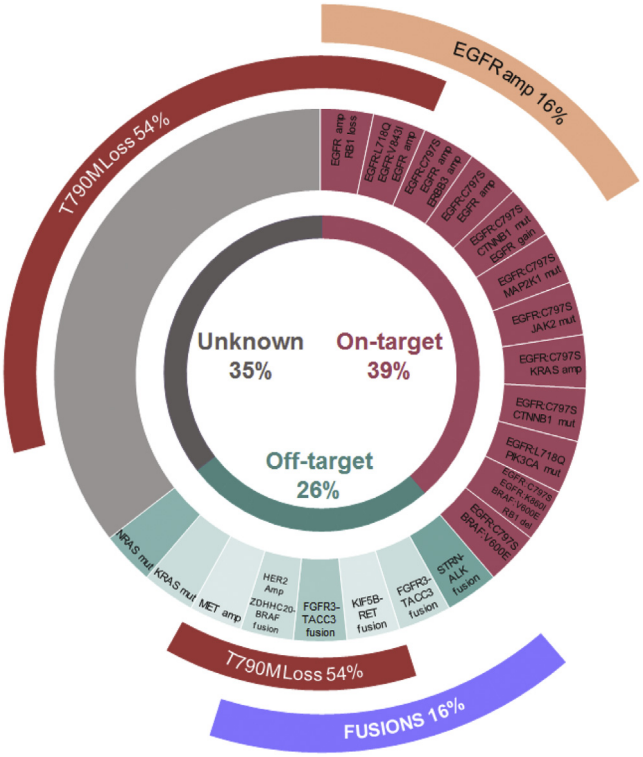

Figure 1. Distribution of resistance alterations to third-generation EGFR tyrosine kinase inhibitor (n = 31). A total of 28 patients tested positive for T790M pre-osimertinib (two received osimertinib as first-line and T790M was not investigated at baseline in one patient). Among them, 15 (54%) lost this mutation at osimertinib progression biopsy. T790M, Thr790Met mutation of the epidermal growth factor receptor *(EGFR)*; *ERBB3*, *Erb-b2 receptor tyrosine kinase*; *CTNNB1*, *catenin beta 1*; *MAP2K1*, *mitogen-activated protein kinase kinase 1*; *JAK2*, *Janus kinase 2*; *PIK3CA*, *phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha*; *RB1*, *retinoblastoma gene*; *STRN*, *striatin gene*; *ALK*, *anaplastic lymphoma kinase*; *FGFR3*, *fibroblast growth factor receptor 3*; *TACC3*, *transforming acidic coiled-coil containing protein 3*; *KIF5B*, *kinesin family member 5B*; *RET*, *ret proto-oncogene*; *HER2 Amp*, *human epidermal growth factor receptor 2 amplification*; *ZDHHC2*, *zinc finger DHHC-type palmitoyltransferase 20*; *MET*; *met proto-oncogene (hepatocyte growth factor receptor)*; *NRAS*, *N-ras proto-oncogene (neuroblastoma RAS viral oncogene homolog)*.

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 [*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 *RET* (n = 6), *ALK* (n = 5), neurotrophic receptor tyrosine kinase 1 (*NTRK1*) (n = 4), *ROS1* (n = 1), and *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.

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 *RET* were also described at EGFR TKI resistance and were successfully reversed by a combination of EGFR and RET TKIs.[13]

The *ALK* partner *STRN*, found in our study, has rarely been established in the EGFR TKI resistance setting. Our patients did not respond to the 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 ALK inhibitors on *STRN-ALK* translocated tumors.[14]

Although *BRAF* alterations in NSCLC are represented mainly by mutations (2%–4%), fusions display another mechanism of BRAF activation, which has also been suggested as a resistance mechanism to EGFR TKIs at a low frequency (~2%).[4] The *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 *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) (cMET). Aberrant GAB1 activity has been associated with resistance mechanisms in *BRAF*-mutant melanomas owing to altered feedback regulation of cMET 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.[8] reported a 50% T790M loss from 10 patients who presented fusions after osimertinib treatment.[8] Furthermore, Oxnard et al.[10] 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*).[10] 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.[2]

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.

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