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The Minority Report: Targeting the Rare Oncogenes in NSCLC

Caroline E. McCoach, MD, PhD¹ and Robert C. Doebele, MD, PhD¹

Robert C. Doebele: Robert.doebele@ucdenver.edu

¹Department of Medicine, Division of Medical Oncology, University of Colorado School of Medicine, 12801 E. 17th Avenue, Aurora, CO 80045 USA

Abstract

Lung cancer is still responsible for the highest number of cancer deaths worldwide. Despite this fact, significant progress has been made in the treatment of non-small cell lung cancer (NSCLC). Specifically, efforts to identify and treat genetic alterations (gene mutations, gene fusions, gene amplification events, etc.) that result in oncogenic drivers are now standard of care (EGFR and ALK) or an intense area of research. The most prevalent oncogenic drivers have likely already been identified; thus, there is now a focus on subgroups of tumors with less common genetic alterations. Interestingly, as we explore these less common mutations, we are discovering that many occur across other tumor types (i.e., non-lung cancer), further justifying their study. Furthermore, many studies have demonstrated that by searching broadly for multiple genetic alterations in large subsets of patients they are able to identify potentially targetable alterations in the majority of patients. Although individually, the rare oncogenic drivers subgroups may seem to occur too infrequently to justify their exploration, the fact that the majority of patients with NSCLC harbor a potentially actionable driver mutation within their tumors and the fact that different types of cancers often have the same oncogenic driver justifies this approach.

Keywords

Non-small cell lung cancer; Oncogenic driver; Mutation; Fusion protein; Tyrosine kinase inhibitor; ROS1; RET; MET; NTRK1; FGFR1; BRAF; HER2; NRG1

Introduction

There are expected to be ~224,000 new cases of lung cancer diagnosed in the United States this year and ~160,000 deaths. Although lung cancer is still responsible for the highest number of deaths across all cancers, the prevention and treatment strategies that have

Correspondence to: Robert C. Doebele, Robert.doebele@ucdenver.edu.

Compliance with Ethics Guidelines

Conflict of Interest

Caroline E. McCoach declares that she has no conflict of interest.

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Human and Animal Rights and Informed Consent

This article does not contain any studies with human or animal subjects performed by any of the authors.

emerged over the past decade have begun to make significant advances in the overall survival of this disease [1, 2]. One of the primary reasons for the improvements in survival observed in some subgroups of patients with lung cancer is the focused effort on a precision medicine approach. This paradigm matches patients whose tumors harbor specific genetic mutations with drugs that specifically target these mutations. This has been driven by the dramatic clinical benefit derived from targeting the tyrosine kinases that are activators of growth and proliferation pathways. Due to this success, mutation analysis for epidermal growth factor receptor (*EGFR*) and anaplastic lymphoma kinase (*ALK*) in lung adenocarcinoma is now recommended in the most recent NCCN guidelines for NSCLC (Version 4.2014). The next logical step is to identify additional subgroups of lung cancer that contain oncogenic drivers and pair these with targeted therapeutics that may provide clinical benefit to patients. In this article we will explore some of the more promising oncogenic drivers that are currently under clinical investigation.

ROS1 Fusions

ROS1 is receptor tyrosine kinase (RTK) encoded by the ROS proto-oncogene 1, receptor tyrosine kinase (*ROS1*) gene, which was discovered by its homology to the v-ros oncogene in the UR2 avian sarcoma virus [3]. The first oncogenic *ROS1* fusion was unveiled in glioblastoma [4]. *ROS1* fusions were later discovered in lung cancer by phosphoproteomic analysis of NSCLC cell lines [5]. When a gene rearrangement occurs in *ROS1*, the extracellular domain (and sometimes the transmembrane domain) is replaced by sequences from an unrelated gene leading to constitutive activation of the ROS1 kinase domain [6]. The HCC78 NSCLC cell line harbors an *SLC34A2-ROS1* fusion and when treated with the ROS1/ALK/MET inhibitor crizotinib shows decreased cell viability [7]. When expressed ectopically in the basal ganglia of mice, it promotes the formation of tumors and a transgenic mouse model that expresses EZR-ROS1 induces lung adenocarcinomas in mice [8, 9]. Activation of downstream pathways (JAK/STAT, PI3K-AKT, RAS/MAPK) by FIG-ROS1 fusions, as well as response to treatment with kinase inhibitors, has been demonstrated in cholangiocarcinoma and glioblastoma [9, 10].

ROS1 fusions are most commonly identified in patient samples using fluorescence *in situ* hybridization (FISH) to demonstrate the presence of a chromosomal rearrangement within the ROS1 gene locus and/or polymerase chain reaction (PCR) or next-generation sequencing (NGS) to identify the translocation partner. Multiple 5' gene partners have been identified for *ROS1* fusions, including *TPM3*, *SDC4*, *LRIG3*, *SLC34A2*, *CD74*, *EZR*, and others [6, 11, 12]. In a case series of 13 Japanese patients, all fusions were associated with adenocarcinomas and were negative for *EGFR* and *KRAS* mutations [11]. Additionally, the presence of *ROS1* fusions has been associated with young age and minimal tobacco history [11]. In a study of 428 NSCLC tumor samples, ROS1 fusion events were identified in 1.2% of tumors [7]. Similar to Takeuchi et al., the patients in this cohort with *ROS1* fusions have low tobacco use histories. Interestingly the cohort had two patients with squamous cell histology, suggesting that this alteration, like *ALK* and *EGFR*, may not be limited to adenocarcinoma [13]. A third cohort of 18 patients (~2% of screened patients) reported by Bergethon et al. also demonstrated a younger median age and never-smoker status and all patients demonstrated adenocarcinoma histology [14]. It is notable, however, that some

sample groups were small, which makes extrapolation to the larger population of NSCLC difficult [11, 14]. Large mutation surveys have since demonstrated similar findings to these smaller studies; the Cancer Genome Atlas Research Network identified *ROS1* fusion events in 4 of 230 (1.7%) tumors and Pan et al. identified 11 *ROS1*-positive tumors in their Asian cohort of 1,139 patients [15•, 16].

The kinase domain of *ROS1* shares significant homology to *ALK*. As noted above, some *ALK* inhibitors also have demonstrated activity in cell lines with *ROS1* fusion proteins. Bergethon et al. demonstrated a dramatic tumor response following crizotinib treatment of a patient with a *ROS1* fusion (and importantly no *ALK* fusion) [14]. Ou et al. evaluated the safety and efficacy of crizotinib in patients with advanced *ROS1* fusion-positive NSCLC. Although this trial is still ongoing (NCT00585195), data from the first 25 evaluable patients demonstrated an objective response rate of 56%; progression-free survival and overall survival have not yet been reported [17]. At this time, multiple, additional studies are evaluating crizotinib as well as other drugs in treatment of patients with *ROS1* fusion-positive NSCLC (Table 1). Currently, there is no U.S. Food and Drug Administration (FDA)-approved treatment for *ROS1* rearrangements in NSCLC.

Similar to *ALK*+ patients (and *EGFR* mutation-positive patients), acquired resistance to inhibitors of *ROS1* has been demonstrated to occur through a secondary kinase domain mutation, G2032R, in *ROS1*. This *ROS1* mutation results in decreased crizotinib binding [18]. Preclinical models also suggest a role for wild-type *EGFR* signaling as another mechanism of acquired resistance [19].

RET Fusions

RET gene fusions have long been described in papillary thyroid carcinomas and radiation-associated thyroid cancers where inversions of chromosome 10 lead to oncogenic activity [20]. *RET* fusions also have been described in chronic myelomonocytic leukemia as the drivers of hematopoietic differentiation to monocytic/macrophage lineage and act in the *RAS* pathway [21]. In lung cancer, *RET* fusions first came to attention as a potential therapeutic target in a study of 1,528 surgical specimens [11]. *RET* fusions were identified by a *KIF5B* split FISH assays to identify alternative fusion partners of *KIF5B*, which is a known fusion partner of *ALK*. After identifying *RET* fusions in 12 samples. Additional investigation led to the identification of another *RET* partner, *CCDC6*, in two additional patients. In total, the frequency of *RET* rearrangements was found to be 0.9% in NSCLCs and 1.2% in the adenocarcinoma subgroup in this cohort [11]. Lipson et al. used NGS to identify clinically actionable genomic alterations in their study. After first identifying a *KIF5B-RET* fusion in their initial cohort, they screened 561 lung adenocarcinomas and identified 11 (2%) additional *KIFB-RET* gene fusions [22]. Both of these studies focused on populations of light or never smokers. Additionally, Lipson et al. found a higher frequency of *RET* fusions in their cohort of 405 Asian patients using NGS, compared with European patients [16]. Another study of 1,139 lung adenocarcinomas from a predominantly Asian population determined the frequency of *RET* fusions to be 1.3% by qRT-PCR; these were found predominantly in younger patients compared with non-*RET* fusion containing samples [16].

The downstream targets of RET activation are thought to be the Ras/Raf/MEK and JAK/STAT pathways [23]. There are at least four FDA-approved tyrosine kinase inhibitors that have activity on the RET tyrosine kinase, ponatinib, sunitinib, vandetanib, and cabozantinib [24–26]. Clinical trials are ongoing to determine the efficacy of these agents in populations of patients with RET fusions (Table 1); preliminary results from these trials are encouraging. Cabozantinib is a multikinase inhibitor and with potent activity against RET. Preliminary results from the first three patients of an ongoing clinical trial (NCT01639508) have demonstrated partial responses in two patients and stable disease in a third patient [26]. Vandetanib is an inhibitor of RET, vascular endothelial growth factor, and EGFR tyrosine kinases, which have demonstrated efficacy in treatment of thyroid cancers. Vandetanib has been shown to be effective in the treatment of RET positive lung cancer and is currently under evaluation in a phase 2 clinical trial for treatment of patients with advanced non-small cell lung cancer (NCT01823068) [27]. Additional clinical trials are currently ongoing for patients with RET gene fusions utilizing other multikinase inhibitors, such as lenvatinib (NCT01877083), dovitinib (NCT01831726), sunitinib (NCT01829217), and ponatinib (NCT01813734). No preclinical or clinical resistance mechanisms to RET inhibitors have yet been described.

***NTRK1* fusions**

Neurotrophic tyrosine kinase, receptor, type 1 (*NTRK1*) fusions are another class of gene rearrangements recently identified in NSCLC that shows potential as a therapeutic target. The *NTRK1* gene encodes the high affinity nerve growth factor receptor or TRKA protein RTK. Currently, two 5' gene fusion partners of *NTRK1* have been identified in tumor samples from NSCLC patients: *MPRIP* and *CD74*, were identified by NGS and FISH assays on tumor tissue from patients who were negative for *EGFR*, *KRAS*, *ALK*, and *ROS1* oncogenic alterations [28]. Of the 91 individual lung adenocarcinoma samples evaluated, 3 (3%) demonstrated evidence of an *NTRK1* gene rearrangement. Vaishnavi et al. reported that these fusion genes demonstrated constitutive TRKA kinase activity and induced tumor formation in nude mice. Fusions of *NTRK1* with other partners have been reported in both colorectal and thyroid cancers [29]. One lung adenocarcinoma patient with an *MPRIP-NTRK1* fusion was treated with crizotinib but only experienced a minor radiographic response and ultimately progressed after 3 months. The lack of a significant response in this patient was consistent with the modest activity of crizotinib as a TRKA inhibitor [28]. The Cancer Genome Atlas project failed to identify *NTRK1* fusions among a group of 230 lung adenocarcinomas [30]. Currently, there are at least three clinical trials of TRK inhibitors in NSCLC (Table 1). Importantly, one of these drugs, RXDX-101 demonstrated an objective tumor response in a colon cancer patient harboring a *TPM3-NTRK1* fusion [31].

MET

MET encodes the cMET RTK, which is typically activated by its cognate ligand, hepatocyte growth factor (HGF). MET gene amplification is estimated to occur in approximately 1–4% of NSCLC as measured by FISH analysis [15•, 32, 33•]. The exact percentage of patients may vary depending on the cutoff used to define gene amplification. Indeed, the precise definition of MET gene amplification appears to have implications for its use as a predictive

biomarker in cancer; recent results from the phase I trial of crizotinib in a cohort of NSCLC patients with *MET* gene amplification demonstrated a higher likelihood of benefit in patients with higher gene amplification [34].

As demonstrated in this review, there exist multiple mechanisms of activation of a proto-oncogene (fusions, point mutations, insertions/deletions, overexpression). Seo et al. identified *MET* exon 14 skipping in 3 samples from a cohort of 87 (3.4%) lung adenocarcinoma patients [30]. Similarly, The Cancer Genome Atlas project identified 10 of 230 lung adenocarcinoma (4.3%) samples with evidence of *MET* exon 14 skipping [15•]. In both cohorts, the *MET* exon 14 alteration occurred only in samples without other known oncogenes. Exon 14 of *MET* encodes the juxtamembrane domain, which regulates endocytosis and degradation of the receptor. Absence of this domain increases expression of *MET*, similar to *MET* gene amplification, leading to ligand-independent activation to downstream signaling and its oncogenic properties [35]. Treatment of cells expressing this abnormal *MET* receptor inhibits MAPK signaling and decreases cell viability. Thus, *MET* gene amplification and *MET* exon 14 skipping may collectively represent 7–8% of NSCLC and are a potential target for *MET* inhibitors.

FGFR1

The majority of targetable oncogenic drivers identified to date are found predominantly in tumors with adenocarcinoma histology. In an attempt to identify oncogenic drivers in squamous cell lung cancer, Weiss et al. screened 155 squamous cell lung cancers with single nucleotide polymorphism (SNP) arrays to uncover additional targetable genomic alterations. They identified fibroblast growth factor receptor 1 (*FGFR1*) gene amplification in 15 of 155 squamous cell lung cancers, but no *FGFR1* gene amplification in 77 lung adenocarcinomas [36]. Using FISH on an independent dataset the authors identified *FGFR1* amplification in 34 of 153 patients with squamous cell carcinoma. In both cohorts, all patients were current or former smokers. Heist et al. had similar results when they looked retrospectively at a cohort of 226 squamous cell lung cancer patients and found that 16% of them had amplifications in gene copy number to a ratio of 2.2 or greater [37].

FGFR1 is a member of a family of RTKs, including FGFR2, FGFR3, and FGFR4, and activates the phospholipase-Cy, RAF-MAPK, and PI3K/AKT pathways when triggered by ligand binding [38]. The oncogenic potential of this amplification also was demonstrated by two groups who showed that inhibition of FGFR1 in NSCLC-FGFR1 amplified cells decreased viability significantly [36, 39]. Further work has demonstrated that mRNA and protein levels may be better predictors of oncogenic potential than gene copy number as increased gene copy number did not always correlate with high expression or drug sensitivity [40•]. Clinical trials of several multikinase inhibitors with activity against FGFR1 are underway. Clinical trials of dovitinib, an FGFR1/multikinase inhibitor, have demonstrated promising results in breast cancer and renal cell carcinoma [40•, 41]. BGJ398, a pan FGFR inhibitor, has recently demonstrated promising results in a phase I trial: of 17 evaluable NSCLC patients with amplifications of *FGFR1*, the ORR was 45% at the data cutoff [42]. The final results from these and other studies of FGFR1 inhibitors are eagerly anticipated. In addition to *FGFR1* gene amplification, oncogenic fusions involving both

FGFR1 and *FGFR3* have been identified in lung cancer and also may predict for susceptibility to FGFR targeted therapies [43, 44]. Table 1 lists active studies of FGFR1 inhibitors.

HER2 Mutations

The human epidermal growth factor receptor 2 (HER2, *ERBB2*) first gained recognition for its importance in breast cancer as a prognostic and predictive marker [45, 46]. The *HER2* gene encodes a transmembrane protein that is a receptor tyrosine kinase in the ERBB family that, when activated, promote cellular proliferation, differentiation, migration, and apoptosis. Despite the demonstrated utility of HER2 protein expression-based treatment in breast and gastric cancers, HER2 targeting in lung cancer has not been proven effective when treatment is based on predictive biomarkers that measure protein expression [46–48]. Importantly, activating mutations within the tyrosine kinase domain of the *HER2* gene were discovered in a small subset of NSCLC patients. Arcila et al. evaluated 1,478 lung adenocarcinoma samples using mass spectrometry analysis (Sequenome) and found that of the group of patients negative for *EGFR* and *KRAS* mutations, 25 of 507 (4.9%) patients demonstrated *HER2* mutations [49]. The overall prevalence in the cohort of 1,478 was 1.8% and in-frame insertions in exon 20 accounted for the majority (96%) of these mutations [49]. Clinically, patients with *HER2* mutations were slightly younger and never smokers, but there were no differences between genders or in the prevalence between Asian and Caucasian patients. They also evaluated 104 squamous cell and six small cell carcinoma samples but found no *HER2* mutations. Buttitta et al. studied a European cohort of 407 lung adenocarcinomas for *HER2* mutations by PCR and direct sequencing methods and identified 9 (2.2%) mutations. They found a predominance of *HER2* mutations in patients whose adenocarcinoma had bronchoalveolar features; however, Arcila et al. did not observe this association. They did find a trend toward younger patients who were never smokers and a slightly higher prevalence in women, although none of these findings were significant [50]. Li et al. utilized direct sequencing in a cohort of 224 Asian lung adenocarcinoma patients and found 8 (3.6%) with *HER2* mutations. In this cohort, *HER2* mutations were associated with women and never smokers [51]. Overall, the group of patients harboring *HER2* mutations comprised <4% of patients with lung adenocarcinoma in these and other studies [33•, 49–51].

Evaluation of HER2-targeted agents in patients both within and outside of clinical trial has yielded significant findings. Interestingly, the positive studies have focused on *HER2* mutation analysis as a predictive biomarker. Afatinib, a potent ERBB family blocker, demonstrated an objective response in all three patients with *HER2* mutations [52]. In a retrospective analysis of 65 patients with stage IV NSCLC with *HER2* mutations, there was an overall response rate of 50% and disease control rate (DCR) of 82% using a variety of HER2-targeted therapies. Trastuzumab combined with chemotherapy or afatinib monotherapy were particularly effective (DCR of 96% and 100%, respectively), but progressive disease was observed in patients treated with lapatinib or masatinib [53]. Currently, there are multiple trials ongoing to identify effective agents that target HER2 mutations in NSCLC (Table 1).

BRAF Mutations and Fusions

Somatic *BRAF* (B1 homolog of the v-raf murine sarcoma viral oncogene) mutations are clinically important components in many types of cancers including thyroid, melanoma, and colorectal [54]. *BRAF* belongs to the RAF kinase family and is in the group of serine-threonine kinases that is important in the RAS/RAF/MAPK signaling pathway. Activating point mutations in *BRAF* result in constitutive activation of this pathway leading to cell growth and proliferation [52]. In NSCLC, the *BRAF* mutation rate is approximately 1–3% [33•, 55]. Chen et al. performed a meta-analysis of 10 studies and found *BRAF* mutations in 98 of 2,224 adenocarcinoma samples (4.04%) and 6 of 1,037 nonadenocarcinomas (0.58%) [56]. Across the studies, a number of different detection techniques were utilized to identify mutations. Within this study, the authors focused on the most common *BRAF* mutation: V600E (53.6%). They found that patients were more likely to be never smokers and that patients were more likely to have adenocarcinoma histology.

Early data on treatment of *BRAF* positive patients with *BRAF* inhibitors has demonstrated strong potential as a therapeutic strategy. A patient with stage IV NSCLC harboring a *BRAF* V600E demonstrated a complete response by PET to vemurafenib [57]. Early results from a phase II study of dabrafenib demonstrated an objective response rate of 54% among 17 NSCLC patients with a *BRAF* V600E mutation; progression-free survival and overall survival have not yet been reported [58]. Based on successful combination of *BRAF* and MEK inhibitors in melanoma patients with *BRAF* V600E, this study has a second arm that is currently still in process to determine the safety and efficacy of dabrafenib in combination with trametinib, a MEK inhibitor [58, 59].

A group investigating mucinous adenocarcinomas recently identified another genetic alteration involving *BRAF*. They identified a constitutively active *BRAF* fusion protein (*TRIM24-BRAF*), similar to the *BRAF* fusions seen in prostate cancer, gastric cancer and melanoma [60, 61]. They demonstrated not only that this aberrant protein activates the *BRAF* kinase but also that it is responsive to sorafenib, a commercially available RAF kinase inhibitor [60]. Clearly, these studies provide compelling results and additional clinical trials are ongoing to determine the clinical benefit of *BRAF* inhibitors in lung cancer patients (Table 1).

NRG1 Gene Fusions

The *NRG1* gene encodes the neuregulin protein, a ligand for ERBB3 (HER3) and ERBB4 (HER4) RTKs. Fernandez-Cuesta et al. utilized gene copy number and transcriptome sequencing analysis to evaluate 25 lung adenocarcinomas from never smokers without *EGFR* or *KRAS* mutations. This analysis yielded a novel gene fusion containing the 5' end of the *CD74* gene in frame with the 3' end of the *NRG1* gene, encoding the EGF-like domain [62]. Follow-up analysis using RT-PCR and FISH in a validation cohort of 102 never-smoker patients who were negative for other identified an additional four cases (3.9%) with *CD74-NRG1* fusions. In sum, all five cases were women with invasive mucinous adenocarcinoma. Another study focusing on invasive mucinous adenocarcinoma used whole transcriptome sequencing to identify 6 of 90 cases with *NRG1* fusions (one *SLC3A2*- and

five *CD74-NRG1*) [60]. As outlined above, this group also identified *BRAF* fusions and another potentially actionable fusion, *ERBB4* (HER4). *NRG1* gene rearrangements were previously identified in breast cancer with a reported prevalence of 4.7–6% of breast cancers and in head and neck cancers and may be a predictive biomarker for lapatinib sensitivity [63–65].

Unlike *ALK*, *ROS1*, *RET*, and *NTRK1* gene fusions where the kinase domain of the respective genes is activated by the fusion event, *NRG1* does not encode a kinase but the ligand for an RTK. Expression of CD74-NRG1 in cell leads to activation of HER2 and HER3 *in vitro*; similarly, all tumor samples harboring these fusions showed high expression and phosphorylation of HER2 and HER3. Thus, this gene fusion leads to high expression of the ligand, inducing an autocrine signaling loop in cancer cells. Inhibition of a cell line model using lapatinib and afatinib inhibited HER2 and HER3 signaling suggesting a possible clinical strategy for treatment of patients harboring these gene fusions [60]. Other potential strategies to target these novel fusions might include the use of HER3 monoclonal antibodies.

Conclusions

The landscape of personalized medicine is constantly evolving. Initially, we found that targeting molecular alterations in signal transduction pathways can generate significant improvements in patient outcomes. With the dramatic success of EGFR TKIs in patients with activating EGFR mutations researchers have demonstrated the value of targeting specific molecular pathways [66]. Treatment of ALK+ patients with crizotinib has lead to similar clinical improvements [67]. As a result of this success, clinical testing of *EGFR* and *ALK* genes are considered part of the standard of care in NSCLC.

Additionally, although age and smoking status often are associated with oncogenic drivers, as noted in many of the examples in this review, this may be more of a reflection of the biases in the sample population than a reflection of the population as a whole. In a study of sequential NSCLC surgical specimens, 40% of those tumors with EGFR mutations were in former or current smokers and there was no significant difference in ages between EGFR positive or negative patients [68]. Wakelee et al. found similar results when studying ALK-positive NSCLC patients. In their survey of 273 ALK+ patients, they found that 33% of patients had a >10 pack year history, 33% were former smokers, and 33% were never smokers. They also found that 52% of ALK-positive patients were aged 65 years or older at diagnosis, indicating that the stereotype of a never-smoker young patient may be imperfect and therefore should not be used to determine which patients will be screened [69].

Currently, both public and private ventures are working to identify additional targetable genetic alterations that may yield similar clinic benefit for patients. The Lung Cancer Mutation Consortium has sought to make mutation detection more efficient by using multiplex analysis to concurrently evaluate samples for genetic alterations. They have recently reported that in a screen for 10 different oncogenic drivers, 64% of lung adenocarcinomas samples screened contained an actionable oncogenic driver [33•]. The Cancer Genome Atlas Research Network recently published their results on the molecular

profiles of 230 resected lung adenocarcinomas. They used a pathway-based approach and were able to demonstrate that a striking 76% of lung adenocarcinomas had oncogenic activities that are drivers of the RTK/RAS/RAF activation pathway [15••]. Thus, although individual mutations might be rare, they add up to a significant number of patients who may benefit from targeted therapy. These studies compliment new clinical trial designs that are oncogene- or pathway-based, instead of cancer-type specific. Hopefully, by using multiplexed genetic analysis to pair patients with targeted therapeutic agents we will be able to maximize the evaluation of new drugs. This new approach supersedes traditional clinical trial design, which is difficult when considering mutations that occur less frequently due to the difficulties of recruiting a sufficient number of patients with a specific cancer type and specific genetic alteration. The unknown variable is whether targeting these mutations will always yield significant clinical benefit, because as we learned from the experience of *BRAF* mutations in melanoma and colon cancer, not all genetic alterations that are identifiable across cancer types respond similarly [70, 71].

Although the new landscape of personalized medicine challenges us to provide selective treatment to patients in smaller subgroups, we can meet this challenge by utilizing multiplexing analysis and new clinical trial designs that take advantage of common oncogenic pathways across tumor types.

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References and Recommended Reading

1. Siegel R, Ma J, Zou Z, Jemal A. Cancer statistics, 2014. *CA Cancer J Clin*. 2014 Jan-Feb;64(1):9–29. [PubMed: 24399786]
2. SEER Stat Fact Sheets: Lung and Bronchus Cancer. [Accessed Aug 14, 2014] Cancer Stat Facts Sheet. 2014. <http://seer.cancer.gov/statfacts/html/lungb.html>.
3. Birchmeier C, Birnbaum D, Waitches G, Fasano O, Wigler M. Characterization of an activated human *ros* gene. *Mol Cell Biol*. 1986 Sep; 6(9):3109–3116. [PubMed: 3785223]
4. Birchmeier C, Sharma S, Wigler M. Expression and rearrangement of the *ROS1* gene in human glioblastoma cells. *Proc Natl Acad Sci U S A*. 1987 Dec; 84(24):9270–9274. [PubMed: 2827175]
5. Rikova K, Guo A, Zeng Q, et al. Global survey of phosphotyrosine signaling identifies oncogenic kinases in lung cancer. *Cell*. 2007 Dec 14; 131(6):1190–1203. [PubMed: 18083107]
6. Davies KD, Doebele RC. Molecular pathways: *ROS1* fusion proteins in cancer. *Clin Cancer Res*. 2013 Aug 1; 19(15):4040–4045. [PubMed: 23719267]
7. Davies KD, Le AT, Theodoro MF, et al. Identifying and targeting *ROS1* gene fusions in non-small cell lung cancer. *Clin Cancer Res*. 2012 Sep 1; 18(17):4570–4579. [PubMed: 22919003]
8. Arai Y, Totoki Y, Takahashi H, et al. Mouse model for *ROS1*-rearranged lung cancer. *PLoS One*. 2013; 8(2):e56010. [PubMed: 23418494]
9. Charest A, Wilker EW, McLaughlin ME, et al. *ROS* fusion tyrosine kinase activates a SH2 domain-containing phosphatase-2/phosphatidylinositol 3-kinase/mammalian target of rapamycin signaling axis to form glioblastoma in mice. *Cancer Res*. 2006 Aug 1; 66(15):7473–7481. [PubMed: 16885344]
10. Gu TL, Deng X, Huang F, et al. Survey of tyrosine kinase signaling reveals *ROS* kinase fusions in human cholangiocarcinoma. *PLoS One*. 2011; 6(1):e15640. [PubMed: 21253578]

11. Takeuchi K, Soda M, Togashi Y, et al. RET, ROS1 and ALK fusions in lung cancer. *Nat Med*. 2012 Mar; 18(3):378–381. [PubMed: 22327623]
12. Le AT, Doebele RC. The democratization of the oncogene. *Cancer Discov*. 2014 Aug; 4(8):870–872. [PubMed: 25092743]
13. Dearden S, Stevens J, Wu YL, Blowers D. Mutation incidence and coincidence in non small-cell lung cancer: meta-analyses by ethnicity and histology (mutMap). *Ann Oncol*. 2013 Sep; 24(9):2371–2376. [PubMed: 23723294] Meta-analysis of 9 genes and their frequencies across different histologies and ethnic groups both individually and as overlapping mutations. Provides a broad view of mutational frequencies.
14. Bergeth K, Shaw AT, Ou SH, et al. ROS1 rearrangements define a unique molecular class of lung cancers. *J Clin Oncol*. 2012 Mar 10; 30(8):863–870. [PubMed: 22215748]
15. Cancer Genome Atlas Research N. Comprehensive molecular profiling of lung adenocarcinoma. *Nature*. 2014 Jul 31; 511(7511):543–550. [PubMed: 25079552] Molecular profiling of 230 lung adenocarcinomas to determine most frequently mutated genes and implicating RTK/RAS/RAF pathway activation in the majority of cases
16. Pan Y, Zhang Y, Li Y, et al. ALK, ROS1 and RET fusions in 1139 lung adenocarcinomas: a comprehensive study of common and fusion pattern-specific clinicopathologic, histologic and cytologic features. *Lung Cancer*. 2014 May; 84(2):121–126. [PubMed: 24629636]
17. Ou SH, Bang YJ, Camidge DR, et al. Efficacy and safety of crizotinib in patients with advanced ROS1-rearranged non-small cell lung cancer (NSCLC). *J Clin Oncol*. 2013; 31 suppl(5s) 2013 abstr 8032.
18. Awad MM, Katayama R, McTigue M, et al. Acquired resistance to crizotinib from a mutation in CD74-ROS1. *N Engl J Med*. 2013 Jun 20; 368(25):2395–2401. [PubMed: 23724914]
19. Davies KD, Mahale S, Astling DP, et al. Resistance to ROS1 inhibition mediated by EGFR pathway activation in non-small cell lung cancer. *PLoS One*. 2013; 8(12):e82236. [PubMed: 24349229]
20. Pierotti MA, Bongarzone I, Borello MG, Greco A, Pilotti S, Sozzi G. Cytogenetics and molecular genetics of carcinomas arising from thyroid epithelial follicular cells. *Genes Chromosomes Cancer*. 1996 May; 16(1):1–14. [PubMed: 9162191]
21. Ballerini P, Struski S, Cresson C, et al. RET fusion genes are associated with chronic myelomonocytic leukemia and enhance monocytic differentiation. *Leukemia*. 2012 Nov; 26(11):2384–2389. [PubMed: 22513837]
22. Lipson D, Capelletti M, Yelensky R, et al. Identification of new ALK and RET gene fusions from colorectal and lung cancer biopsies. *Nat Med*. 2012 Mar; 18(3):382–384. [PubMed: 22327622]
23. Qian Y, Chai S, Liang Z, et al. KIF5B-RET fusion kinase promotes cell growth by multilevel activation of STAT3 in lung cancer. *Mol Cancer*. 2014 Jul 21; 13(1):176. [PubMed: 25047660]
24. Mologni L, Redaelli S, Morandi A, Plaza-Menacho I, Gambacorti-Passerini C. Ponatinib is a potent inhibitor of wild-type and drug-resistant gatekeeper mutant RET kinase. *Mol Cell Endocrinol*. 2013 Sep 5; 377(1–2):1–6. [PubMed: 23811235]
25. Saito M, Ishigame T, Tsuta K, Kumamoto K, Imai T, Kohno T. A mouse model of KIF5B-RET fusion-dependent lung tumorigenesis. *Carcinogenesis*. 2014 Jul 26.
26. Drilon A, Wang L, Hasanovic A, et al. Response to Cabozantinib in patients with RET fusion-positive lung adenocarcinomas. *Cancer Discov*. 2013 Jun; 3(6):630–635. [PubMed: 23533264]
27. Gautschi O, Zander T, Keller FA, et al. A patient with lung adenocarcinoma and RET fusion treated with vandetanib. *J Thorac Oncol*. 2013 May; 8(5):e43–e44. [PubMed: 23584301]
28. Vaishnavi A, Capelletti M, Le AT, et al. Oncogenic and drug-sensitive NTRK1 rearrangements in lung cancer. *Nat Med*. 2013 Nov; 19(11):1469–1472. [PubMed: 24162815]
29. Alberti L, Carniti C, Miranda C, Roccato E, Pierotti MA. RET and NTRK1 proto-oncogenes in human diseases. *J Cell Physiol*. 2003 May; 195(2):168–186. [PubMed: 12652644]
30. Seo JS, Ju YS, Lee WC, et al. The transcriptional landscape and mutational profile of lung adenocarcinoma. *Genome Res*. 2012 Nov; 22(11):2109–2119. [PubMed: 22975805]
31. De Braud FG, Pilla L, Nigam M, et al. Phase 1 open label, dose escalation study of RXDX101, an oral pan-trk, ROS1, and ALK inhibitor, in patients with advanced solid tumors with relevant molecular alterations. *J Clin Oncol*. 2014; 32 suppl(5s) abstr 2502.

32. Cappuzzo F, Marchetti A, Skokan M, et al. Increased MET gene copy number negatively affects survival of surgically resected non-small-cell lung cancer patients. *J Clin Oncol*. 2009 Apr 1; 27(10):1667–1674. [PubMed: 19255323]
33. Kris MG, Johnson BE, Berry LD, et al. Using multiplexed assays of oncogenic drivers in lung cancers to select targeted drugs. *JAMA*. 2014 May 21; 311(19):1998–2006. [PubMed: 24846037] This study found actionable driver mutations in 64% of lung adenocarcinomas demonstrating that by combining oncogenic mutations it is possible to find potential therapeutic targets for the majority of patients.
34. Camidge DR, Ou SH, Shapiro G, et al. Efficacy and safety of crizotinib in patients with advanced c-MET-amplified non-small cell lung cancer (NSCLC). *J Clin Oncol*. 2014; 32 suppl(5s) abstr 8001.
35. Kong-Beltran M, Seshagiri S, Zha J, et al. Somatic mutations lead to an oncogenic deletion of met in lung cancer. *Cancer Res*. 2006 Jan 1; 66(1):283–289. [PubMed: 16397241]
36. Weiss J, Sos ML, Seidel D, et al. Frequent and focal FGFR1 amplification associates with therapeutically tractable FGFR1 dependency in squamous cell lung cancer. *Sci Transl Med*. 2010 Dec 15.2(62):62ra93.
37. Heist RS, Mino-Kenudson M, Sequist LV, et al. FGFR1 amplification in squamous cell carcinoma of the lung. *J Thorac Oncol*. 2012 Dec; 7(12):1775–1780. [PubMed: 23154548]
38. Knights V, Cook SJ. De-regulated FGF receptors as therapeutic targets in cancer. *Pharmacol Ther*. 2010 Jan; 125(1):105–117. [PubMed: 19874848]
39. Dutt A, Ramos AH, Hammerman PS, et al. Inhibitor-sensitive FGFR1 amplification in human non-small cell lung cancer. *PLoS One*. 2011; 6(6):e20351. [PubMed: 21666749]
40. Wynes MW, Hinz TK, Gao D, et al. FGFR1 mRNA and protein expression, not gene copy number, predict FGFR TKI sensitivity across all lung cancer histologies. *Clin Cancer Res*. 2014 Jun 15; 20(12):3299–3309. [PubMed: 24771645] This study highlights the importance of utilizing the right biomarker to determine treatment sensitivity.
41. Andre F, Bachelot T, Campone M, et al. Targeting FGFR with dovitinib (TKI258): preclinical and clinical data in breast cancer. *Clin Cancer Res*. 2013 Jul 1; 19(13):3693–3702. [PubMed: 23658459]
42. Nogova L, Sequist LV, Cassier PA, et al. Targeting FGFR1-amplified lung squamous cell carcinoma with the selective pan-FGFR inhibitor BGJ398. *J Clin Oncol*. 2014; 32 suppl(5s) abstr 8034.
43. Wang R, Wang L, Li Y, et al. FGFR1/3 Tyrosine Kinase Fusions Define a Unique Molecular Subtype of Non-Small Cell Lung Cancer. *Clin Cancer Res*. 2014 Aug 1; 20(15):4107–4114. [PubMed: 24850843]
44. Wu YM, Su F, Kalyana-Sundaram S, et al. Identification of targetable FGFR gene fusions in diverse cancers. *Cancer Discov*. 2013 Jun; 3(6):636–647. [PubMed: 23558953]
45. Berger MS, Locher GW, Saurer S, et al. Correlation of c-erbB-2 gene amplification and protein expression in human breast carcinoma with nodal status and nuclear grading. *Cancer Res*. 1988 Mar 1; 48(5):1238–1243. [PubMed: 2893663]
46. Pegram MD, Lipton A, Hayes DF, et al. Phase II study of receptor-enhanced chemosensitivity using recombinant humanized anti-p185HER2/neu monoclonal antibody plus cisplatin in patients with HER2/neu-overexpressing metastatic breast cancer refractory to chemotherapy treatment. *J Clin Oncol*. 1998 Aug; 16(8):2659–2671. [PubMed: 9704716]
47. Bang YJ, Van Cutsem E, Feyereislova A, et al. Trastuzumab in combination with chemotherapy versus chemotherapy alone for treatment of HER2-positive advanced gastric or gastro-oesophageal junction cancer (ToGA): a phase 3, open-label, randomised controlled trial. *Lancet*. 2010 Aug 28; 376(9742):687–697. [PubMed: 20728210]
48. Lara PN Jr, Laptalo L, Longmate J, et al. Trastuzumab plus docetaxel in HER2/neu-positive non-small-cell lung cancer: a California Cancer Consortium screening and phase II trial. *Clin Lung Cancer*. 2004 Jan; 5(4):231–236. [PubMed: 14967075]
49. Arcila ME, Chaft JE, Nafa K, et al. Prevalence, clinicopathologic associations, and molecular spectrum of ERBB2 (HER2) tyrosine kinase mutations in lung adenocarcinomas. *Clin Cancer Res*. 2012 Sep 15; 18(18):4910–4918. [PubMed: 22761469]

50. Buttitta F, Barassi F, Fresu G, et al. Mutational analysis of the HER2 gene in lung tumors from Caucasian patients: mutations are mainly present in adenocarcinomas with bronchioloalveolar features. *Int J Cancer*. 2006 Dec 1; 119(11):2586–2591. [PubMed: 16988931]
51. Li C, Sun Y, Fang R, et al. Lung adenocarcinomas with HER2-activating mutations are associated with distinct clinical features and HER2/EGFR copy number gains. *J Thorac Oncol*. 2012 Jan; 7(1):85–89. [PubMed: 22071781]
52. De Greve J, Teugels E, Geers C, et al. Clinical activity of afatinib (BIBW 2992) in patients with lung adenocarcinoma with mutations in the kinase domain of HER2/neu. *Lung Cancer*. 2012 Apr; 76(1):123–127. [PubMed: 22325357]
53. Mazieres J, Peters S, Lepage B, et al. Lung cancer that harbors an HER2 mutation: epidemiologic characteristics and therapeutic perspectives. *J Clin Oncol*. 2013 Jun 1; 31(16):1997–2003. [PubMed: 23610105]
54. Davies H, Bignell GR, Cox C, et al. Mutations of the BRAF gene in human cancer. *Nature*. 2002 Jun 27; 417(6892):949–954. [PubMed: 12068308]
55. Chen Q, Xia C, Deng Y, et al. Immunohistochemistry as a quick screening method for clinical detection of BRAF(V600E) mutation in melanoma patients. *Tumour Biol*. 2014 Jun; 35(6):5727–5733. [PubMed: 24563339]
56. Chen D, Zhang LQ, Huang JF, et al. BRAF Mutations in Patients with Non-Small Cell Lung Cancer: A Systematic Review and Meta-Analysis. *PLoS One*. 2014; 9(6):e101354. [PubMed: 24979348]
57. Peters S, Michielin O, Zimmermann S. Dramatic response induced by vemurafenib in a BRAF V600E-mutated lung adenocarcinoma. *J Clin Oncol*. 2013 Jul 10; 31(20):e341–e344. [PubMed: 23733758]
58. Planchard D, Mazieres J, Riely GJ, et al. Interim results of phase II study BRF113928 of dabrafenib in BRAF V600E mutation-positive non-small cell lung cancer (NSCLC) patients. *J Clin Oncol*. 2013; (suppl) abstr 8009.
59. Ribas A, Gonzalez R, Pavlick A, et al. Combination of vemurafenib and cobimetinib in patients with advanced BRAF(V600)-mutated melanoma: a phase 1b study. *Lancet Oncol*. 2014 Aug; 15(9):954–965. [PubMed: 25037139]
60. Nakaoku T, Tsuta K, Ichikawa H, et al. Druggable oncogene fusions in invasive mucinous lung adenocarcinoma. *Clin Cancer Res*. 2014 Jun 15; 20(12):3087–3093. [PubMed: 24727320]
61. Palanisamy N, Ateeq B, Kalyana-Sundaram S, et al. Rearrangements of the RAF kinase pathway in prostate cancer, gastric cancer and melanoma. *Nat Med*. 2010 Jul; 16(7):793–798. [PubMed: 20526349]
62. Fernandez-Cuesta L, Plenker D, Osada H, et al. CD74-NRG1 Fusions in Lung Adenocarcinoma. *Cancer Discov*. 2014 Apr; 4(4):415–422. [PubMed: 24469108]
63. Huang HE, Chin SF, Ginestier C, et al. A recurrent chromosome breakpoint in breast cancer at the NRG1/neuregulin 1/hereregulin gene. *Cancer Res*. 2004 Oct 1; 64(19):6840–6844. [PubMed: 15466169]
64. Prentice LM, Shadeo A, Lestou VS, et al. NRG1 gene rearrangements in clinical breast cancer: identification of an adjacent novel amplicon associated with poor prognosis. *Oncogene*. 2005 Nov 10; 24(49):7281–7289. [PubMed: 16027731]
65. Wilson TR, Lee DY, Berry L, Shames DS, Settleman J. Neuregulin-1-mediated autocrine signaling underlies sensitivity to HER2 kinase inhibitors in a subset of human cancers. *Cancer Cell*. 2011 Aug 16; 20(2):158–172. [PubMed: 21840482]
66. Mok TS, Wu YL, Thongprasert S, et al. Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma. *N Engl J Med*. 2009 Sep 3; 361(10):947–957. [PubMed: 19692680]
67. Shaw AT, Kim DW, Nakagawa K, et al. Crizotinib versus chemotherapy in advanced ALK-positive lung cancer. *N Engl J Med*. 2013 Jun 20; 368(25):2385–2394. [PubMed: 23724913]
68. D'Angelo SP, Janjigian YY, Ahye N, et al. Distinct clinical course of EGFR-mutant resected lung cancers: results of testing of 1118 surgical specimens and effects of adjuvant gefitinib and erlotinib. *J Thorac Oncol*. 2012 Dec; 7(12):1815–1822. [PubMed: 23154553]
69. Wakelee HA, M S, Zhang J, et al. Description of ALK+ NSCLC patient characteristics and ALK testing patterns. *J Clin Oncol*. 2014; 32 suppl(5s) abstr 8062.

70. Chapman PB, Hauschild A, Robert C, et al. Improved survival with vemurafenib in melanoma with BRAF V600E mutation. *N Engl J Med*. 2011 Jun 30; 364(26):2507–2516. [PubMed: 21639808]
71. Prahallad A, Sun C, Huang S, et al. Unresponsiveness of colon cancer to BRAF(V600E) inhibition through feedback activation of EGFR. *Nature*. 2012 Mar 1; 483(7387):100–103. [PubMed: 22281684]

Table 1

Characteristics of targetable mutations in NSCLC and active clinical trials

Gene	Frequency (%) [*]	Predominant histology	Mechanism	Detection method	Clinical trials
<i>ROS1</i> [7,4,15,16]	1–2%	AdenoCA	Fusion	FISH, NGS, PCR	NCT01945021 NCT02186821 NCT02183870 NCT02097810 NCT01970865 NCT01964157 NCT02034981 NCT01922583
<i>RET</i> [11,16,22]	1.2–2%	AdenoCA	Fusion	FISH, NGS, PCR	NCT01813734 NCT01639508 NCT01823068 NCT01829217 NCT01877083 NCT01831726 NCT01582191 NCT01922583 NCT02029001
<i>NRK1</i> [28]	<0.5%	AdenoCA	Fusion	FISH, NGS, PCR	NCT02048488 NCT02097810 NCT01804530
<i>MET</i> [15,32,33]	1–4%	AdenoCA	Gene amplification, (exon 14 splicing)	IHC, PCR, FISH	NCT02132598 NCT01610336 NCT01911507 NCT01324479 NCT02154490 NCT01039948 NCT00585195 NCT01014936 NCT02055066 NCT01472016 NCT01253707 NCT01468922 NCT01773018

Gene	Frequency (%) [*]	Predominant histology	Mechanism	Detection method	Clinical trials
<i>FGFR</i> [36,37]	10–20%	SCC	Gene amplification, (Fusion)	SNP array, FISH, IHC, qPCR	NCT01831726 NCT01935336 NCT02160041 NCT01004224 NCT01948297 NCT01795768 NCT01283945 NCT02109016 NCT01976741 NCT02052778 NCT01868022 NCT02154490 NCT01676714 NCT02133157 NCT02117167
<i>HER2</i> [33,49–51]	1–3%	AdenoCA	Insertion/deletion	PCR, direct sequencing, mass spectrometry	NCT01922583 NCT02029001 NCT01827267 NCT01542437 NCT02183883 NCT01148849 NCT01526473 NCT02117167 NCT01306045
<i>BRAF</i> [33,55]	1–3%	AdenoCA	Point mutation (Fusion)	PCR, direct sequencing, MALDI-TOF, mass spec, SSCA, HRMA	NCT01531361 NCT01306045 NCT01922583 NCT02029001 NCT01336634 NCT02012231
<i>NRG1</i> [62]	3.9%	Mucinous AdenoCA	Fusion	RT-PCR, FISH, gene copy number, transcriptome sequencing	None

AdenoCA, adenocarcinoma; FISH, fluorescence in situ hybridization; NGS, next generation sequencing; PCR, polymerase chain reaction; IHC, immunohistochemistry; SNP, single nucleotide polymorphism; qPCR, quantitative PCR; MALDI-TOF, matrix-assisted laser desorption/ionization time of flight; Mass spec, mass spectrometry; SSCA, single strand conformational analysis; HRMA, high-resolution melting analysis; RT-PCR, reverse transcriptase PCR.

^{*} Frequencies listed are derived from individual studies with different cohort characteristics and therefore are not comparable across mutation type.