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Solid Fusion Expression

Patient Name:		Ordered By	
Date of Birth:		Ordering Physician:	
Gender (M/F):		Physician ID:	
Client:		Accession #:	
Case #:		Specimen Type:	
Body Site:		Specimen ID:	

Ethnicity:				Family History:	
MRN:				Indication for Testing:	
Collected Date:		Time:		Reason for Referral:	Malignant Neoplasm of Lung
Received Date:		Time:		Tumor Type:	Lung
Reported Date:		Time:		Stage:	T2B

Test Description:

This is a next generation sequencing (NGS) test to identify molecular abnormalities in RNA in 55 genes implicated in solid tumors. Whenever possible, clinical relevance and implications of detected abnormalities are described below.

Detected Genomic Alterations				
Level 1 (FDA-Approved)	Level 2 (Standard of Care)	Level 3 (Clinical Evidence)	Level 4 (Biological Relevance)	Other
ALK: EML4-ALK Fusion	KIT: T670I			TP53: I255S

Tumor Heterogeneity
KIT mutations is detected in small subclone as compared to TP53

Expression	
CD79A	High
MYC	Low

Prognostic Implications	
ALk	Good

FDA-Approved Therapeutics	
ALK: EML4-ALK Fusion	Crizotinib, Ceritinib, Brigatinib, Alectinib

Relevant Alteration Associated with Resistance
NONE

FDA-Approved Therapeutics in Other Tumor Types	
KIT: T670I	Regoraenib, Imatinib, Sunitinib

Results Summary

- Fusion involving the ALK gene (EML4-ALK) is detected.
- The presence of ALK rearrangement suggests response to ALK inhibitors.

Biological Relevance of Detected Alterations

- ALK amplifications, fusions and mutations have been shown to be driving events in non-small cell lung cancer. While crizontinib has demonstrated efficacy in treating the amplification, mutations in ALK have been shown to confer resistance to current tyrosine kinase inhibitors. Second-generation TKI's have seen varied success in treating these resistant cases, and the HSP90 inhibitor 17-AAG has been shown to be cytostatic in ALK-altered cell lines.
- c-KIT activation has been shown to have oncogenic activity in gastrointestinal stromal tumors (GISTs), melanomas, lung cancer, and other tumor types. The targeted therapeutics nilotinib and sunitinib have shown efficacy in treating KIT overactive patients, and are in late-stage trials in melanoma and GIST. KIT overactivity can be the result of many genomic events from genomic amplification to overexpression to missense mutations. Missense mutations have been shown to be key players in mediating clinical response and acquired resistance in patients being treated with these targeted therapeutics.
- TP53 mutations are universal across cancer types. The loss of a tumor suppressor is most often through large deleterious events, such as frameshift mutations, or premature stop codons. In TP53 however, many of the observed mutations in cancer are found to be single nucleotide missense variants. These variants are broadly distributed throughout the gene, but with the majority localizing in the DNA binding domain. There is no single hotspot in the DNA binding domain, but a majority of mutations occur in amino acid positions 175, 245, 248, 273, and 282 (NM_000546) (Olivier et al., 2010). To fulfill its proper biological function four TP53 polypeptides must form a tetramer which functions as a transcription factor, therefore even if one out of four polypeptides has inactivating mutation it may lead to dominant negative phenotype of variable degree. While a large proportion of cancer genomics research is focused on somatic variants, TP53 is also of note in the germline. Germline TP53 mutations are the hallmark of Li-Fraumeni syndrome, and many (both germline and somatic) variants have been found to have a prognostic impact on patient outcomes. The significance of many polymorphisms for susceptibility and prognosis of disease is still very much up for debate.

Drug Information

Crizotinib

A selective c-Met/Alk tyrosine kinase inhibitor in clinical development as an anticancer agent.
An orally bioavailable agent belonging to the class of c-met/hepatocyte growth factor receptor (HGFR) tyrosine kinase inhibitors with potential antineoplastic activity. MET tyrosine kinase inhibitor PF-02341066 inhibits the membrane receptor MET and activation of

the MET signaling pathway, which may block tumor cell growth, migration and invasion, and tumor angiogenesis in susceptible tumor cell populations.
XALKORI is indicated for the treatment of patients with metastatic non-small cell lung cancer (NSCLC) whose tumors are anaplastic lymphoma kinase (ALK) or ROS1-positive as detected by an FDA-approved test

Ceritinib

An anaplastic lymphoma kinase inhibitor.
ZYKADIA® is indicated for the treatment of patients with metastatic non-small cell lung cancer (NSCLC) whose tumors are anaplastic lymphoma kinase (ALK)-positive as detected by an FDA-approved test.

Brigatinib

An anaplastic lymphoma kinase inhibitor.
An orally available inhibitor of receptor tyrosine kinases anaplastic lymphoma kinase (ALK) and the epidermal growth factor receptor (EGFR) with potential antineoplastic activity. Brigatinib binds to and inhibits ALK kinase and ALK fusion proteins as well as EGFR and mutant forms. This leads to the inhibition of ALK kinase and EGFR kinase, disrupts their signaling pathways and eventually inhibits tumor cell growth in susceptible tumor cells. In addition, brigatinib appears to overcome mutation-based resistance. ALK belongs to the insulin receptor superfamily and plays an important role in nervous system development; ALK dysregulation and gene rearrangements are associated with a series of tumors. EGFR is overexpressed in a variety of cancer cell types.
ALUNBRIG is indicated for the treatment of patients with anaplastic lymphoma kinase (ALK)-positive metastatic non-small cell lung cancer (NSCLC) who have progressed on or are intolerant to crizotinib.
This indication is approved under accelerated approval based on tumor response rate and duration of response. Continued approval for this indication may be contingent upon verification and description of clinical benefit in a confirmatory trial.

Alectinib

An anaplastic lymphoma kinase (Alk) inhibitor
An orally available inhibitor of the receptor tyrosine kinase anaplastic lymphoma kinase (ALK) with antineoplastic activity. Upon administration, alectinib binds to and inhibits ALK kinase, ALK fusion proteins as well as the gatekeeper mutation ALKL1196M known as one of the mechanisms of acquired resistance to small-molecule kinase inhibitors. The inhibition leads to disruption of ALK-mediated signaling and eventually inhibits tumor cell growth in ALK-overexpressing tumor cells. ALK belongs to the insulin receptor superfamily and plays an important role in nervous system development. ALK dysregulation and gene rearrangements are associated with a series of tumors
ALECENSA is indicated for the treatment of patients with anaplastic lymphoma kinase (ALK)-positive metastatic non-small cell lung cancer (NSCLC) as detected by an FDA-approved test.

Potential Clinical Trials

Trial #	Status	Title	Disease	Drug	Sites
NCT02468661	Recruiting	Phase I/II Trial of Alectinib and Bevacizumab in Patients With Advanced, Anaplastic Lymphoma Kinase (ALK)-Positive, Non-Small Cell Lung Cancer	Non-Small Cell Lung Cancer	Drug: Alectinib Drug: Bevacizumab	Massachusetts General Hospital Boston, Massachusetts, United States Beth Israel Deaconess Medical Center Boston, Massachusetts, United States

Detailed Results

Single Nucleotide Variant (SNV)								
Gene name	Hgvsp	Hgvsc	Aminoacids	Codons	Consequence	Allele frequency	Read depth	Predicted effect on protein
TP53	NP_000537.3:p.Ile255Asn	NM_000546.5:c.764T>A	I/N	aTc/aAc	missense_variant	34.2	268	deleterious (0)
KIT	NP_000213.1:p.Trp557Arg	NM_000222.2:c.1669T>C	W/R	Tgg/Cgg	missense_variant	17.4	401	deleterious (0)

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Copy Number Variant
NA

Fusion (traslocation)	
Gene Name	Fusion Reads (%)
EML4-ALK	51%

Heterogeneity within the Tumor

The most dominant clone caries the TP53 mutation. Kit mutation appears to be in subclone.

Methodology and Test Background

This is a next generation sequencing (NGS) test that analyzes targeted RNA with a focus on 55 genes. It is based on hybrid capture of targeted RNA. Duplicates are excluded for levels measurements. While the major focus of the analysis is the detection of fusion mRNA, mutations in the expressed RNA of the analyzed genes are also analyzed and reported. mRNA expression levels are evaluated, and only significant high expression of specific genes are relatively reported. CD274 (PD-L1) mRNA levels are reported when they are significantly elevated. If requested, detailed expression levels will be provided as a research data and not for clinical use. All detect fusion transcripts are reported. This test specifically covers translocations that lead to the expression of fusion RNA. Translocations that lead to deregulation of expression can be addressed by this test if compared to the expression proper normal control. The sensitivity of this assay in detecting fusion mRNA is between 1% and 5%. This assay is not designed to detect minimal residual disease and should be used for diagnosis when neoplastic cells are >10% of the analyzed cells. The Universal Human Reference (UHR) RNA is used as control.

Tested genes

RNA Fusions/Expression

Fusion/Expression													
ABL1	ALK	BRAF	CREBBP	EPOR	ETV5	FGFR2	FOXO1	JAK2	MAP3K1	NOTCH1	NUP214	PCM1	PICALM
ABL2	BCL1	CBFB	CRLF2	ERG	ETV6	FGFR3	FUS	KMT2A	MECOM	NTRK1	NUP98	PDGFRA	PML
AKT3	BCL2	CBL	CSF1R	ETV1	EWSR1	FIP1L1	GLI1	KRT18P6	MYC	NTRK2	P2RY8	PDGFRB	PTK2B
ALK	BCL6	CIC	EGFR	ETV4	FGFR1	FLT3	IKZF3	LYN	MYH9	NTRK3	PBX1	PD-L1	RARA

References

1. First-line crizotinib versus chemotherapy in ALK-positive lung cancer. Solomon BJ, Mok T, Kim DW, Wu YL, Nakagawa K, Mekhail T, Felip E, Cappuzzo F, Paolini J, Usari T, Iyer S, Reisman A, Wilner KD, Tursi J, Blackhall F; PROFILE 1014 Investigators. N Engl J Med. 2014 Dec 4;371(23):2167-77. doi: 10.1056/NEJMoa1408440. Erratum in: N Engl J Med. 2015 Oct 15;373(16):1582.
2. PF-06463922 is a potent and selective next-generation ROS1/ALK inhibitor capable of blocking crizotinib-resistant ROS1 mutations. Zou HY, Li Q, Engstrom LD, West M, Appleman V, Wong KA, McTigue M, Deng YL, Liu W, Brooun A, Timofeevski S, McDonnell SR, Jiang P, Falk MD, Lappin PB, Affolter T, Nichols T, Hu W, Lam J, Johnson TW, Smeal T, Charest A, Fantin VR. Proc Natl Acad Sci U S A. 2015 Mar 17;112(11):3493-8. doi: 10.1073/pnas.1420785112. Epub 2015 Mar 2.
3. Crizotinib in ROS1-rearranged non-small-cell lung cancer. Shaw AT, Ou SH, Bang YJ, Camidge DR, Solomon BJ, Salgia R, Riely GJ, Varella-Garcia M, Shapiro GI, Costa DB, Doebele RC, Le LP, Zheng Z, Tan W, Stephenson P, Shreeve SM, Tye LM, Christensen JG, Wilner KD, Clark JW, Iafrate AJ. N Engl J Med. 2014 Nov 20;371(21):1963-71. doi: 10.1056/NEJMoa1406766. Epub 2014.
4. Crizotinib: in locally advanced or metastatic non-small cell lung cancer. Curran MP. Drugs. 2012 Jan 1;72(1):99-107. doi: 10.2165/11207680-000000000-00000. Review.
5. Crizotinib versus chemotherapy in advanced ALK-positive lung cancer. Shaw AT, Kim DW, Nakagawa K, Seto T, Crinó L, Ahn MJ, De Pas T, Besse B, Solomon BJ, Blackhall F, Wu YL, Thomas M, O'Byrne KJ, Moro-Sibilot D, Camidge DR, Mok T, Hirsh V, Riely GJ, Iyer S, Tassell V, Polli A, Wilner KD, Jänne PA. N Engl J Med. 2013 Jun 20;368(25):2385-94. doi: 10.1056/NEJMoa1214886. Epub 2013 Jun 1. Erratum in: N Engl J Med. 2015 Oct 15;373(16):1582.
6. The ALK inhibitor ceritinib overcomes crizotinib resistance in non-small cell lung cancer. Friboulet L, Li N, Katayama R, Lee CC, Gainor JF, Crystal AS, Michellys PY, Awad MM, Yanagitani N, Kim S, Pferdekamper AC, Li J, Kasibhatla S, Sun F, Sun X, Hua S, McNamara P, Mahmood S, Lockerman EL, Fujita N, Nishio M, Harris JL, Shaw AT, Engelman JA. Cancer Discov. 2014 Jun;4(6):662-673. doi: 10.1158/2159-8290.CD-13-0846. Epub 2014 Mar 27.A
7. safety assessment of crizotinib in the treatment of ALK-positive NSCLC patients. Dikopf A, Wood K, Salgia R. Expert Opin Drug Saf. 2015 Mar;14(3):485-93. doi: 0.1517/14740338.2015.1007040. Epub 2015 Feb 7. Review.
8. Cabozantinib overcomes crizotinib resistance in ROS1 fusion-positive cancer. Katayama R, Kobayashi Y, Friboulet L, Lockerman EL, Koike S, Shaw AT, Engelman JA, Fujita N. Clin Cancer Res. 2015 Jan 1;21(1):166-74. doi: 10.1158/1078-0432.CCR-14-1385. Epub 2014 Oct 28.
9. Alectinib shows potent antitumor activity against RET-rearranged non-small cell lung cancer. Kodama T, Tsukaguchi T, Satoh Y, Yoshida M, Watanabe Y, Kondoh O, Sakamoto H. Mol Cancer Ther. 2014 Dec;13(12):2910-8. doi: 10.1158/1535-7163.MCT-14-0274. Epub 2014 Oct 27.
10. Alectinib: a review of its use in advanced ALK-rearranged non-small cell lung cancer. McKeage K. Drugs. 2015 Jan;75(1):75-82. doi: 10.1007/s40265-014-0329-y. Review. Erratum in: Drugs. 2015 Feb;75(2):241.
11. Antitumor activity of the selective ALK inhibitor alectinib in models of intracranial metastases. Kodama T, Hasegawa M, Takanashi K, Sakurai Y, Kondoh O, Sakamoto H. Cancer Chemother Pharmacol. 2014 Nov;74(5):1023-8. doi: 10.1007/s00280-014-2578-6. Epub 2014 Sep 10.
12. Selective ALK inhibitor alectinib with potent antitumor activity in models of crizotinib resistance. Kodama T, Tsukaguchi T, Yoshida M, Kondoh O, Sakamoto H. Cancer Lett. 2014 Sep 1;351(2):215-21. doi: 10.1016/j.canlet.2014.05.020. Epub 2014 Jun 2.
13. Two novel ALK mutations mediate acquired resistance to the next-generation ALK inhibitor alectinib. Katayama R, Friboulet L, Koike S, Lockerman EL, Khan TM, Gainor JF, Iafrate AJ, Takeuchi K, Taiji M, Okuno Y, Fujita N, Engelman JA, Shaw AT. Clin Cancer Res. 2014 Nov 15;20(22):5686-96. doi: 10.1158/1078-0432.CCR-14-1511. Epub 2014 Sep 16.
14. Alectinib salvages CNS relapses in ALK-positive lung cancer patients previously treated with crizotinib and ceritinib. Gainor JF, Sherman CA, Willoughby K, Logan J, Kennedy E, Brastianos PK, Chi AS, Shaw AT. J Thorac Oncol. 2015 Feb;10(2):232-6. doi: 10.1097/JTO.0000000000000455.
15. Alectinib: a selective, next-generation ALK inhibitor for treatment of ALK-rearranged non-small-cell lung cancer. Santarpia M, Altavilla G, Rosell R. Expert Rev Respir Med. 2015 Jun;9(3):255-68. doi: 10.1586/17476348.2015.1009040. Epub 2015 Feb 5.

Electronic Signature

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The Technical Component Processing, Analysis and Professional Component of this test was completed at GTC Laboratories, 21 Technology Dr. #100, Irvine, CA / 92618/ Medical Director: Maher Albitar, M.D. .

The performance characteristics of this test have been determined by GTC Laboratories. This test has not been approved by the FDA. The FDA has determined such clearance or approval is not necessary. This laboratory is CLIA certified to perform high complexity clinical testing.