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Detection of crizotinib-sensitive lung adenocarcinomas with *MET*, *ALK* and *ROS1* genomic alterations via comprehensive genomic profiling

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Abstract

Introduction—Crizotinib is an oral multitargeted tyrosine kinase inhibitor (TKI) with activity against lung cancers driven by *ALK*-rearrangements, *ROS1*-rearrangements and *MET*-amplification. Comprehensive genomic profiling (CGP) based on clinical next generation sequencing (NGS) can detect crizotinib-sensitive genomic changes. We describe use of CGP to identify tumors responsive to crizotinib.

Methods—Retrospective review of representative lung adenocarcinomas treated with crizotinib and assayed with a clinical NGS assay.

Results—We report 3 cases of lung adenocarcinoma; one each identified to harbor an *ALK*-rearrangement (*EML4-ALK*), *ROS1*-rearrangement (*SDC4-ROS1*) and *MET*-amplification by genomic profiling. Notably, the *MET*-amplification was only detected by CGP as subsequent FISH testing did not show amplification. CGP also revealed other common genomic changes (somatic mutations [*TP53* in 2 cases], deletions [*CDKN2A* in 1 case], amplifications [*MCL1* in 1 case] and variants of unknown significance) in these cases. All patients received crizotinib 250 mg twice daily and achieved radiographic tumor reduction for months. The case harboring *MET*

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amplification of 10 copies achieved partial response and is one of the first *MET*-amplified lung cancer responsive to crizotinib in which the sole detection method was CGP.

Conclusions—CGP holds the promise of detecting predictive genomic alterations (somatic mutations, copy number changes and rearrangements) that may underlie tumor dependency in an oncogene and govern response to clinically-available TKIs for lung adenocarcinomas.

Keywords

mutation; lung cancer; next generation sequencing; genomic profiling; *MET*; *ALK*; *ROS1*; crizotinib

INTRODUCTION

The multitargeted tyrosine kinase inhibitor (TKI) crizotinib was developed as an oral anti-cancer drug with appropriate pharmacokinetic/pharmacodynamics (1, 2) parameters and preclinical activity against anaplastic lymphoma kinase (*ALK*), hepatocyte growth factor receptor (*MET*) and c-ros oncogene 1 (*ROS1*) plus cells driven by these driver oncogenes (3–5). This TKI has had a significant impact in the care of advanced non-small-cell lung cancers (NSCLCs); heterogeneous cancers often characterized by mutations in oncogenes (6, 7). A substantial proportion of NSCLCs - often lung adenocarcinomas (8) - harbor *ALK*-rearrangements (5% of adenocarcinomas), *ROS1*-rearrangements (1–2% of adenocarcinomas) or high level amplification of *MET* (1% of adenocarcinomas); numbers that correspond to more than fifteen thousand new cases of lung cancer yearly in the United States (9). The clinical evidence for use of crizotinib has been well established for *ALK*-rearranged lung adenocarcinomas, where the drug is superior to cytotoxic chemotherapy and has been Food and Drug Administration (FDA) approved since 2011 (10, 11). Significant evidence for use of crizotinib for *ROS1*-rearranged lung adenocarcinoma also exists from clinical trials showing impressive anti-tumor responses (12). The clinical evidence for use of crizotinib in *MET*-amplified lung adenocarcinoma is more modest and based mostly in few case reports and an ongoing expansion cohort of a phase I clinical trial (6, 13).

Despite the building clinical evidence that *ALK*, *ROS1* and *MET* genomic aberrations are predictive of the benefit of crizotinib, most current clinical guidelines for the care of lung cancer only recommend using a single gene assay (fluorescence in situ hybridization [FISH]) for *ALK*-rearrangement detection (14). In addition, most reports and trials attempting to identify *ROS1* and *MET* changes in tumors use technically challenging FISH assays done at central laboratories that have not been validated (12). Therefore, a more robust and integrated method of detection for mutation, insertion/deletions, copy number changes and rearrangements in lung adenocarcinomas is warranted. Herein, we describe the use of a comprehensive genomic profiling (CGP) assay based on hybrid capture-based next generation sequencing (NGS) capable of simultaneously identifying *ALK*-rearrangements, *ROS1*-rearrangements and *MET*-amplification in tumors; and provide index cases that these cancers are indeed responsive to crizotinib.

METHODS

Patient selection and data collection

Patients seen at Beth Israel Deaconess Medical Center with a diagnosis of NSCLC and whose tumors were submitted for genomic profiling were identified through an ongoing Institutional Review Board-approved study (as of October 31st 2014 a total of 643 tumors had been genotyped for at least one genomic change and 31 cases were analyzed using NGS-based CGP [4 cases using FoundationOne]); with the selection of three representative cases in which CGP was performed for this report. Data was collected by retrospective chart review and managed using REDCap electronic data capture hosted at BIDMC.

Tumor genotype

Following diagnosis, tumor material in formalin-fixed paraffin-embedded (FFPE) tissue blocks were submitted for genomic analyses. An assay for *ALK*-rearrangement was performed using the Vysis *ALK* break-apart FISH probe (Abbott Molecular, Inc., Des Plaines, IL) by a commercial vendor. A *ROS1* break-apart FISH assay was performed as previously described (12). *MET* copy number changes were inferred using a dual-color probe FISH assay for *MET* (7q31) with a control probe (CEP7) to evaluate copy number gain (8, 13). A commercially-available CGP assay based on clinical NGS (FoundationOne [Foundation Medicine, Cambridge, MA]) was used to analyze the tumors described here. This assay uses deoxyribonucleic acid (DNA) isolated from FFPE blocks to interrogate 315 cancer-related genes and 28 introns of genes involved in rearrangements using massively parallel DNA sequencing that characterizes base substitutions, short insertions/deletions, copy number alterations and rearrangements; as described previously (15). *MET* copy number gain is ascertained by assessing the coverage ratio of the entire coding sequence of *MET* between the patient sample and a diploid process-matched control sample (15).

RESULTS

Patient and tumor characteristics

We identified three cases of crizotinib-sensitive lung adenocarcinoma profiled by CPG assays from FFPE specimens (Tables 1 and 2).

CGP results

The *EML4-ALK*-rearranged tumor also harbored a tumor protein p53 (*TP53*) gene mutation and additional somatic mutations plus variants of unknown clinical/preclinical significance (Table 2). The *SDC4-ROS1*-rearranged adenocarcinoma contained additional mutations involving *TP53*, an amplification of myeloid cell leukemia 1a (*MCL1*), a deletion of cyclin-dependent kinase inhibitor 2A (*CDKN2A*), and additional somatic mutations, amplifications and variants of unknown clinical/preclinical significance (Table 2). The *MET*-amplified cancer specimen harbored additional somatic mutations plus variants of unknown clinical/preclinical significance (Table 2). The additional genomic changes identified (Table 2) in conjunction with the driver oncogenes (*ALK*, *ROS1* and *MET*) are not known to be associated with preclinical resistance to crizotinib and exclude co-existence of other clinically-validated driver oncogenes in each sample (6).

Response to crizotinib monotherapy

Case 1 was that of a patient with an *ALK*-rearranged lung adenocarcinoma that had received multiple cytotoxic chemotherapies prior to being enrolled on a clinical trial of crizotinib (Table 1). The original method of detection of *ALK* was performed using *ALK* FISH and subsequently the tumor was confirmed to have *EML4-ALK*-E13;A20 using CGP (Table 2). The patient tolerated crizotinib 250 mg twice daily with minimal changes in vision, gastrointestinal problems (diarrhea) and edema as adverse events. Within a month of crizotinib, his baseline cardio-pulmonary symptoms improved and he attained radiographic improvement of his cancer-related lesions. Using Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1, sum of target lesion diameters decreased by 14.2% and non-target lesions improved significantly; a scenario best classified as stable disease. This clinical and radiographic response was sustained for 17 months of crizotinib, upon which the patient experienced central nervous system and systemic progression.

Case 2 consisted of a never smoker with a *ROS1*-rearranged tumor initially recognized using FISH (Table 1) and subsequently identified as harboring the *SDC4-ROS1* using CGP (Table 2). After progression on first line therapy with carboplatin-pemetrexed, the patient was enrolled on a clinical trial of crizotinib (12). He started crizotinib 250 mg twice daily and developed minimal visual and gastrointestinal (diarrhea) effects. Within weeks of therapy, his baseline cardio-pulmonary status and performance status improved remarkably. This improvement was accompanied by radiographic improvement of lymphangitic tumor spread (non-target lesion) and a decreased of 26.8% in RECIST target lesions; classified as stable disease (and just under the threshold for a partial response). The response lasted for 4 months when the patient experienced acquired resistance with worsening dyspnea and pathologically-confirmed malignant pericardial effusion.

Case 3 refers to a 72-year old former smoker (12 pack-years) woman whose tumor burden had progressed after initial response to carboplatin and pemetrexed (Table 1). Genomic profiling revealed *MET*-amplification (all 20 exons were amplified to an estimated copy number of 10) as the main oncogenic driver (Table 2). However, as part of screening for a clinical trial of crizotinib (NCT00585195), *MET* FISH failed to show amplification (*MET*:*CEP7* ratio of 1:1) in the same tissue sample and the patient was ineligible for trial inclusion. Therefore, off label crizotinib 250 mg twice daily was prescribed. The patient initially tolerated crizotinib without adverse events. Within a week of therapy, she noted improvement in baseline cardio-pulmonary complaints, hoarseness and previously palpable lymphadenopathy had diminished in size. Radiographic assessment after 1 and 2 months of therapy disclosed significant improvement of nodal and pulmonary tumor burden, with a decrease in 38.7% of target lesions; a partial response by RECIST (Table 1). This response is ongoing for over 5 months of clinical follow-up after initiation of crizotinib.

DISCUSSION

The management of advanced lung adenocarcinomas is increasingly dictated by the genomic profile of the individual tumor. The College of American Pathologists among other associations in 2013 endorsed guidelines for rapid single gene assays for epidermal growth factor receptor (*EGFR*) mutations and *ALK*-rearrangements for all cases of metastatic

adenocarcinomas (14). Accordingly, the FDA labels for approved EGFR TKIs (erlotinib and afatinib) and ALK TKIs (crizotinib and ceritinib) in 2015 require the presence of a mutation in *EGFR* or rearrangement in *ALK*, respectively, detected by FDA-approved single gene assays (6). However, lung adenocarcinomas as a class have a long tail of driver genomic alterations beyond just alterations in *EGFR* and *ALK* (6) that may also predict for response to TKIs (6, 8, 10–13). As the list of potential predictive biomarkers for use of TKIs increase, so does the need for clinically-oriented assay platforms that can identify these genomic alterations. CGP offered by commercial vendors are clinically-feasible when based on a targeted panel of genes that identify alterations in known oncogenes and tumor suppressor genes with a turn around time acceptable for the practicing oncologist. Some of these NGS assays have been adjusted to allow for analysis of DNA purified from either cytologic biopsies or surgical procedures, with resulting tumor preserved as FFPEs as to be compatible with pathology workflow (15). The major advantage of CGP is the ability to simultaneously screen for a multitude of DNA base substitutions, short insertions/deletions, copy number alterations and rearrangements; information that cannot be obtained by parceling a clinical FFPE specimen into multiple aliquots for single gene assays (15).

We describe the potential of CGP in the course of clinical care to identify targets for the multitargeted ALK/ROS1/MET TKI crizotinib. A comprehensive genomic profiling assay probing over hundreds of cancer-related genes (15) reliably identified *ALK*-rearrangement, *ROS1*-rearrangement and *MET*-amplification in the crizotinib-responsive cases portrayed here. In specific, the patient harboring the *MET*-amplified lung adenocarcinoma had significant tumor reduction when given crizotinib 250 mg twice daily and represents one of the first reported cases of *MET*-amplified lung cancer responsive to crizotinib in which the singular method of identification was genomic profiling and not *MET* FISH (8, 13). It is possible that the single *MET* FISH probe at position may not reproduce the extended exon analysis of CGP (15).

CONCLUSION

The CGP used for these patients - as well as other evolving NGS technologies - can detect genomic alterations that may underlie tumor dependency in an oncogenic pathway and predict response to clinically-available TKIs (such as crizotinib) for lung adenocarcinomas. Further research into the use of CGP based on clinical NGS for routine oncology clinical practice is warranted.

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CLINICAL PRACTICE POINTS

- *ALK* and *ROS1* rearrangements, and MET amplification occur in lung cancer
- These genomic changes predict for response to the kinase inhibitor crizotinib
- Targeted next generation sequencing can identify simultaneously crizotinib-responsive genotypes
- Comprehensive genomic profiling may hold the promise of detecting multiple predictive genomic alterations (somatic mutations, copy number changes and rearrangements) that may underlie tumor dependency in an oncogene and govern response to clinically-available TKIs for lung adenocarcinomas.

Table 1

Clinical, pathologic and genomic characteristics plus tumor response of patients with *ALK* rearranged, *ROS1* rearranged and *MET* amplified lung adenocarcinomas treated with crizotinib.

	Clinical and pathologic characteristics	Major driver oncogene detected by NGS	Major driver oncogene detected by FISH assay	RECIST response to crizotinib	Duration of response
Case no. 1					
ALK rearrangement	male/White/38 years-old	EML4-ALK E13:A20	ALK FISH break-apart positive	stable disease	17 months
	former smoker (5 pack-years)			(dose: 250mg twice daily)	
	adenocarcinoma			–14.2% sum target lesions	
	recurrent/metastatic				
	prior therapies: carboplatin-paclitaxel-bevacizumab, pemetrexed, docetaxel, vinorelbine, gemcitabine				
Case no. 2					
ROS1 rearrangement	male/Asian/41 years-old	SDC4-ROS1	ROS1 FISH break-apart positive	stable disease	4 months
	never smoker			(dose: 250mg twice daily)	
	adenocarcinoma			–26.8% sum target lesions	
	stage IV				
	prior therapies: carboplatin-pemetrexed				
Case no. 3					
MET amplification	female/White/72 years-old	MET amplification	MET FISH copy number negative	partial response	> 5 months
	former smoker (12 pack-years)			(dose: 250mg twice daily)	(ongoing)
	adenocarcinoma			–38.7% sum target lesions	
	stage IV				
	prior therapies: carboplatin-pemetrexed				

Genomic aberrations in *ALK* rearranged, *ROS1* rearranged and *MET* amplified lung adenocarcinomas using a targeted next generation sequencing assay.

Table 2

	Rearrangements	Somatic mutations	Amplifications *	Deletions	Somatic variants of unknown significance **
Case no. 1					
	ALK rearrangement				
	EMIL4-ALK E13;A20	TP53 V143M	no amplifications	no deletions	AKT1 D46E
		SETD2 R1625H			AXL R368W
					BARD1 R529Q
					ERBB3 L1177I
Case no. 2					KDM2B K930del
					MLL2 P692T
					NCOR1 S2219T + P1536S
					NKX2-1 A57D
					SETBP1 R498Q
					SETD2 R950H
	ROS1 rearrangement	TP53 G187fs*21	MCL1	CDKN2A	MITF S218C
		NOTCH2 P6fs*27	APH1A		NF1 I826V
					PRSS8 S26P
Case no. 3					RUNX1 D332N
					SETBP1 N280S
					SGK1 M32L
					TRRAP S2321G
					ZRSR2 R440Q
Case no. 3					
	MET amplification				
		GRIN2A F183I	MET	no deletions	ASXL1 G121C + S48I
		KDM5C P380fs*50	(copy number: 10)		BARD1 P358_S364del
		PBRM1 R1010*			BRCA1 A224S + K223N
					CEBPA P39H
					CHD2 P405L

	Rearrangements	Somatic mutations	Amplifications *	Deletions	Somatic variants of unknown significance **
					FLT1 I423A
					GNAS Q161H
					GS3B R316Q
					IGFR IR G92V
					JAK2 A598T
					KIT S692L
					NTRK2 V272L
					RET A349fs *64 + T1078M
					ROS1 A573S + V946F
					STK11 E145Q
					ZNF217 I59_D456>N

*
copy number > 8
**
percent reads > 20%