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REVIEW

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Alterations in genes other than *EGFR/ALK/ROS1* in non-small cell lung cancer: trials and treatment options

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ABSTRACT During the last decade, we have seen tremendous progress in the therapy of lung cancer. Discovery of actionable mutations in *EGFR* and translocations in *ALK* and *ROS1* have identified subsets of patients with excellent tumor response to oral targeted agents with manageable side effects. In this review, we highlight treatment options including corresponding clinical trials for oncogenic alterations affecting the receptor tyrosine kinases MET, FGFR, NTRK, RET, HER2, HER3, and HER4 as well as components of the RAS-RAF-MEK signaling pathway.

KEYWORDS RAS; RAF; MEK; receptor tyrosine kinases (RTK); fibroblast growth factor receptor (FGFR); non-small cell lung cance (NSCLC)

Introduction

Lung cancer is the leading cause of cancer-related mortality globally with an estimated 1.6 million deaths annually[1]. Targeted therapies have revolutionized the treatment of many cancers, including lung cancer in the past decade. Their superior efficacy and generally favorable side effect profile relative to many conventional chemotherapy regimens have improved treatment outcomes for lung cancer patients whose tumors have particular genetic aberrations. Adenocarcinoma of the lung is the commonest type of lung cancer with studies showing actionable mutations in as many as 64% of these patients[2]. Clinical experience with successfully developed therapies directed against *EGFR*, *ALK*, and *ROS1* had been at the forefront for many years. In this article, we describe the various actionable genetic alterations in non-small cell lung cancer (NSCLC) other than *EGFR*, *ALK*, and *ROS1* and delineate the clinical trials and treatment options associated with these pathways. **Table 1** lists a summary of the genetic alterations discussed in this review.

A systematic analysis of literature was performed by using a Mesh (Medical subject headings) search in PubMed using the term NSCLC or with any of the following words: oncogenes, receptor tyrosine kinase, mutation, amplification, copy number, translocation, and inhibitor. The search was limited to English language articles between 2011 and 2016. One of the three authors reviewed the titles, abstracts, full text journal articles and cross-references in published articles

Table 1 Summary of the different molecular aberrations and their frequency

Target	Alteration	Frequency
MET	Amplification	5%-22% NSCLC
	Exon 14 mutation	3% LUAD
	MET fusion	<1% LUAD
FGFR	FGFR1 amplification	10%-20% LUSC
	FGFR2, 3 mutation	6% NSCLC
	FGFR1-3 fusion	5% NSCLC
RET	Mutation	1%-5% NSCLC
	RET fusion	1%-2% LUAD
NTRK	NTRK2, 3 mutation	33% LNEC
	NTRK1-3 fusion	1%-2% LUAD
HER3	NRG1 fusion	7% IMAs
HER4	HER4 fusion	1% IMAs
BRAF	V600E mutation	1.5% LUAD
	BRAF fusion	1% IMAs
	BRAF kinase duplication	<1% NSCLC

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were identified for potential relevance. Abstracts from the American Society of Clinical Oncology (ASCO), American Association for Cancer Research (AACR), and European Society of Medical Oncology (ESMO) and World Conference on Lung Cancer during this time frame were also manually reviewed. Candidate alterations were rated and chosen based on clinical relevance in terms of available therapeutic agents, reported clinical outcomes and applicable findings in other malignancies.

RAS-RAF-MEK pathway

RAS

RAS belongs to a family of related proteins involved in signal transduction through their GTPase function. There are three RAS oncogenes that have been implicated in human malignancies, namely *HRAS*, *KRAS*, and *NRAS*. *KRAS* is the most common oncogenic alteration, occurring in about 25% of lung adenocarcinoma patients[2]. Mutations usually occur in codons 12, 13 and less commonly in codon 61, leading to constitutive activation of multiple signaling pathways including MAPK, PI3K/mTOR and RALGDS pathways. *NRAS* alterations have also been recently discovered in NSCLC, present in <1% of cases, predominantly in lung adenocarcinomas[3]. Of interest is that while majority of patients with *NRAS* mutations were former or current smokers, the frequency of smoking-related transversion mutations were significantly less frequent compared to *KRAS* mutant NSCLC[3]. Analysis of TCGA database show that *HRAS* alterations are similarly clustered in codons 12, 13 and 61 but in comparison to *KRAS* and *NRAS*, appear to occur in slightly higher frequency in the squamous cell subtype (<3%) compared to adenocarcinoma subtype in NSCLC (<1%)[4].

While numerous attempts to therapeutically target *RAS* have been attempted, successful pharmacologic approach with direct competitive inhibition has been elusive to date as *RAS* GTPase binds to its substrate (GTP) with very high affinity. While recent research efforts, as exemplified by the development of allosteric inhibitors of KRAS[5], are gaining momentum, these agents are not yet clinically available and have yet to demonstrate *in vivo* efficacy. Hence the vast majority of efforts targeting *KRAS* to date have instead focused on blocking downstream or parallel pathways that cross-signal in mediating proliferation, anti-apoptosis, angiogenesis and metastasis. MEK inhibitors demonstrated antitumor efficacy in early preclinical studies in both *KRAS* and *NRAS* mutants and have thus been most investigated in the clinical setting at this time[3,6]. Selumetinib, a MEK inhibitor in combination with docetaxel has shown increased response rate and progression-free survival (PFS) in *KRAS* mutant patients but did not meet the primary end point of overall survival(OS). In addition, the combination caused increased side effects[7]. Recent studies have shown that co-existing alterations such as in p53, STK 11 or CDKN2A/B may not only impact prognosis but also modify responses to therapy which provides an explanation for the variability in clinical outcomes and response to therapy[8,9]. Ongoing and future trials specifically focusing on the subset of *KRAS* mutant NSCLC will need to incorporate co-mutation status in study enrollment and data analysis.

Nonetheless, due to clinical activity seen in some patients with MEK inhibition, studies are ongoing with MEK inhibitors, as listed in **Table 2** below. The focus is on combination therapy based on synthetically lethal or synergistic activity shown in preclinical models, such as combination treatment with cyclin-dependent kinase (CDK) inhibitors. Other agents with recently reported clinical trial data in the last quarter of 2015 include the evaluation of the focal adhesion kinase inhibitor defactinib based on preclinical experiments demonstrating potent antitumor activity of this agent when *KRAS* mutation co-occurs with loss of p53 or INK4/p16[10]. Interim results from a phase 2 Simon two-stage study enrolling *KRAS* mutant NSCLC (regardless of co-mutation status) showed that median PFS was 11.7 weeks, with approximately 25% of patients

Table 2 Selected RAS-targeted clinical trials

Drug	Target	Phase	Study population	Clinicaltrials.gov
Selumetinib	MEK	3	Second line stage 4 or stage 3B NSCLC	NCT01933932
MEK162+erlotinib	MEK	1	Metastatic, failed prior therapies	NCT01859026
Trametinib+momelotinib	MEK+JAK1/2	1	Metastatic, progression after platinum based chemotherapy	NCT02258607
PALBOCICLIB+PD-0325901	CDK4/6+MEK	1/2	Metastatic, failed prior therapies	NCT02022982
AZD2014+selumetinib	mTOR+MEK	1/2	Metastatic, failed prior therapies	NCT02583542

demonstrating some degree of tumor shrinkage although partial response (PR) rate by RECIST was technically only 10% in the 42 evaluable patients[11]. There did not appear to be correlation between drug efficacy and co-mutation status of *INK4/p16* or *p53*. Lastly, while the HSP90 inhibitor ganetespib showed cytotoxic activity in combination with MEK inhibitors in several *KRAS* mutant lung cancer cell lines[12], its further clinical development in non-ALK translocation NSCLC is uncertain with the termination of the phase 3 GALAXY-2 trial in late October 2015 due to futility in demonstrating OS improvement with the combination of ganetespib to docetaxel as second-line therapy in NSCLC compared to docetaxel alone.

RAF

BRAF is one of the three members of the RAF family of serine/threonine protein kinases (the other two are ARAF and CRAF, also known as RAF1) and plays an important role in the regulation of the MAPK/ERK pathway. *BRAF* activates ERK which in turn activates downstream transcription factors leading to cell differentiation, growth, proliferation and apoptosis[13]. Mutations in BRAF are reported in 4.9% of lung adenocarcinomas and 5%-20% of *BRAF* mutations are found concurrently with *EGFR* mutations. V600E is the most frequent mutation found in 50%-60% of NSCLC patients[14,15]. It is commonly associated with poor OS, more commonly seen in females and are found in both smokers and never smokers.

Vemurafenib and dabrafenib are two commercially available *BRAF* inhibitors, approved by the FDA for melanoma, with higher selectivity for the mutant activated V600E kinase relative to wildtype *BRAF* and *CRAF*. Initial case reports had been reported demonstrating significant clinical activity with the use of either agent in BRAF V600 mutant NSCLC as early as 2012. In a phase 2 study of dabrafenib administered 150 mg twice daily in patients with BRAF V600E mutant advanced NSCLC, the predominant histology of patients enrolled was adenocarcinoma. The overall response rate was 32% (25/78 evaluable patients) and the median duration of response was 12 months thus demonstrating significant anti-tumor activity[16]. Similarly, in a phase 2 basket study of vemurafenib as therapy for nonmelanoma cancers with *BRAF V600* mutations, patients enrolled in the NSCLC cohort experienced a response rate of 42% with median PFS of 7.3 months[17]. To delay emergence of treatment resistance and in light of clinical experience with the therapy of melanoma patients whose tumors harbor *BRAF V600E*, combination of dabrafenib with the MEK inhibitor trametinib was conducted in a phase 2 study among patients with *BRAF V600E* mutated NSCLC. The overall response rate was 63% (15 of 24 evaluable patients) and the disease control rate for >12 weeks was 88%[18].

However, 40%-50% of *BRAF* mutations in NSCLC are non-V600 alterations where, in contradistinction with the increased kinase activity of the V600 alteration, many of these mutations may have impaired kinase function (e.g. D594A, G466V) that nonetheless still retain oncogenic activity either through transactivating the catalytically competent RAS or CRAF by facilitating dimerization or through abrogation of autoinhibition thus leading to constitutive kinase activity[19]. Due to the low frequency of these mutations, efficacy of first-generation BRAF mutant selective inhibitors is mostly characterized in preclinical models with non-V600 mutants (e.g. K601E showing sensitivity) although there maybe anecdotal documentation of clinical response (e.g. drug resistance with G469L mutation) specifically in NSCLC[20] or in a different tumor type (e.g. L597 mutation in melanoma sensitive to vemurafenib but this mutation has not been described to date in NSCLC)[21]. Although MEK inhibitors have demonstrated activity against some of these non-V600 alterations in the clinic in melanoma patients[22,23], clinical experience in NSCLC is sparse to date. There is also limited evidence that dasatinib may have clinical activity in NSCLC against kinase-inactivating *BRAF* mutations, i.e. Y472C[24]. Other genetic alterations of *BRAF* described in NSCLC include *BRAF* inframe deletions which promote BRAF dimerization and downstream signaling activation. Such deletion mutants are resistant to vemurafenib but sensitive to the RAF dimer inhibitor, LY3009120[25]. Novel fusion gene products with BRAF that demonstrate constitutive kinase activity and increased downstream ERK signaling sensitive to MEK inhibition preclinically have also been described[26,27]. Also recently reported is an intrachromosomal duplication of the BRAF kinase domain, mutually exclusive with other known oncogenic alterations, which was first described in gliomas[28] but can also be found in multiple tumor types including lung cancer (author's experience). It represents 0.5% of *BRAF* alterations and clinical response to the multikinase inhibitor regorafenib was documented in a case report.

Alterations in *ARAF* or *CRAF* are even less common. *ARAF* and *CRAF* mutations are found in less than 1%-2% of NSCLC. The *ARAF* somatic mutation S214C had been described to be oncogenic and thought to be the driver mutation underlying clinical response to sorafenib in a patient with lung adenocarcinoma[29]. The *CRAF* somatic mutations S257 and S259 have also been described in lung

cancer specimens. These mutations demonstrate transforming effects and are sensitive to sorafenib and MEK inhibition *in vitro*[29]. Both these specific ARAF and CRAF mutations are thought to abrogate negative regulation of RAF1 activation as they are located in the CR2 domain that negatively regulates RAF1 activation.

MEK

Somatic activating mutations in exon 2 of *MEK1*, a dual-specificity serine/threonine and tyrosine kinase, were first described in 2008 to occur in <1% of lung adenocarcinomas[30]. These alterations are mutually exclusive with other drivers (EGFR, KRAS, NRAS, BRAF, HER2, PIK3CA, and AKT)[31] and are predominantly transversions associated with smoking. The most common mutation is K57N (approximately 60%) followed by Q56P (20%). Preclinically, these mutations are sensitive to MEK inhibitors.

Receptor tyrosine kinases (RTK)

MET

MET (mesenchymal-epithelial transition factor) is a proto-oncogene present on chromosome 7q21-q31 which encodes the *MET* receptor tyrosine kinase. The MET receptor binds the hepatocyte growth factor(HGF), causing activation of several downstream signaling pathways including RAS/MAPK, PI3K and SRC kinase pathways[32]. Increased levels of HGF and MET expression have been associated with aggressive tumor biology and portend a poor prognosis. Aberrations in the HGF/MET signaling pathway occur through a variety of mechanisms including MET amplification, gene rearrangements, and mutations or through HGF or MET overexpression. These alterations may potentially be sensitive to various MET inhibitors in clinical development, such as those listed in **Table 3**.

MET amplification is one of the mechanisms of acquired resistance to EGFR tyrosine kinase inhibitors which can occur in up to 22% of treated patients[33]. It has also been reported to cause resistance to selective ALK inhibitors[34]. Whether immunohistochemistry (IHC) or fluorescence *in situ* hybridization (FISH) platform for testing is a better predictor of response to MET therapy is not well established. Onartuzumab, a humanized antibody to the MET receptor, did not improve survival endpoints in a phase 3 study of NSCLC patients with MET-positive tumors as defined by IHC. In comparison, early reports by Camidge et al.[35] evaluating the oral multikinase ALK and MET inhibitor crizotinib showed that when MET high amplification is defined as a MET/centromere ratio of >5, a higher response rate of 50% was seen compared to response rates in patients with lower ratio. However, frequency of high amplification as defined occurs in only 0.8% of 800 screened consecutive samples.

MET exon 14 skipping mutations occur in about 3% of lung adenocarcinoma and tumors harboring them respond significantly to anti-MET targeted therapy as the splice site alteration renders it less susceptible to ubiquitination and subsequent protein degradation[36]. Recently Liu et al.[37]

Table 3 Examples of MET inhibitors in clinical trials

Drug	Target	Phase	Study population	Clinicaltrials.gov
Selective MET inhibitors				
Capmatinib (INC280)	MET	1	Dose expansion MET-aberrant solid tumors	NCT01324479
	MET	2	EGFR wild type NSCLC after one or two prior lines of chemotherapy	NCT02414139
SAR125844	MET	2	MET-amplified tumors	NCT02435121
Multikinase inhibitors				
Crizotinib	MET, ALK, ROS1, NTRK	2	CREATE: ALK/MET Cross-tumor	NCT01524926
Cabozantinib	MET, AXL, VEGFR, RET, ROS1, KIT, NTRK1-3	2	NSCLC with brain metastasis+/-MET amplification	NCT01232598
			Genotypically selected NSCLC	NCT01639508
MGCD516	MET, AXL, VEGFR, RET, PDGFR, DDR2, KIT, NTRK1-3	1	Dose expansion in Genotypically selected NSCLC	NCT02219711
Altiratinib (DCC-2701)	MET, VEGFR2, NTRK1-3	1	Solid tumors	NCT02228811

reported that MET exon 14 skipping alterations were present in 22% of pulmonary sarcomatoid carcinoma and could be used as a potential target for therapy. Oncogenic gene fusion as produced by chromosomal rearrangements is another mechanism for aberrantly activating the pathway. Stransky et al.[38] reported a novel kinase fusion KIF5B-MET in lung adenocarcinoma with the predicted chimeric protein following the classic activation paradigm of fusing dimerization motifs to an intact kinase domain, thus also representing a possible target for drug therapy.

FGFR

The FGFR (fibroblast growth factor receptor) family is composed of 4 tyrosine kinase receptors (FGFR1-4) and 22 ligands. FGF ligand binding causes receptor activation leading to receptor dimerization and auto-phosphorylation activating key downstream regulators through the RAS/RAF/MAPK and PI3K/AKT pathway. The FGFR signaling pathway is constitutively activated by point mutations, gene amplification and chromosomal translocations leading to altered cell proliferation, angiogenesis, cell migration and tumorigenesis[39]. Activation of the FGFR pathway has also been shown to mediate resistance to other targeted therapies like BRAF, VEGR, HER2, MET and EGFR[40].

Focal amplification of FGFR1 is predominantly found in squamous cell lung cancer (21%-22%) and less frequently in adenocarcinoma of lung (around 3%)[41,42]. A subset of squamous cell carcinoma with FGFR1 amplification with increased MYC expression (40% cases) was found to be more susceptible to FGFR inhibition indicating a potential response-predictive biomarker[43]. Liao et al.[44] described *FGFR2* and *FGFR3* mutations in 3% of lung squamous cell carcinoma samples respectively. Using cell culture and xenograft models they showed that these mutations were oncogenic in nature and are sensitive to the receptor tyrosine kinase inhibitor pazopanib. Mutations in *FGFR* can occur both in the extracellular domain (ECD) and in the kinase domains and are transforming in nature. *FGFR* ECD mutants facilitate covalent dimerization through the formation of intermolecular disulfide bonds[44]. *FGFR* fusions have also been described in lung cancer such as FGFR2-KIAA1967 fusion reported in lung squamous cell carcinoma and *FGFR2-CIT* fusion reported in lung adenocarcinomas. Smoking and tumor size were independent predictors of *FGFR* fusions[45]. The *FGFR* fusion product, *FGFR3-TACC3* is most commonly reported in around 3% of lung squamous cell carcinomas but occurs in <1% of lung adenocarcinomas[46].

Although various multi kinase anti-angiogenic inhibitors include FGFR as a drug target, these first-generation agents were non-selective and have low potency. As FGFR was increasingly recognized as an important therapeutic target in multiple malignancies including lung cancer, next generation agents were developed for their selective FGFR inhibitory activity. Hyperphosphatemia is a mechanism-based toxicity caused by potent FGFR kinase inhibitors due to inhibition of the FGF23/Klotho signaling axis, thereby causing a decrease in renal phosphate excretion[47]. FGF ligand trap agents however that spare "hormonal" FGF23, such as FP-1039, may potentially avoid this particular toxicity.

AZD4547 and BGJ398 are selective anti *FGFR* 1-3 inhibitors with potent preclinical anti-tumor activity in *FGFR1* amplified squamous cell lung cancer cell lines and patient derived xenografts. However, clinical activity seen to date even in genotype-selected patients appears to be modest. In a phase 1 multicenter expansion study of AZD4547 enrolling previously treated advanced FGFR amplified squamous cell lung cancer (defined as: FGFR1 CEP8 >2), 7 patients hadhigh amplification (FISH ratios >2.8) and the remaining 8 had low amplification (FISH ratios between 2-2.8). Only one patient (tumor had high *FGFR* amplification) achieved PR. The most common adverse effects were central serous retinopathy (20%), and dehydration. Exon sequencing of the 283 cancer-related genes in 6 available patient tumors did not show any clear response modifiers. The increase in serum phosphate concentration observed in this study provides evidence that AZD4547 at the dose of 80 mg orally twice daily caused pharmacologic target inhibition. The patient who achieved PR had no other somatic mutation. Patients with disease progression or stable disease as best response on the other hand had multiple additional genetic alterations[48]. Similarly for BGJ398, a phase 1 study dose escalation study of 21 patients with *FGFR1* amplified lung squamous cell cancer showed low response rates despite patient selection. PR was noted in 2 patients lasting 8 and 3 months respectively[49]. Exploratory biomarker analysis revealed that four patients with high FGFR gene amplification by FISH did not derive any clinically meaningful efficacy outcome. In other tumors, additional *RTK* amplifications were seen[50]. **Table 4** lists examples of FGFR inhibitors in clinical trials.

RET

Mutations in *RET* cause the multiple endocrine neoplasia type 2 syndrome and sporadic medullary thyroid cancer[51].

Table 4 Examples of FGFR inhibitors in clinical trials

Drug	Target	Phase	Study population	Clinicaltrials.gov
Multikinase FGFR inhibitors				
Dovitinib	VEGFR, FGFR, PDGFR, KIT, FLT3, RET, NTRK	2	FGFR1 amplified NSCLC	NCT01861197
Pazopanib	VEGFR, FGFR, PDGFR, KIT	2	Solid tumors with FGFR2 alterations	NCT02450136
Lucitanib	VEGFR, FGFR, PDGFR	2	Genotypically selected NSCLC/SCLC	NCT02109016
Nintedanib	VEGFR, FGFR, PDGFR, RET, FLT3	2	Genotypically selected NSCLC	NCT02299141
			FGFR biomarker analysis in LUSC	NCT01948141
Selective FGFR inhibitors				
BAY1163877	FGFR1-3	1/2	FGF/FGFR-aberrant solid tumors or MM	NCT02052778
TAS-120	FGFR1-4	1	Solid tumors	NCT01752920
ARQ087	FGFR1-4, mut FGFR2	1b	FGFR-aberrant solid tumors	NCT01752920
JNJ-42756493	FGFR1-4	1	Dose expansion LUSC, SCLC	NCT01703481
FP-1039 (GSK3052230)	FGF-ligand trap (anti-FGFR1)	1	Solid tumors with aberrant FGF signaling	NCT01868022

RET fusion genes are present in 1%-2% of lung adenocarcinomas[52] and are more likely to occur in patients younger than 60 years, non-smokers, in tumors with lymph node metastasis and in poorly differentiated tumors. Wang et al.[52] studied 936 surgically resected NSCLCs and detected KIF5B-RET, CCDC6-RET and NCOA4 RET fusions in 13 patients. Although there are currently no selective RET inhibitors in the clinic, a number of multikinase inhibitors in clinical use include RET in their spectrum of drug targets. For example, alectinib, a selective ALK inhibitor also has potent anti-RET activity[53]. Sunitinib and vandetanib had been reported to induce rapid tumor response or long-term disease stabilization in RET-rearranged NSCLC patients in case reports[54-56]. More recently, results from a phase 2 study of cabozantinib administered 60 mg daily to patients with RET-rearranged lung adenocarcinoma were reported. The rate of PR at the end of 12 weeks was 33% (5/15); the overall response rate was 28% with 5 confirmed PRs. The stable disease rate was 72% (13/18) with a median PFS of 7 months. Toxicities were mostly grade 1 or 2 fatigue, diarrhea, transaminitis, thrombocytopenia and palmar-plantar erythrodysesthesia[57]. Other multikinase inhibitors being testing in NSCLC with RET translocation include ponatinib and lenvatinib.

Neurotrophic tyrosine receptor kinase (NTRK)

The NTRK pathway has been implicated in the pathogenesis of lung cancer. Marchetti et al.[58] investigated the presence of *NTRK* mutations in 538 primary lung carcinomas, including 17 typical carcinoids, A total of 10 atypical carcinoids, 39 small cell lung carcinomas, 29 large cell neuroendocrine carcinomas (LCNEC) and 443 NSCLCs by single-strand conformation polymorphism and sequencing of the tyrosine kinase domain of *NTRK1*, *NTRK2* and *NTRK3*. A total of 10 somatic mutations were detected in *NTRK2* and *NTRK3* located mostly in the activating and the catalytic loops. The mutations were restricted to the LCNEC histotype which accounted for 31% of cases. These mutations play an important role in tumorigenesis and could possibly have important implications in selection of patients for targeted therapy.

Vaishnavi et al.[59] identified novel gene fusions in patients with lung cancer harboring the kinase domain of the *NTRK1* gene which encodes TRKA protein, a high-affinity nerve growth factor receptor. The *MPRIP-NTRK1* and *CD74-NTRK1* fusion products are oncogenic and lead to constitutive TRKA kinase activity. Targeting the *NTRK1* fusions with TRKA kinase inhibitors inhibited auto phosphorylation. Next generation sequencing or fluorescence in situ hybridization demonstrated *NTRK1* fusions in 3.3% of patient lung cancer tumor samples without known oncogenic alterations. These newly discovered fusions are clinically relevant in lung cancer as drugs targeting *NTRK* are in early clinical development. One patient with stage 4 lung adenocarcinoma with an *SQSTM1-NTRK1* fusion transcript who was enrolled in a clinical trial evaluating entrectinib

(RDX-101), a multikinase ALK/ROS1/NTRK1-3 inhibitor, demonstrated a RECIST-defined PR and complete response of all brain metastasis, demonstrating entrectinib's significant anti-tumor activity in NSCLC with *NTRK1* gene rearrangements[60]. Treatment response was durable and ongoing at more than 6 months at the time of publication. In this study, investigators screened 1,378 cases, identifying *NTRK1* gene rearrangements at a frequency of 0.1%. **Table 5** lists several agents that are in development.

HER2-4

Human epidermal growth factor receptor plays a crucial role in normal homeostasis and dysregulation of this pathway results in carcinogenesis. The HER family consists of 4 structurally related tyrosine kinase receptors, HER1 (EGFR), HER2, HER3 and HER4. They form homodimers and heterodimers by interacting with specific ligands (except HER2) leading to auto phosphorylation of tyrosine residues and activating various downstream signaling pathways including PI3K/AKT/MTOR, STAT pathway and the RAS/RAF/MAPK pathway[61]. Alterations in *HER2* in lung cancer are described in approximately 3% of adenocarcinomas, more commonly in nonsmokers and can be either mutations or amplifications[62]. While *HER2* amplification is a valid target in breast cancer, it does not seem to be predictive of response to anti-HER agents in NSCLC[63,64]. In contrast, *HER2* exon 20 mutations seem to confer sensitivity to trastuzumab-based therapies and oral pan-HER inhibitors such as afatinib, dacomitinib and lapatinib[65-68].

On the other hand, fusion gene products have been recently identified that may aberrantly activate the HER3/HER4 pathway. Whole transcriptome sequencing of 32 invasive mucinous adenocarcinoma (IMA) including 27 cases without *KRAS* mutations revealed that oncogenic fusions occurred mutually exclusively with *KRAS* mutations. NRG1 fusions were present in approximately 18% of KRAS-negative IMAs. The NRG1 fusion proteins (e.g. CD74-NRG1) retain the EGF-like domain of the NRG1 ligand protein (also known as neuregulin or heregulin) which activates juxtacrine HER2:HER3 signaling. EZR-ERBB4 fusions are present in 1% of IMAs and activate HER4 via the coiled-coiled domain of EZR facilitating homodimerization of the kinase function of HER4[27]. These fusion products are shown to be suppressed by pan-HER inhibitors such as afatinib and lapatinib.

Conclusions

We have outlined the salient features characterizing potentially druggable genetic alterations in NSCLC. Many of these are rare in occurrence. On the other hand, there are multiple drugs being developed competing for the same patient population. Conventional phase 2 or phase 3 trial designs to evaluate drug efficacy and obtain FDA approval are thus becoming less feasible in the face of this bottleneck. Trial designs in this era of personalized medicine to address such issues are currently biomarker-driven, either incorporating multiple treatment arms for different

Table 5 NTRK inhibitors in clinical testing

Drug	Target	Phase	Study population	Clinicaltrials.gov
Multikinase NTRK inhibitors				
Cabozantinib	MET, AXL, VEGFR, RET, ROS1, KIT, AXL, NTRK1-3	2	Genotypically selected NSCLC	NCT01639508
MGCD516	MET, AXL, VEGFR, RET, PDGFR, DDR2, KIT, NTRK1-3	1	Dose expansion in genotypically selected NSCLC	NCT02219711
Entrectinib (RXDX-101)	NTRK1-3, ROS1, ALK	1/2	Genotypically selected solid tumors	NCT02097810
TSR-011	ALK, NTRK1-3	1/2	ALK/NTRK-altered solid tumors and lymphoma	NCT02048488
Altiratinib (DCC-2701)	MET, VEGFR2, NTRK1-3	1	Solid tumors	NCT02228811
DS-6051b	ROS, NTRK1-3	1	ROS1/NTRK-altered solid tumors	NCT02279433
Selective NTRK inhibitors				
PLX7486	NTRK1-3	1	NTRK-altered solid tumors	NCT01804530
LOXO-101	NTRK1-3	1	NTRK-altered solid tumors	NCT02122913

drug/target pairs in "umbrella" studies for one specific disease type or enrolling different cancer types harboring a pre-specified biomarker in "basket" studies. The Lung-MAP (S1400) trial for patients with squamous cell lung cancer is an example of the former whereas the NCI Molecular Analysis for Therapy Choice Trial (NCI-MATCH) protocol is an example of the latter. It is important to remember, however, that these studies may include biomarkers that have not been comprehensively validated yet at the time of study launch and thus 'failure' of a drug treatment arm to achieve study endpoints maybe reflective of incorrect biomarker choice or testing platform to a similar degree as inherent lack of drug efficacy. On the other hand, we have seen increasing number of drug approvals based on early phase 1 study data, thus supporting the current trend in phase 1 trial designs of incorporating biomarker validation and genotype-driven patient selection for targeted therapies during early phase clinical testing.

Conflict of interest statement

No potential conflicts of interest are disclosed.

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