Review Article on Looking for Chimeras in NSCLC: Widen Therapeutic Options Targeting Oncogenic Fusions

Emerging oncogenic fusions other than *ALK*, *ROS1*, *RET*, and *NTRK* in NSCLC and the role of fusions as resistance mechanisms to targeted therapy

Kenichi Suda, Tetsuya Mitsudomi

Division of Thoracic Surgery, Department of Surgery, Kindai University Faculty of Medicine, Osaka-Sayama, Japan

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Correspondence to: Kenichi Suda, MD, PhD. Associate Professor, Division of Thoracic Surgery, Department of Surgery, Kindai University Faculty of Medicine, 377-2 Ohno-higashi, Osaka-Sayama 589-8511, Japan. Email: ascaris@surg2.med.kyushu-u.ac.jp.

Abstract: Recent evidence has shown that gene fusions caused by chromosomal rearrangements are frequent events in the initiation and during progression of solid tumors, including non-small cell lung cancers (NSCLCs). Since the discoveries of *ALK* and *ROS1* fusions in 2007 and the subsequent successes of pharmacological targeting for these fusions, numerous efforts have identified additional oncogenic driver fusions in NSCLCs, especially in lung adenocarcinomas. In this review, we will summarize recent advances in this field focusing on novel oncogenic fusions other than *ALK*, *ROS1*, *NTRK*, and *RET* fusions, which are summarized in other articles in this thematic issue. These novel gene fusions include *neuregulin-1* (*NRG1*) fusions, *MET* fusions, fusion genes involving *fibroblast growth factor receptor* (*FGFR*) family members, *EGFR* fusions, and other rare fusions. In addition, evidence has suggested that acquisition of gene fusions by cancer cells can be a molecular mechanism of acquired resistance to targeted therapies. Most of the current data are from analyses of resistance mechanisms to EGFR tyrosine kinase inhibitors in lung cancers with oncogenic *EGFR* mutations. However, a few recent studies suggest that gene fusions can also be a resistance mechanism to *ALK*-tyrosine kinase inhibitors in lung cancers with oncogenic *ALK* fusions. Detection, validation, and pharmacological inhibition of these fusion genes are becoming more important in the treatment of NSCLC patients.

Keywords: Neuregulin-1 (NRG1), MET, fibroblast growth factor receptors (FGFRs), molecular targeted therapies, acquired resistance

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Introduction

Oncogenic driver mutations are known to play important roles in carcinogenesis and tumor progression in some non-small cell lung cancers (NSCLCs), especially in lung adenocarcinomas. *Epidermal growth factor receptor* (*EGFR*) mutations or *anaplastic lymphoma kinase* (*ALK*) fusions represent such oncogenic driver mutations in NSCLC, and pharmacologic inhibition using specific tyrosine kinase inhibitors (TKIs) in NSCLC patients harboring these oncogenic driver mutations has revolutionized treatment in advanced stage diseases (1). In addition to these two "classical" oncogenic driver mutations, *ROS1* fusion and *BRAF* V600E mutation are predictive biomarkers already approved for clinical use, and *neurotrophic receptor tyrosine kinase* (*NTRK*) fusions, *MET* exon 14 skipping mutations, *ERBB2* exon 20 insertion mutations, *RET* fusions, and *KRAS* G12C mutation have joined the list of treatable oncogenic driver mutations in

NSCLCs (2-7).

Recent advances in sequencing technology have shown that gene fusion, caused by chromosomal rearrangements, is one of the frequent hallmarks of cancer genome aberrations. For example, a detailed analysis of The Cancer Genome Atlas (TCGA) dataset identified 20,731 gene fusions in 9,966 well-characterized cancer samples across 33 cancer types (after filtering against a list of 3,838 transcript "fusions" detected in a panel of 648 non-neoplastic samples) (8). Another study that analyzed 9,624 tumors from TCGA identified a total of 25,664 fusions and suggested that fusions drive the development of 16.5% of cancer cases and function as the sole oncogenic driver in more than 1% of cancer cases (9). In this review, we will summarize and discuss novel gene fusions, other than *ALK*, *ROS1*, *NTRK*, and *RET* fusions, that are considered to be oncogenic drivers in NSCLCs, especially for lung adenocarcinomas. These rare but potentially important fusions include *neuregulin-1* (*NRG1*) fusions, *MET* fusions, fusion genes involving *fibroblast growth factor receptor* (*FGFR*) family members, *EGFR* fusions, and *BRAF* fusions. Some studies reported that rare primary pulmonary tumors have specific fusion genes, e.g., *synaptotagmin 1* (*SYT*)-*SSX1* or *SYT-SSX2* fusions in synovial sarcoma (10) and *EWS RNA binding protein 1* (*EWSR1*)-*cAMP responsive element binding protein 1* (*CREB1*) fusion in pulmonary myxoid sarcoma (11); however, we will not include these rare tumors in this review.

Mutational processes of gene fusions in lung adenocarcinomas

In considering the mutational processes of lung adenocarcinomas, it is important to classify lung adenocarcinomas into two groups: lung adenocarcinomas unrelated to smoking and those related to smoking (12). A recent study by Lee *et al*. (13) used a mutational signature 4 (a C:G>>A:T-dominant signature, related to exposure to smoking carcinogen) (14) as a negative marker for lung adenocarcinomas unrelated to smoking. In their analyses, lung adenocarcinomas unrelated to smoking were further classified into two groups: tumors with oncogene mutations (*EGFR*, *KRAS* G12D or G12A, *ERBB2*, and *MET* exon 14 skipping) and those with oncogenic gene fusions (*ALK*, *ROS1*, *RET*, *fibroblast growth factor receptor 2* (*FGFR2*), *neuregulin-1* (*NRG1*), *MET*, and *AXL*). In the analysis of tumors with oncogenic gene fusions, the authors found that 26% of oncogenic fusion genes were generated by simple rearrangements such as large deletions (e.g., *EZR-ROS1*), reciprocal inversions (e.g., *EML4-ALK* and *KIF5B-RET*), and reciprocal translocations (e.g., *CD74-ROS1*). In contrast, 74% of oncogenic gene fusions, including all *NRG1*, *AXL*, *FGFR2*, and *MET* fusions, were complex and involved a median of 20 rearrangement breakpoints (range, 4–281). These complex rearrangements were considered to be generated by chromoplexy (15) or chromothripsis, a mutational process involving catastrophic chromosomal shattering followed by stochastic rejoining of the DNA segments (16). In addition, careful reconstruction of the complex rearrangements provided evidence of secondary complex rearrangements in some cases superimposed on the oncogenic fusion gene-generating chromoplexy. These results indicate that there are numerous possibilities for oncogenic fusion genes in lung adenocarcinomas unrelated to smoking. In the following sections, we mainly focus on the recurrent oncogenic gene fusions in lung adenocarcinomas that can be targetable using molecular targeted drugs.

NRG1 fusions

NRG1 is a ligand for ERBB3 and ERBB4 receptor tyrosine kinases (17) that is proteolytically cleaved and secreted. NRG1 is involved in a diverse spectrum of cellular processes primarily in, but not limited to, neural and cardiac development. The possible occurrence of the *NRG1* fusion in lung cancer was described in 2004 (18), the same year when activating *EGFR* mutations were discovered in NSCLCs (19,20). The authors focused on a recurrent chromosome breakpoint in breast cancer at the *NRG1* gene, and the study included 11 NSCLC specimens, with one positive case (with squamous cell histology).

A decade later, Fernandez-Cuesta and colleagues discovered a novel chimeric transcript that fused CD74 to the EGF-like domain of the NRG1 III-beta3 isoform in lung adenocarcinoma cases with invasive mucinous subtype (21). Mechanistically, part of CD74 replaced the transmembrane domain of wild-type NRG1 III-beta3 but preserved the membrane-tethered extracellular EGF-like domain of NRG1 III-beta3, thereby providing a ligand for ERBB3/ERBB2 receptor complexes. Mechanistically, it is considered that binding of the EGF-like domain of the NRG1 fusion to ERBB3 in an autocrine, paracrine, or juxtacrine fashion triggers the activation of ERBB2/ERBB3 complex and the downstream signaling (*Figure 1*) (22). Therefore, it is reasonable that the authors observed that

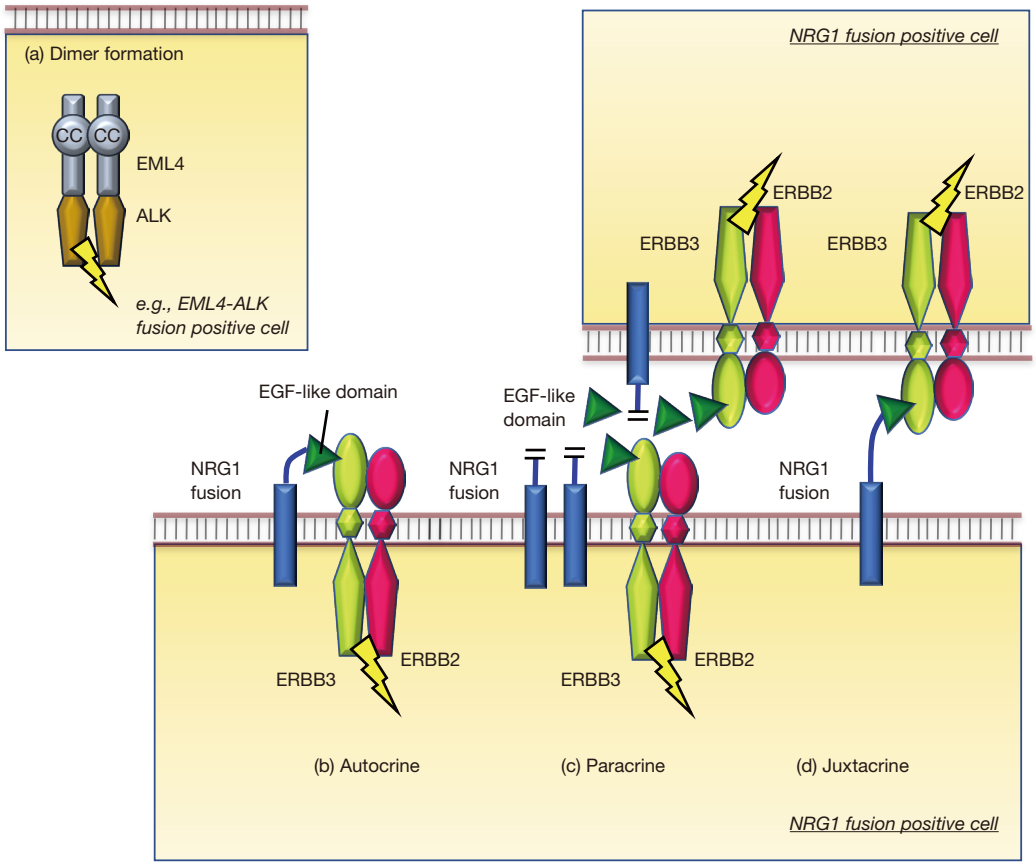

Figure 1 Oncogenic mechanisms of *NRG1* fusions. (a) Most of the fusion genes (e.g., *EML4-ALK* fusion) have constitutive kinase (ALK in this figure) activation due to self-association through the coiled-coil domain (CC) of a fusion partner (EML4 in this figure). However, NRG1 fusions act on cellular biology in a different way. *NRG1* fusion gene products possess both transmembrane domain and the EGF-like domain of NRG1. Binding of the EGF-like domain to ERBB3 in an autocrine (b), paracrine (c), or juxtacrine (d) fashion activates ERBB2/ERBB3 complexes and then triggers oncogenic signaling.

ERBB2 and ERBB3 expression was high along with high phosphorylated ERBB3 levels in tumors bearing the *CD74-NRG1* fusion (21). After this initial study, three additional groups reported the presence of *NRG1* fusions in NSCLCs in 2014, expanding NRG1 fusion partners to *solute carrier family 3 member 2* (*SLC3A2*), *syndecan 4* (*SDC4*), and others (23-25). To date, while many genes have been reported as the partners of *NRG1* fusions (26), *CD74-NRG1* accounts for approximately half of cases in NSCLCs with *NRG1* fusions (26,27).

Intensive analyses showed that the incidence of *NRG1* fusions in NSCLC is very rare. A recent large-scale study by Jonna and colleagues evaluated the incidence of *NRG1* fusions in 21,858 solid tumor specimens profiled at a genomics laboratory, Caris Life Sciences, from September 2015 to December 2018. The authors found that only 0.3% of NSCLCs, predominantly tumors that contain invasive mucinous part (32%), had *NRG1* fusions (27). The incidence of *NRG1* fusion was recently reported as even lower in Chinese patients (0.16%, 18 of 10,966 NSCLC patients) (28), while another recent report from China found an incidence similar with rates in Caucasian populations (0.36%, 6 of 1,681 lung adenocarcinomas) (29). The study by Jonna and colleagues (27) also reported that *NRG1* fusions could be detected at a low incidence across multiple tumor types, e.g., 0.5% of gallbladder cancers, 0.5% of pancreatic ductal adenocarcinomas, 0.4% of ovarian cancers, and 0.2% of breast cancers; however, *CD74* was not detected as the partner gene in tumors other than NSCLCs.

Because *NRG1* fusions are considered to bind ERBB3, and ERBB3/ERBB2 heterodimers activate downstream signaling, molecular targeted drugs that inhibit this pathway are anticipated to show efficacy in tumors with this fusion. The first attempt, as case studies, was performed in 2017 (30-32) by three independent groups using afatinib, an irreversible pan-ERBB TKI (EGFR, ERBB2, and ERBB4), which has been approved in the treatment of NSCLCs with activating *EGFR* mutations (1). Four *NRG1* fusion positive lung adenocarcinoma patients (two with *CD74-NRG1*, one with *SLC3A2-NRG1*, and one with *SDC4-NRG1*) received afatinib monotherapy, which led to clinical benefit lasting from 26 weeks to 12 months (*Table 1*). Other studies reported that GSK2849330, an anti-ERBB3 monoclonal antibody (mAb) (33), MCLA-128 (zenocutuzumab), a bispecific ERBB2/3 antibody (34), and the combination of lumretuzumab (anti-ERBB3 mAb) plus erlotinib (35) showed clinical efficacy in one or a few NSCLC patients with *NRG1* fusions. It should be noted that, in contrast to aforementioned case reports of afatinib, some of these patients experienced progressive disease following afatinib monotherapy (*Table 1*). Notably, an in vitro study using afatinib, pertuzumab (anti-ERBB2 mAb), and lumretuzumab against an *SLC3A2-NRG1* fusion model reported that the combination treatment with two mAbs or the combination treatment of one of the drugs plus taxol was more effective than each of the single agents alone (45). These in vitro studies will be important to evaluate the effectiveness of potential treatment strategies, although the results should be confirmed in clinical settings.

MET fusions

MET gene aberration by exon 14 skipping has become an important therapeutic target in lung adenocarcinomas and possibly in pleomorphic carcinomas of the lung (4). In addition, several studies reported MET activation in lung adenocarcinomas by *MET* gene fusions.

MET gene fusions with *KIF5B* is a recurrent fusion, although the incidence is quite low, as reported by some independent research groups (36,37,46,47). A recent study by Gow and colleagues reported the oncogenic activity of *KIF5B-MET* by soft agar colony formation assays and a xenograft mouse model, and the authors found that crizotinib effectively inhibited the growth of tumors harboring these fusions in vitro and in vivo (47). Notably, two patients with *KIF5B-MET* fusion and one patient with *STARD3NL-MET* fusion received crizotinib (*Table 1*) and all three patients showed clinical benefit (36,37). Recently, additional novel fusions involving *MET*, *CD47-MET* (29), *HLA-DRB1-MET* (48) and *MET-ATXN7L1* (49) were also reported in a common-driver negative lung adenocarcinoma patient.

FGFR fusions

Gene fusions involving *FGFR* family members were discovered in glioblastoma multiforme in 2012, such as *FGFR1* and *FGFR3* fusions with *transforming acidic coiled-coil containing protein 1* (*TACC1*) and *TACC3*, respectively (50), and in bladder carcinomas in 2013 (*FGFR3-TACC3* fusion) (51). Subsequent analysis across multiple tumor cohorts (52) revealed that *FGFR* fusions are present in a wide variety of tumors including lung squamous cell carcinomas, as summarized below. Although the *FGFR3-TACC3* fusion is more common in lung squamous cell carcinomas, this *FGFR3-TACC3* fusion has also been identified in lung adenocarcinomas (38,53,54). In addition, a recent report observed the presence of *FGFR1* fusions (*BAG4-FGFR1*, which has been previously reported in lung squamous cell carcinomas (54), and *FGFR1-CIT*), *FGFR2* fusions (*FGFR2-KIAA1598*, which was previously described in cholangiocarcinoma (55), *FGFR2-CIT*, *FGFR2-ERC1*, *FGFR2-LZTFL1*, *FGFR2-POC1B*, *FGFR2-SORBS1*, *FGFR2-TP73*, *FGFR2-TXLNA*), *FGFR3* fusions other than *FGFR3-TACC3* (*FGFR3-PHLDB3* and *WHSC1-FGFR3*), and *FGFR4* fusions (*ANO3-FGFR4* and *NSD1-FGFR4*) in lung adenocarcinomas, adenosquamous cell carcinomas, or NSCLC not otherwise specified (38). Among patients with these *FGFR* fusions, one invasive mucinous adenocarcinoma patient with *FGFR2-LTZTFL1* fusion was treated with erdafitinib, a pan-FGFR inhibitor, in a clinical trial; he attained a partial response with 60% tumor shrinkage after 2 months of therapy and continued to receive this drug for a total of 11 months (38) (*Table 1*).

Other rare fusions in lung cancers

Through detailed analyses, such as through RNA-based next-generation sequencing and fusion assay, of selected patients with tumors without known driver mutations (e.g., *EGFR*, *KRAS*, *ERBB2*, *BRAF V600E*, *ALK*, *ROS1*) or tumors with invasive mucinous phenotype (enriched cohort for *NRG1* fusions), several rare fusions have been identified. Nakaoku and colleagues conducted whole-transcriptome sequencing for 32 invasive mucinous adenocarcinoma tissues without

Table 1 Summary of clinical efficacy of targeted therapies for novel rare fusions in NSCLCs

Fusion genes	Age/sex/smoking status	Histology	Fusion partners	Targeted therapies	Duration of response	Ref.
NRG1	43/Female/Never	AC	SDC4	Afatinib	12 months	(30)
	62/Female/Never	AC	CD74	Afatinib	26 weeks	(31)
	42/Male/Never	AC	SLC3A2	Afatinib	12 months	(32)
	62/Male/Never	AC	CD74	Afatinib	10 months	(32)
	81/Male/1 year cigar use	AC	CD74	Afatinib	13 weeks (SD)	(33)
	56/Female/2PY	AC	SDC4	Afatinib	PD	(33)
	51/Male/<1PY	AC	CD74	Afatinib	PD	(33)
	86/Male/Never	AC	CD74	GSK2849330 (anti-ERBB3 mAb)	19 months	(33)
				→ Afatinib	PD	
	54/Male/NR	NSCLC	CD74	Afatinib	PD	(34)
				→ MCLA-128 (anti-ERBB2/3 mAb)	Response >3 months	
	55/Female/Never	AC	SLC3A2	Erlotinib	8.1 months	(35)
				→ Lumretuzumab (anti-ERBB3 mAb) + erlotinib	16.4 weeks	
				→ Afatinib	PD	
	42/Female/Never	AC	SCL3A2	Lumretuzumab (anti-ERBB3 mAb) + erlotinib	16.3 weeks	(35)
				→ Afatinib	PD	
MET	51/Female/Never	AC	KIF5B	SAIT301 (anti-MET mAb)	PD	(36)
				→ Crizotinib	10 months	
	33/Female/10PY	AC	KIF5B	Crizotinib	>8 months	(37)
	62/Female/Never	AC	STARD3NL	Crizotinib	>12 months	(37)
FGFR2	72/Male/NR	AC	LZTFL1	Erdafitinib (pan-FGFR inhibitor)	11 months	(38)
FGFR3	NR	SQ	NR	AZD4547 (pan-FGFR inhibitor)	No response	(39)
EGFR	35/Female/Never	AC	RAD51	Erlotinib	8 months	(40)
	21/Female/3PY	AC	RAD51	Erlotinib	5 months	(40)
	43/Female/10PY	AC	PURB	Erlotinib	>20 months	(40)
	38/Male/3PY	AC	RAD51	Erlotinib	>6 months	(40)
	48/Male/Smoker[c]	AC	RAD51	Erlotinib	>5 months	(41)
	62/Female/Never	AC	RAD51	Afatinib	>6 months	(42)
	26/Male/Never	AC	RAD51	Icotinib	>15 months	(43)
BRAF	60/Male/Never	AC	TRIM24	Vemurafenib	3.5 months	(44)

NSCLC, non-small-cell lung cancer; PY, pack-year; NR, not reported; AC, adenocarcinoma; SQ, squamous cell carcinoma; SD, stable disease; PD, progressive disease. c, no data reported about the amount of smoking.

KRAS mutation and detected one *EZR-ERBB4* fusion and one *TRIM24-BRAF* fusion in addition to six *NRG1* fusions and one novel *RET* fusion (*KIAA1468-RET*) (23). The tumorigenicity of NIH-3T3 cells expressing *EZR-ERBB4* or *TRIM24-BRAF* fusion cDNAs was confirmed through experiments using nude mine. A 60-year-old male lung adenocarcinoma patient with *TRIM24-BRAF* fusion was reported to respond to vemurafenib; however, the duration of response was only 3.5 months (44) (*Table 1*). A recently reported technique involving a single-tube, dual-template assay and an integrated bioinformatics pipeline for relevant variant calling identified the *BBS9-BRAF* fusion in lung adenocarcinomas (48). Another study reported five *SND1-BRAF* fusions in lung adenocarcinoma tissues; however, the role of *SND1-BRAF* fusion as an oncogenic driver is unclear, since four out of five detected fusions co-existed with other driver mutations (two *EGFR* exon 20 mutations, one *ERBB2* YVMA insertion, and one *EML4-ALK* fusion) (56). The *SND1-BRAF* fusion gene was also reported as a potential resistance mechanism to a MET inhibitor, PF-04217903, in GTL16 gastric adenocarcinoma cells with *MET* gene amplification through MAPK activation (57).

Activation of EGFR by a recurrent gene fusion (*EGFR-RAD51* fusion) was reported in 2016 in 4 out of ~10,000 lung adenocarcinomas (40). Tumors bearing this *EGFR-RAD51* fusion, as well as those with *EGFR-PURB* fusion, were markedly sensitive to EGFR-TKIs (40), which was confirmed by recent case studies (41-43) (*Table 1*). Recent reports identified several rare fusion genes for *EGFR*, such as *SEPT14-EGFR*, *EGFR-KDD*, *EGFR-YAP1*, *EGFR-SHC1*, and others, in NSCLCs at frequencies of 0.0–0.13% (29,42,43). Another group reported the presence of *EGFR-ANXA2* and *EGFR-RAD51* double fusion mutations in a 36-year-old female patient with lung adenocarcinoma (58).

Fusion genes in lung squamous cell carcinomas

Fusion genes are also frequently found in lung squamous cell carcinomas (59); however, their potential as therapeutic targets are largely unknown. One exception is the *FGFR3-TACC3* fusion, which was identified by independent research groups in 0.6–1.3% of lung squamous cell carcinomas (9,38,52,54,60,61). Interestingly, the *FGFR3-TACC3* fusion is also found in head and neck squamous cell carcinomas, oral cancers, cervical squamous cell carcinomas, and bladder cancers (9,52) as well as lung adenocarcinomas, as described above (38). In vivo experiments of mice harboring tumors derived from RT4 and SW780 bladder cancer cells, containing *FGFR3-TACC3* and *FGFR3-BAIAP2L1* fusion, respectively, showed that a FGFR inhibitor, PD173074, inhibited tumor growth in a dose-dependent manner (52). However, in a phase II study of a FGFR inhibitor, AZD4547, in previously treated patients with lung squamous cell carcinoma with *FGFR* aberration(s), only one patient had *FGFR3* fusion among those included in this study, and the patient did not respond to this novel agent (39) (*Table 1*). Several phase I studies of pan-FGFR inhibitors have been performed in solid tumors with genetic aberration(s) of FGFRs (gene amplifications, mutations, and fusions); however, the response rates of lung squamous cell carcinomas or NSCLCs were as low as 5% compared with 46% and 27% in urothelial carcinoma and cholangiocarcinomas, respectively (62). Other fusion genes as possible oncogenic drivers in lung squamous cell carcinomas include *BAG4-FGFR1* fusion (52,54) and *FGFR2-KIAA1967* fusion (52). The above-mentioned *NRG1* fusions were also detected in two lung squamous cell carcinomas out of 9,592 NSCLCs (27).

Fusions as a mechanism of acquired resistance to TKIs

The first choice of treatment for advanced NSCLCs with driver mutations, such as *EGFR* mutations, *ALK* fusions, or *ROS1* fusions, is TKI monotherapy that targets specific molecules (1). However, despite initial dramatic clinical responses, the emergence of acquired resistance to these molecular targeted therapies is almost inevitable (63). Analyses of the molecular mechanisms underlying the acquired resistance to TKIs have been extensively performed in NSCLCs. These resistance mechanisms can be classified into several groups including (I) a secondary mutation/amplification of the targeted molecule, such as T790M secondary mutation in *EGFR*, (II) activation of a bypass pathway, such as *MET* gene amplification, (III) activation of a downstream pathway, such as *PTEN* loss, in tumors with *EGFR* mutation, and (IV) histological/morphological transformation including small cell lung cancer transformation and epithelial to mesenchymal transition (63).

The increasing use of comprehensive genomic testing and wide application of re-biopsy (including liquid biopsy) at the time of resistance has expanded our understanding of TKI resistance mechanisms in NSCLCs. Despite initial belief that oncogenic fusion genes such as *ALK* fusion (64)

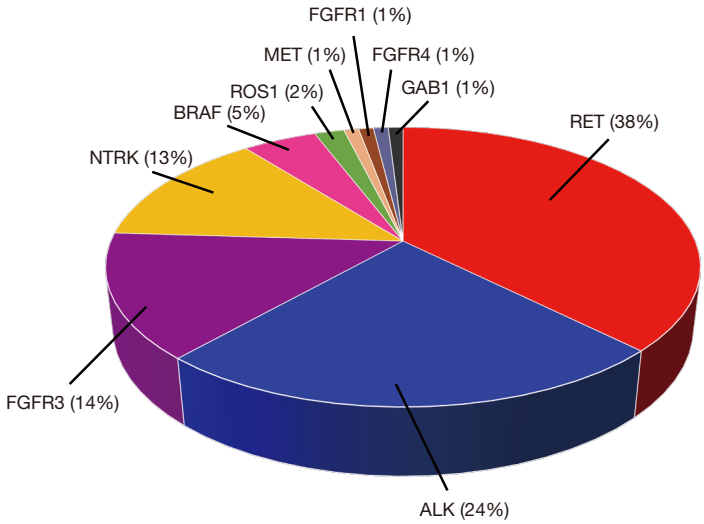

Figure 2 Distribution of reported gene fusions as a resistance mechanism to EGFR tyrosine kinase inhibitors in lung cancers with activating *EGFR* mutations.

or *ROS1* fusion (65) are mutually exclusive with other oncogenic driver mutations in NSCLCs, since 2015 (66), many reports have suggested that gene fusion sometimes causes acquisition of resistance to TKI monotherapy through the activation of bypass pathways (67).

How, then, do fusion genes occur in lung adenocarcinoma patients with *EGFR* mutation? The aforementioned study by Lee *et al*. that traced gene fusions in the mutational history of lung adenocarcinomas has provided a possible answer for this question (13). As described above, the authors classified lung adenocarcinomas unrelated to smoking [low S4 signature (14)] into tumors with oncogenic mutations and those with oncogenic gene fusions. Interestingly, the authors observed a significantly larger burden of rearrangements in tumors with oncogenic mutations than in those with oncogenic gene fusions (211 versus 87; P=0.0009). *EGFR* activating mutation, as well as other oncogenic mutations, occurs in the early stage of carcinogenesis, and these oncogenic mutations are suggested to accelerate the occurrence of chromosomal rearrangements in the later stage of tumor development. Some rearrangements accidentally involve oncogenes such as *RET* or *ALK*, and minor clones with *RET* or *ALK* fusion will be dominant upon EGFR-TKI treatment.

The roles of receptor tyrosine kinase (RTK) fusions as a resistance mechanism to EGFR-TKIs in NSCLCs with *EGFR* mutation have been comprehensively summarized in a very recent review by Zhu and colleagues (67). In this review, the authors identified a total of 86 cases with RTK gene fusion that acquired resistance to EGFR-TKI(s). Acquired RTK gene fusions were observed at progression across all three generations of EGFR-TKIs, but were apparently enriched after osimertinib therapy (3.7%, 15/409 cases) compared with after 1st or 2nd generation EGFR-TKI therapy (1.8%, 3/167 cases). This difference would be reasonable, since more than half of patients who progress against 1st or 2nd generation EGFR-TKIs harbor a T790M secondary mutation as a resistance mechanism (68).

In addition to the 86 cases summarized in the above review (67), our literature search identified an additional thirteen patients who acquired resistance to EGFR-TKIs and harbored gene fusions (38,69-72), including *BRAF* fusions. We could not find *NRG1* fusion as the mechanism of acquired resistance to EGFR-TKIs. The rates of fusion genes as a resistance mechanism to EGFR-TKIs are summarized in *Figure 2*, with the highest incidence in *RET* gene fusions, followed by *ALK*, *FGFR3*, and *NTRK* fusions. As reported in the review by Zhu and colleagues (67), interestingly, *KIF5B* accounts for only 2% of the fusion partners of acquired resistance *RET* fusions, whereas it accounts for 54% in a survey of 106 Chinese NSCLC patients with *RET* fusions. In contrast, *CCDC6-RET* accounts for 17% of the de novo *RET* fusions in NSCLCs compared with 58% of the *RET* fusions related to EGFR-TKI resistance. Although the data are limited, safety and

clinical efficacy of a dual blockade of acquired gene fusions and mutated *EGFR* (founder) were reported in 10 cases (67).

Acquired gene fusion as a resistance mechanism to TKIs may occur in patients with a founder *ALK* fusion who received ALK-TKIs. Through comprehensive analyses for resistance mechanisms to ALK-TKIs in 43 patients with founder *ALK* fusions, McCoach and colleagues identified a *RALGAPA1-NRG1* fusion in a post-alectinib tumor sample and a *CCDC6-RET* fusion in a post-brigatinib biopsy tissue that was not detected on the pre-brigatinib biopsy performed after alectinib treatment. In vitro analysis showed that CRISPR-induced *RALGAPA1-NRG1* fusion conferred crizotinib resistance in the *ALK*-positive lung cancer cell line, H3122, and addition of afatinib re-sensitized H3122 cells with *RALGAPA1-NRG1* fusion to crizotinib (73). The authors also evaluated 12 patients with founder *ROS1* fusions; however, no acquired gene fusion was detected (73). Recently, Boyle and colleagues reported an *AGK-BRAF* fusion as a potential resistance mechanism to ALK inhibitor therapy in a lung adenocarcinoma patient with founder *EML4-ALK* fusion (74).

Conclusions

The increasing use of comprehensive genomic profiling in research as well as the clinical setting has contributed to accumulation of numerous data regarding gene fusions. However, we should recognize that some comprehensive panel tests analyze both DNA and RNA samples while others examine DNA only, and that the latter may not be able to detect rare novel fusions often due to the large intronic regions. We now know that gene rearrangement is a frequent event in the initiation and progression of NSCLCs. Several novel fusions, in addition to *ALK*, *ROS1*, *RET*, and *NTRK* fusions, are considered to be oncogenic drivers in NSCLCs. In addition, evidence has suggested that acquisition of gene fusion is a molecular mechanism of acquired resistance to targeted therapies. Detection, validation, and pharmacological inhibition of these fusion genes are becoming more important in the treatment of NSCLC patients.

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