Emerging Biomarkers in Personalized Therapy of Lung Cancer

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Abstract The two clinically validated and Food and Drug Administration approved lung cancer predictive biomarkers (epidermal growth factor receptor mutations and anaplastic lymphoma kinase (*ALK*) translocations) occur in only about 20 % of lung adenocarcinomas and acquired resistance develops to first generation drugs. Several other oncogenic drivers for lung adenocarcinoma have emerged as potentially druggable targets with new predictive biomarkers. Oncologists are requesting testing for *ROS*1 translocations which predict susceptibility to crizotinib, already approved for *ALK* positive lung cancers. Other potential biomarkers which are currently undergoing clinical trials are RET, MET, HER2 and BRAF. Detection of these biomarkers includes fluorescent in situ hybridization and/or reverse transcriptase polymerase chain reaction (ROS1, RET, HER2), mutation analysis (*BRAF*) and immunohistochemistry (MET). Screening by immunohistochemistry may be useful for some biomarkers (ROS1, BRAF). Targeted next generation sequencing techniques may be useful as well. These five biomarkers are under consideration for inclusion in revised lung cancer biomarker guidelines by the College of American Pathologists, International Association for the Study of Lung Cancer and Association for Molecular Pathology.

Keywords ROS1 • RET • MET • HER2 • BRAF • Multikinase inhibitors • Fluorescent in situ hybridization • Crizotinib • Reverse transcriptase polymerase chain reaction • Immunohistochemistry

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1 Introduction

Two predictive biomarkers for personalized therapy of non-small cell lung cancers (NSCLC) have been well validated in clinical trials and approved by the Federal Drug Administration (FDA): epidermal growth factor receptor (EGFR) mutations and anaplastic lymphoma kinase (ALK) translocations [1]. These two biomarkers have been the subject of the first lung cancer biomarkers guidelines from the College of American Pathologists (CAP), International Association for the Study of Lung Cancer (IASLC) and Association for Molecular Pathology (AMP) [2] as well as the CAP Lung Cancer Biomarker Reporting Template [3].

The frequency of EGFR mutations found in non-small cell lung cancers (NSCLC), more specifically in adenocarcinomas, ranges from about 15 % of whites and Hispanics to about 19 % of African Americans to about 30 % of Asian patients [4–7]. ALK translocations creating fusion genes occur in about 4–5 % of adenocarcinomas [8–12]. Lung cancers that initially respond to first generation EGFR TKIs or to crizotinib eventually develop drug resistance and relapse, typically within a year [13–19]. Since about 80 % of adenocarcinomas lack *EGFR* mutations or *ALK* translocations and since lung cancers with these abnormalities develop acquired resistance to current therapies, there has been a robust search for additional oncogenic drivers in lung cancers that might be actionable. Investigations have not yet discovered drugs that target *KRAS*, the most frequent oncogenic driver in lung adenocarcinomas, occurring in about 30 % of cases [1, 2]. Oncogenic drivers have not yet been identified in a substantial number of lung adenocarcinomas and, of the additional drivers that have been identified, investigations of several are sufficiently advanced that they are being considered for revisions to the CAP/IASLC/AMP lung cancer biomarker guidelines and CAP lung cancer biomarker reporting template (See Fig. 1).

2 ROS1

Chromosomal rearrangements of the receptor tyrosine kinase gene c-ros oncogene 1 (*ROS1*) are found in approximately 1–2 % of lung cancers with adenocarcinoma histology, or about 2000–4000 new cases of ROS1 positive lung cancer each year in the United States [20–24]. ROS1 has considerable amino acid homology with ALK [25]. In 2012, Bergethon et al. [20] reported sensitivity of a *ROS*1 positive lung cancer cell line and *ROS*1 transfected cell lines to the small molecule multikinase inhibitor crizotinib. They also reported a near complete response of a *ROS*1 positive lung cancer to crizotinib in a single patient enrolled in an expansion cohort of an early phase study [20]. In an expansion of the PROFILE 1001 study, Shaw et al. [26] reported one complete response, six partial responses and four stable disease in thirteen patients with *ROS*1 positive lung cancers at 8 weeks of treatment with crizotinib. These observations indicating *ROS*1 positive lung adenocarcinomas might respond to crizotinib, a drug that already had FDA approval for treatment of

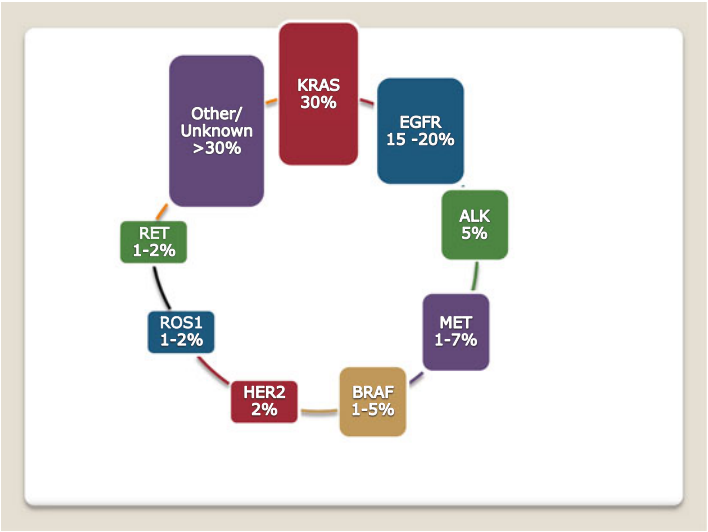

Fig. 1 Diagram showing the actionable and potentially actionable biomarkers in lung adenocarcinoma. *KRAS* mutation is the most common oncogenic driver, but no drugs specifically targeted to *KRAS* mutation are yet available. *EGFR* mutation and *ALK* translocation are clinically validated as predictive biomarkers for FDA approved TKI therapy. Emerging as biomarkers currently in clinical trials at this time are *ROS*1, *RET*, *MET*, *Her*2 and *BRAF*

ALK positive lung cancers, produced requests for ROS1 biomarker testing by medical oncologists for lung adenocarcinomas. Typically, this has been as part of an algorithm or after adenocarcinomas were reported negative for EGFR and ALK. As a result, ROS1 has moved to the forefront of new biomarkers for lung cancer.

Similar to *ALK* rearrangements, *ROS1* rearrangements with any of several fusion partners result in oncogenic kinase activation and the resultant oncogenic fusion kinase is susceptible to the multikinase inhibitor crizotinib [24, 27–31]. *ROS*1 positive adenocarcinomas share histologic and demographic features with *ALK* positive adenocarcinomas. *ROS*1 translocations tend to occur in adenocarcinomas with solid, papillary, cribriform or signet ring cell histologic patterns, tend to produce mucin and tend to arise in patients who are younger and never smokers. There are many exceptions to these general tendencies. As with other oncogenic drivers identified in lung adenocarcinomas, ROS1 translocation most often excludes the presence of other oncogenic drivers in the same tumor [20, 21, 28, 32–36].

Like *ALK* rearrangements, *ROS1* rearrangements can be detected by a break-apart fluorescent in situ hybridization (FISH) probe that is not dependent on the specific fusion partner [20, 22, 37, 38]. Specific fusion partners are detected by reverse transcriptase polymerase chain reaction (RT-PCR), including *CD74-ROS1, SDC4-ROS1, EZR-ROS1, SLC34A22-ROS1* and *FIG-ROS1* [20, 21, 29, 31, 34, 38–41]. Immunohistochemistry (IHC) can be used to screen for ROS1 positivity which can then be confirmed by FISH. IHC is performed on formalin-fixed, paraffin-embedded

sections using clone D4D6 from Cell Signaling Technology. As a screening tool, IHC is reported to be highly sensitive (100 %) for ROS1 positive lung cancers confirmed by FISH and/or RT-PCR with strong diffuse staining. False positive immunostaining is reported to occur in some *ROS*1 negative lung cancers with considerable variability depending on the study [35, 37, 38, 42].

As with *ALK* positive adenocarcinomas, acquired resistance to crizotinib has been observed in *ROS*1 positive adenocarcinomas. Acquired resistance of a ROS1 positive lung cancer to crizotinib has been reported with a proposed mechanism of EGFR pathway activation [43] and, in another case, due to a mutation in *CD74-ROS*1 [44]. Therefore, similar to the situation with other oncogenic drivers of lung cancers, new drugs are under investigation for inhibiting ROS1. Davare et al. [45] reported preclinical studies which demonstrated that foretinib is a potent ROS1 inhibitor.

3 RET

The rearranged during transfection (*RET*) gene encodes for the RET receptor tyrosine kinase. Chromosomal rearrangements of the *RET* gene result in an oncogenic fusion kinase in about 1–2 % of lung cancers with adenocarcinoma histology. The majority are *KIF5B-RET* fusion genes with a lesser number of *CCDC6-RET, NCOA4* and *TRIM33* fusion genes reported [27, 34, 46–55]. Preclinical studies have reported that RET-positive lung cancer cell lines are sensitive to the multikinase inhibitors vandetanib, sunitinib, and sorafenib [56, 57]. One patient with *RET* positive advanced adenocarcinoma has been reported to respond to vandetanib [58]. Preliminary results from a phase II trial of the multikinase inhibitor cabozantinib were partial responses in two of three patients and stable disease in the third patient [54]. Therefore, oncologists may order *RET* tests for lung adenocarcinomas for possible enrollment of a patient in a clinical trial or RET may be detected in a lung cancer using next generation sequencing techniques.

Translocations of *RET* which result in oncogenic fusion kinases in lung adenocarcinomas have a tendency to occur in the same demographic and histologic groups as the reported tendencies for oncogenic fusion kinases from ROS1 and ALK translocations. Patients tend to be younger and never smokers and the adenocarcinomas tend to have solid, papillary and lepidic patterns and more often produce mucin. As with *ALK* and *ROS*1 positive adenocarcinomas, there are many exceptions to these general histologic and demographic tendencies for *RET* positive adenocarcinomas. Also, identification of a *RET* translocation usually excludes the presence of other oncogenic drivers such as *EGFR*, *ALK* and *ROS*1 in the same cancer [34, 48, 52, 54, 55].

RET translocations may be detected by FISH, by RT-PCR or by next generation sequencing [34, 48, 52, 54, 55, 59]. Immunohistochemistry for RET has had variable results and, currently, is not popular for identification of *RET* positive lung adenocarcinomas [52, 59].

4 MET

The MNNG-HOS transforming (*MET*) gene encodes a receptor tyrosine kinase and binding of its ligand hepatocyte growth factor (HGF) causes a conformational change in the MET receptor that facilitates receptor activation. *MET* can be activated in lung cancers by amplification and/or overexpression [60–67]. About 18 % of cases of acquired resistance to EGFR TKIs are associated with overexpression and/or amplification of *MET* or HGF, but prevalence of *MET* amplification in NSCLC patients who have not received treatment is 1–7 % [68].

Onartuzumab (MetMAb) is a recombinant, humanized, monovalent monoclonal antibody that targets MET [69]. In a phase II study patients with previously treated NSCLC were evaluated for therapy with onartuzumab plus erlotinib versus placebo plus erlotinib [70]. Patient lung cancer samples were classified as positive for MET expression or negative for MET expression by IHC using a cut-off of 50 % of malignant cells with moderate and/or strong staining intensity for classification as MET positive. The combination of onartuzumab and erlotinib resulted in improved progression free survival (PFS) and overall survival (OS) compared to placebo plus erlotinib in MET positive cases whereas the opposite was true in MET negative cases. Therefore, this IHC test provides the biomarker for MET treatment in this setting and is being considered as a companion diagnostic for onartuzumab in combination with erlotinib for treatment of lung cancer [71]. The phase II study is being followed by the MetLung phase III study [72].

ARQ 197 or tivantinib is a TKI that inhibits MET. The MARQUEE (**M**et Inhibitor **ARQ** 197 pl**u**s **E**rlotinib vs. **E**rlotinib plus placebo in NSCLC) phase III trial of tavantinib plus erlotinib in previously treated patients with locally advanced or metastatic non-squamous NSCLC was stopped not meet its primary endpoint of improved overall survival [73, 74]. Cabozantinib and ficlatuzumab, an anti-HGF monoclonal antibody, have undergone investigation in clinical trials for lung cancer combined with EGFR TKIs as well [75]. None of these drugs is currently approved for lung cancer therapy.

5 HER 2

HER2/ERBB2/NEU is a receptor tyrosine kinase of the epidermal growth factor family. Amplification or overexpression of HER2 is well known as a biomarker that predicts breast cancer response to targeted therapies. *HER*2 activation in lung cancer is associated with mutations, mostly insertions in exon 20, which are independent of *HER2* gene amplification. These mutations are not seen in breast cancer. *HER*2 mutations are found in 2 % of lung adenocarcinomas. *HER*2 mutations are more prevalent in lung adenocarcinomas from patients who are never smokers and perhaps are more common in Asians and women. Adenocarcinomas with *HER*2 mutations generally lack other oncogenic drivers such as *EGFR*, *ALK* and *KRAS* [76–83].

Clinical trials in patients with NSCLC that have *HER*2 mutations have shown promising early results for therapy with afatinib [83, 84], trastuzumab [83], dacomitinib [85, 86] and neratinib plus temsirolimus [87, 88]. Therefore, detection of *HER*2 mutations is a potential biomarker for a small subset of lung adenocarcinomas.

HER2 expression in lung cancers by IHC has not yet proven to be a successful biomarker for selecting patients for therapy [89]. *HER*2 gene amplification is found in approximately 2 % of NSCLCs identified by FISH using the criteria for HER2 amplification in breast cancer [90]. Grob et al. [91] detected *HER*2 amplification by FISH in 3 % of NSCLC, overwhelmingly adenocarcinomas, with high-level amplification in 2 %. They also reported that HER2 amplification in lung cancer may be heterogeneous, thus impacting the outcomes of trastuzumab or other HER2 therapies based on *HER*2 amplification. *HER*2 amplification also sometimes plays a role in acquired resistance to EGFR TKIs in lung cancer patients who initially respond to these therapies [92].

6 BRAF

The *BRAF* gene encodes for a nonreceptor serine/threonine kinase that is activated downstream of the Ras protein. About 50 % of melanomas have *BRAF* mutations which activate the BRAF kinase and increase phosphorylation of downstream targets, particularly MEK, and about 80–90 % are V600E mutations. The FDA has approved vemurafenib for the treatment of *BRAF V600E* mutation-positive, inoperable or metastatic melanoma [93, 94] and approved the cobas 4800 BRAF V600 Mutation Test as the companion diagnostic for the biomarker [95]. IHC using the primary mouse monoclonal antibody VE1, specific for BRAF p.V600E has been studied as a screening tool for the *BRAF V600E* mutation [96–98]. Dabrafenib, a mutant-*BRAF* kinase inhibitor [99], and trametinib, a MEK inhibitor [100], have also been approved for treatment of *BRAF V600E* positive unresectable or metastatic melanoma.

BRAF mutations occur in about 1–5 % of lung cancers. In contrast to melanomas, *V600E* mutations account for 50–60 % of these mutations and non-*V600E* mutations account for the remainder. With few exceptions, *BRAF* positive lung cancers are adenocarcinomas and, in some series, patients are more likely to be current or former smokers [101–105]. Marchetti et al. [103] reported that *V600E* mutations occurred more frequently in women and never smokers and were associated with micropapillary pattern whereas non-*V600E* mutations occurred in smokers.

Cases have been reported of *BRAF V600E* mutated lung adenocarcinomas which responded to vemurafenib [106–108], whereas a *BRAF* G469L mutated lung adenocarcinoma did not [109] which anecdotally suggests that *BRAF V600E* mutation is a predictive biomarker for therapy of lung adenocarcinoma with vemurafenib. Two patients with BRAF V600E mutated lung NSCLC, at least one an adenocarcinoma, are reported to have had a partial responses to dabrafenib [110, 111]. In these cases, patients have developed acquired resistance similar to what is observed with targeted

therapies with the other biomarkers. Clinical trials with vemurafenib [94], dabrafenib [99] and trametinib [100] will hopefully validate these therapies for BRAF V600E mutated lung NSCLC.

Testing for *BRAF V600* mutations can be done by Sanger sequencing and various molecular techniques. As previously noted, the cobas 4800 BRAF V600 Mutation Test has been approved by the FDA as the companion diagnostic for *BRAF V600E* testing for vemurafenib therapy in melanoma [95]. *BRAF V600* mutations can be detected with targeted next generation sequencing [112, 113]. IHC using the aforementioned VE1 antibody has also been reported as a successful screening tool for BRAF V600E mutation in lung adenocarcinomas [114, 115].

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