Original Study

Multiple Biomarker Testing Tissue Consumption and Completion Rates With Single-gene Tests and Investigational Use of Oncomine Dx Target Test for Advanced Non–Small-cell Lung Cancer: A Single-center Analysis

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Abstract

The increasing number of genes relevant to non–small-cell lung cancer treatment, combined with small lung tissue samples, has heightened the need for tissue stewardship to enable multiple biomarker testing. The present retrospective analysis evaluated 3659 single-gene tests across 1402 clinician-submitted samples and 169 investigational Oncomine Dx Target Tests. Compared with single-gene testing, the Oncomine Dx Target Test could facilitate successful multiple biomarker testing of small samples.

Introduction: First-line targeted therapies have been developed for advanced non–small-cell lung cancer (NSCLC). However, small biopsy samples pose a challenge to testing all relevant biomarkers. The present study characterized clinician-ordered single-gene lung cancer testing and evaluated tissue stewardship and the ability to successfully determine mutation status with single-gene testing or investigational use of the Oncomine Dx Target Test. **Materials and Methods:** Clinician-submitted orders for 3659 single-gene tests (*EGFR*, *ALK*, *ROS1*, *BRAF*, *KRAS*, *ERBB2*, *MET*, *RET*, *FGFR1*) across 1402 samples at a large US-based commercial reference laboratory and 169 investigational Oncomine Dx Target Tests were retrospectively evaluated. The testing success rates and tissue consumption were evaluated by sample type, test type, and number of single-gene tests per sample. **Results:** The large majority of lung tissue samples submitted for clinical testing were small (70.5% core needle biopsies; 10.0% fine needle aspirations). With single-gene testing, mutation status was successfully reported for $\geq$ 1 biomarker for 88.4% of the clinical samples. The success rates decreased and tissue consumption increased with testing of additional biomarkers. Investigational Oncomine Dx Target Tests were permitted 1 tissue slide each and demonstrated success rates similar to single-gene testing for $\geq$ 5 biomarkers on core needle biopsies, $\geq$ 4 biomarkers on fine needle aspirations, and $\geq$ 2 biomarkers on surgical resection specimens. **Conclusion:** Tissue stewardship is important to enable successful completion of genetic testing and informed NSCLC treatment decisions. Preliminary assessment of the investigational Oncomine Dx Target Test suggests it could facilitate access to multiple biomarker testing using small tissue samples to support therapy decisions for patients with advanced NSCLC.

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Introduction

In the United States, lung cancer was expected to be newly diagnosed in 222,500 people and lead to 155,870 deaths in 2017.[1] Most lung cancer cases have been non–small-cell lung cancers (NSCLC), and > 60% of cases were regional or metastatic disease at diagnosis.[2,3] For patients with stage IIIB or IIIC NSCLC at diagnosis, the 5-year cancer-specific survival has ranged from 26% or 24% (for clinically staged) to 46% or 52% (for pathologically staged).[4] The 5-year overall survival (OS) rates for patients with advanced NSCLC (aNSCLC) patients have been reported at 26% for stage IIIB, 13% for stage IIIC, 10% for stage IVA, and 1% for stage IVB.[5]

The clinical practice guidelines have recommended genetic testing to guide first-line treatment for aNSCLC.[6] For patients with activating genetic mutations, targeted therapies have been shown to increase progression-free survival (PFS), and, more recently, OS compared with nontargeted chemotherapy.[7,8] The small lung cancer tissue samples available for testing can make it difficult for patients and physicians to access the mutation status across multiple relevant genes to guide treatment-decisions using traditional, sequential, single-gene testing. A study at the University of Pittsburgh Medical Center found that only 67% of computed tomography-guided core needle biopsies (CNBs) and 46% of fine needle aspirations (FNAs) had sufficient tumor to successfully determine the *EGFR*, *ALK*, and *KRAS* mutation status.[9] With the continuing development and approval of targeted therapies for NSCLC (eg, crizotinib for *ROS1*$^+$ patients, dabrafenib plus trametinib for *BRAF*$^+$ patients), physicians and patients selecting appropriate treatments face the challenge of the increasing number of genes that must be tested using small amounts of available tumor tissue.

The feasibility of next generation sequencing (NGS) for multiple biomarker testing and targeted treatment guidance for lung cancer and other solid tumors is being investigated in clinics and cancer trials. The ongoing NCI-MATCH (National Cancer Institute Molecular Analysis for Therapy Choice) trial has used a customized NGS assay and the Ion Torrent platform (Thermo Fisher Scientific, Waltham, MA) and reported a testing completion rate of 85% at the interim analysis across all tumor biopsy samples. This success rate increased to 94% in later updated reports, when adjustments to the protocol required that supplemental cytology specimens be submitted with the biopsy tissue sample.[10,11]

The Oncomine Dx Target Test (Ion Torrent PGM Dx Sequencer; Thermo Fisher Scientific) is an NGS panel for NSCLC testing approved by the US Food and Drug Administration (FDA) in June 2017. It is a qualitative, in vitro diagnostic test that uses high-throughput parallel sequencing technology to detect sequence variations in 23 genes on DNA and RNA isolated from formalin-fixed paraffin-embedded (FFPE) specimens. The diagnostic is validated against standard references, including clinical concordance, limit of blank, limit of detection, and variant-level detection accuracy and reproducibility.[12] The results from completed clinical studies have supported the concordance and clinical efficacy of the Oncomine Dx Target Test for 3 companion diagnostic genetic targets: *BRAF*, *EGFR*, and *ROS1* (overall clinical concordance, 100%, 99%, and 96.5%, respectively), with corresponding accuracy and reproducibility across standard measures. Likewise, analytical performance has been established for 4 additional variants, and the Oncomine Dx Target Test's performance for outstanding gene targets has been validated using a representative method.

As more gene targets are identified and recognized in clinical practice guidelines for aNSCLC, physicians and patients need to be able to test more biomarkers on small amounts of available tissue to make fully informed treatment decisions. As such, tissue stewardship is critical to enable testing; however, little evidence has been reported on the rates of successful determination of mutation status across multiple biomarkers for a single patient comparing single-gene testing or NGS methods or on the amounts of tissue needed to support these methods. The present study characterized the current single-gene testing paradigm in the molecular assessment for lung cancer patients and evaluated the success rates and tissue consumption with clinician-ordered single-gene testing and investigational use of the Oncomine Dx Target Test across different types of lung tumor tissue samples.

Materials and Methods

The present retrospective study analyzed the records from a large, US-based, Clinical Laboratory Improvement Amendments–certified, commercial reference laboratory. This laboratory conducted clinical molecular assessment of physician-submitted FFPE lung tissue samples and performed investigational testing using the Oncomine Dx Target Test before FDA approval on archival FFPE lung tissue samples. Investigational use of the Oncomine Dx Target Test was conducted in accordance with the FDA-approved protocol, except that only 1 tissue slide was permitted per test. The laboratory data contained the status (successful completion or reason for failure) of a test procedure but not the patient's mutation status. Because the laboratory data were accessed retrospectively and provided to the researchers such that the subjects could not be identified, directly or through identifiers linked to the subject, no patient consent forms or institutional review board approval was necessary.[13] The present study evaluated the proportion of tests that were successful (able to report results) and the tissue stewardship (number of slides cut for testing). Both outcomes were assessed by test type, sample type, and, for samples evaluated using single-gene testing, the number of tests per sample.

Clinical Single-gene Testing

The clinical single-gene testing paradigm was characterized using data from physician-ordered genetic tests on lung tumor samples submitted to the laboratory from September 2015 through October 2016. Single-gene tests ordered for activating mutations relevant to first-line treatment for aNSCLC were included: therascreen *EGFR* RGQ PCR Kit (QIAGEN Manchester Ltd, Manchester, UK); Vysis IntelliFISH for *ALK* (Abbott Laboratories, Abbott Park, IL); cobas 4800 *BRAF V600* Mutation Test (Roche Molecular Systems, Pleasanton, CA); and laboratory-developed tests (LDTs) for *BRAF*, *KRAS*, *MET* amplification, *RET*, *ERBB2*, *FGFR1*, and *ROS1*. The *BRAF* and *KRAS* LDTs used real-time, or quantitative, polymerase chain reaction. All other LDTs used fluorescent in situ hybridization.

Testing for *CD274* gene expression (ie, programmed cell death ligand 1 expression), was not offered at this laboratory until nearly halfway through the study period (earliest test was in March 2016). Therefore, these tests were not included in the present study. Clinical samples were excluded if any type of NGS panel test was initiated or if a *CD274* gene expression test was initiated, because the use of sample tissue to attempt these tests this could have affected the ability to complete other single-gene tests using the sample.

Data on each clinical test order included the type of sample submitted for testing: CNB, FNA, surgical resection, or cell block. The single-gene tests ordered per sample, the status of each ordered test showing whether test results were successfully reported, and the reason for not reporting results, if applicable, were also available from the data.

Lung tissue samples with ≥ 1 study-eligible single-gene test were included in the present analysis. Cancelled tests were excluded from the present study. A test was considered cancelled if the test status showed that the reason for not reporting results was cancellation by the client, duplication of the order, or conversion of the order to NGS. A test was considered unsuccessful if the test status was any of the following: PCR amplification failure, insufficient DNA quantity, poor DNA quality, high background, no hybridization, decalcification failure, wrong tumor type, insufficient tumor quantity, or poor tumor quality. This included tests that were ordered by physicians but were not initiated, which could have occurred if the sample's tumor content did not meet the minimum testing requirements as defined by the laboratory's protocol for single-gene tests. Although numerical data on the tumor content percentage were missing for some clinical samples, all samples included in the present analysis had $> 0\%$ tumor content. A clinical single-gene test was considered successfully completed if the test status indicated that results were reported.

The number of slides cut from the sample for each single-gene test was used to inform the analyses of tissue consumption. In some cases, these data fields were blank, which could indicate that no slides had been cut (ie, sample rejection) or that the data were missing. The tests for which these data was not reported—whether that was because of sample rejection or missing data—were excluded from the tissue consumption analyses.

Investigational Use of the Oncomine Dx Target Test

The investigational use of the Oncomine Dx Target Test was conducted on archival FFPE lung cancer samples as part of a previous study conducted by the laboratory from April 2016 to July 2016. The tests were conducted using the Ion Torrent PGM Dx platform. The panel included 23 genes: *AKT1*, *ALK*, *BRAF*, *CDK4*, *DDR2*, *EGFR*, *ERBB2*, *ERBB3*, *FGFR2*, *FGFR3*, *HRAS*, *KIT*, *KRAS*, *MAP2K1*, *MAP2K2*, *MET*, *MTOR*, *NRAS*, *PDGFRA*, *PIK3CA*, *RAF1*, *RET*, and *ROS1* (the list of specific gene variants tested is provided in Supplemental Table 1; available in the online version). The investigational protocol was identical to the protocol for the FDA-approved Oncomine Dx Target Test, except for the number of tissue slides used per test.[14] The pre-established protocol for the investigational study allowed only 1 slide per test, and the protocol for the FDA-approved test recommended ≥ 2 slide-mounted 5-μm sections of surgical resection samples or 9 slide-mounted 5-μm sections of CNB samples.

Investigational Oncomine Dx Target Tests were included in the present analysis if the archival sample was a CNB, FNA, or surgical resection sample. The laboratory data included the type of sample tested and the date stamps for completion of the procedural steps, if successful. Investigational Oncomine Dx Target Tests were considered successfully completed if a date stamp for completion of sequencing was available. The lack of a date stamp for sequencing completion indicated an unsuccessful Oncomine Dx Target Test. A successfully completed Oncomine Dx Target Test sequenced and reported the genetic results for all the target genes; however, an unsuccessful test would not be able to report the genetic results for any target genes.

Statistical Analysis

The success rates and tissue consumption in clinical single-gene testing were evaluated by type of single-gene test and by the total number of single-gene tests ordered per sample. The success rate was defined as the percentage of physician-ordered tests or physician-submitted samples for which the biomarker status could be reported, regardless of test initiation or sample rejection. Each success rate was evaluated only if ≥ 10 applicable tests or samples were available in the data set.

Analyses stratified by the number of tests per clinical sample were conducted to evaluate both the resources required to test increasing numbers of single-gene tests and the ability of the current single-gene testing paradigm to provide information to support first-line treatment decisions for aNSCLC. The total tissue consumption per sample was evaluated by the number of tests on that sample with data available on the number of slides cut. The average number of slides required to attempt a certain number of single-gene tests was assessed across samples that had slides cut for that exact number of tests. The probability of successfully completing a certain number of single-gene tests was evaluated among samples on which at least that many included tests had been ordered. For example, the probability that 5 single-gene tests could be successfully completed on a single sample was evaluated among the clinical samples for which ≥ 5 single-gene tests had been ordered.

Overall analyses were conducted across all clinical samples to describe the success rates and tissue consumption of the single-gene testing paradigm in general. Differences between the success rates for increasing numbers of single-gene tests were evaluated using the χ^2 test if the number of samples was > 5 and the Fisher exact test otherwise. The success rates for > 1 single-gene test were compared to the success rate of completing 1 single-gene test. The differences in sequential success rates were also evaluated (eg, 2 vs. 3 tests, 3 vs. 4 tests, etc.). Statistical significance was considered present at $P < .05$. Statistical analyses were conducted using SAS, version 9.4 (SAS Institute, Cary, NC).

The success rates and tissue consumption with the investigational Oncomine Dx Target Test were not assessed overall across all the archived samples but were assessed for different lung tumor sample subcategories. Subanalyses by sample type and tumor content were conducted for both the clinical single-gene testing and the investigational use of the Oncomine Dx Target Test.

Table 1 Tissue Sample Characteristics

Characteristic	Sample Type							
	CNB				FNA	Resection	Cell Block	All Sample Types
	Any	Tumor Content						
		< 25%	≥ 25%	NA				
Clinical samples submitted for single-gene testing								
Samples, n (% of total)	988 (70.5)	209 (14.9)	685 (48.9)	94 (6.7)	140 (10.0)	167 (11.9)	107 (7.6)	1.402 (100.0)
Patients, n	971	209	677	92	140	165	104	1,368
Tumor content of samples								
Samples with tumor content data, n	894	209	685	0	112	166	82	1,254
Tumor proportion, %								
Mean	45.2	18.3	53.4	NA	53.2	56.2	42.3	47.2
Range	2-100	2-22	25-100	NA	5-100	5-100	1-100	1-100
Tests ordered, n (% of samples)								
EGFR therascreen	947 (95.9)	201 (96.2)	663 (96.8)	83 (88.3)	131 (93.6)	153 (91.6)	102 (95.3)	1,333 (95.1)
ALK Vysis	745 (75.4)	160 (76.6)	542 (79.1)	43 (45.7)	113 (80.7)	128 (76.6)	71 (66.4)	1,057 (75.4)
ROS1 LDT	480 (48.6)	100 (47.8)	345 (50.4)	35 (37.2)	61 (43.6)	73 (43.7)	49 (45.8)	663 (47.3)
BRAF cobas	66 (6.7)	13 (6.2)	47 (6.9)	6 (6.4)	3 (2.1)	5 (3.6)	5 (4.7)	79 (5.6)
BRAF LDT	16 (1.6)	2 (1.0)	14 (2.0)	0 (0.0)	2 (1.4)	2 (1.2)	1 (0.9)	21 (1.5)
KRAS LDT	146 (14.8)	33 (15.8)	105 (15.3)	8 (8.5)	12 (8.6)	41 (24.6)	10 (9.3)	209 (14.9)
MET LDT	101 (10.2)	18 (8.6)	79 (11.5)	4 (4.3)	5 (3.6)	16 (9.6)	7 (6.5)	129 (9.2)
RET LDT	99 (10.0)	17 (8.1)	78 (11.4)	4 (4.3)	5 (3.6)	13 (7.8)	8 (7.5)	125 (8.9)
FGFR1 LDT	29 (2.9)	5 (2.4)	22 (3.2)	2 (2.1)	2 (1.4)	4 (2.4)	3 (2.8)	38 (2.7)
ERBB2 LDT	4 (0.4)	0 (0.0)	3 (0.4)	1 (1.1)	1 (0.7)	0 (0.0)	0 (0.0)	5 (0.4)
Archival samples for investigational use of Oncomine Dx Target Test								
Samples, n	69 (40.8)	41 (24.3)	28 (16.6)	0 (0.0)	13 (7.7)	87 (51.5)	0 (0.0)	169 (100.0)
Tumor, %				NA			NA	
Mean	29.0	15.0	49.5		28.9	42.4		35.9
Range	1-90	1-20	25-90		1-90	5-90		1-90

Abbreviations: CNB = core needle biopsy; FNA = fine needle aspiration; LDT = laboratory developed test.

Subanalyses were conducted for the sample types present among both the clinical and the archival samples: CNB, FNA, or surgical resection. Because most tissue samples submitted for aNSCLC testing were CNBs, further subanalyses were conducted to evaluate both the clinical and the archival CNB samples stratified by tumor content. CNB samples for which tumor content data were available were evaluated in 2 subgroups: $< 25\%$ and $\geq 25\%$ tumor content. CNB samples for which data on tumor content were missing were evaluated as a separate "unknown" category. Just as with the overall analysis of clinical single-gene testing, the success rates in the subanalyses were only evaluated if ≥ 10 tests or samples were available.

Results

Data from the reference laboratory on 3659 physician-ordered clinical single-gene tests were included in the present analysis. The clinical single-gene tests were ordered on 1402 samples from 1368 patients. The clinical samples for testing were predominantly from biopsies, with CNBs comprising 70.5% of the submitted samples and FNAs comprising an additional 10.0% (Table 1). The range in tumor content was as high as 100% for all sample types and as low as 2% among CNBs, 5% among FNAs, 5% among surgical resection samples, and 1% among cell blocks. Of the CNB samples, 21.2% had $< 25\%$ tumor content recorded.

The laboratory data also included 169 investigational use cases of the Oncomine Dx Target Test. Compared with the clinical samples, the archival samples used for the investigational Oncomine Dx Target Tests included a greater proportion of surgical resection samples (11.9% of clinical samples vs. 51.5% of archival samples) and a lower proportion of CNBs (70.5% of clinical samples vs. 40.8% of archival samples). Regardless of sample type, the archival samples tended to have lower tumor content than the clinical samples. The range in tumor content among the archival samples was $\leq 90\%$ across all sample types and as low as 1% among the CNBs or FNAs and 5% among the surgical resection samples.

Across all clinical samples submitted for testing, the single-gene tests most commonly ordered by physicians were *EGFR* therascreen (95.1%), *ALK* Vysis (75.4%), and *ROS1* LDT (47.3%; Figure 1). Among the clinical samples on which single-gene tests for ≥ 3 biomarkers were ordered, a large majority included tests for *EGFR*, *ALK*, and *ROS1*. When single-gene tests for ≥ 4 biomarkers were ordered on a single sample, the tests for *MET*, *RET*, *KRAS*, or *BRAF* were commonly included.

The proportion of physician-ordered single-gene tests for which mutation status was successfully reported varied by test type from 62.4% for *RET* LDT to 89.1% for *ALK* Vysis (Figure 2A). Evaluations by sample type found greater success rates on surgical resection samples and lower success rates on FNA samples across nearly all the single-gene tests (Figure 2B). The exception was *RET* LDT, for which the success rates were lower from the surgical resection samples than from the CNB samples.

Tissue consumption analyses included 3314 single-gene tests on 1258 samples. The number of slides needed to run a single-gene test was greatest for the *BRAF* LDT, with a mean tissue consumption of 7.1 slides per test (n = 20 tests). The *KRAS* LDT was the second-most tissue-intensive test, consuming 6.8 slides per test on average (n = 180). The *EGFR* therascreen consumed 2.7 slides per test on average (n = 1107). The *MET* LDT, *RET* LDT, *ROS1* LDT, *FGFR1* LDT, and *ALK* Vysis tests consumed an average of 2.0 (n = 117), 1.6 (n = 112), 1.3 (n = 614), 1.3 (n = 32), and 1.1 (n = 979) slides per test, respectively. *BRAF* cobas (n = 67) and *ERBB2* LDT (n = 2) each consumed 1.0 slide per test.

Some clinical samples did not have sufficient tissue to allow slides to be cut for all the single-gene tests requested by the ordering physician. For those samples and tests for which slides could be cut, the average number of slides consumed per sample increased steadily with the number of biomarker tests attempted (Figure 3A). This likely reflects histotechnologist standard operating procedures for uniformity of sectioning and not pathologic evaluation of the amount of tumor present in the different types of samples. When slides could be cut to run tests, tissue consumption increased from 2.4 slides to run 1 single-gene test, to 8.7 to run 4 single-gene tests, to 17.0 slides to run 8 single-gene tests on a single sample.

When multiple single-gene tests were ordered for the same clinical sample, the probabilities of successfully reporting the molecular status for multiple biomarkers decreased as the number of biomarkers increased (Figure 3B). Across all submitted clinical samples, 88.4% had successfully reported mutation status results from ≥ 1 single-gene test. Among the samples on which ≥ 4 tests were ordered, the probability of having successfully reported the mutation status for ≥ 4 biomarkers was 76.6%. The success rates for ≥ 2 tests through ≥ 7 tests were all significantly lower than the success rate for ≥ 1 test ($P < .05$ for ≥ 2 tests; $P < .0001$ for ≥ 3-7 tests). Furthermore, the probability of successfully completing ≥ 3 tests was significantly lower than the probability of successfully completing ≥ 2 tests ($P < .0001$). Subsequent sequential differences in the success rates (eg, ≥ 3 vs. ≥ 4 tests) did not reach statistical significance, although the difference in the success rates for ≥ 4 versus ≥ 5 tests did show marginal statistical significance ($P = .052$). Even so, the observed success rates continued to show a trend downward for greater numbers of single-gene tests.

The evaluations stratified by sample type found that the numbers of slides cut to run multiple single-gene tests on clinical samples were similar across CNBs, FNAs, and surgical resection samples, again likely reflecting histotechnologist standard operating procedures for uniformity of sectioning and not pathologic evaluation of the amount of tumor present in the different sample types (Figure 4A). Investigational use of the Oncomine Dx Target Test was artificially restricted to 1 slide per test, regardless of the sample type, in accordance with the pre-established protocol specific to the investigational use testing study for which they were conducted. Similar to clinical single-gene testing, the success rates with the Oncomine Dx Target Test were lowest for the FNA samples and greatest for the surgical resection samples (Figure 4B). The success rates with the investigational use Oncomine Dx Target Test was 69.2% for the FNA samples, 75.4% for the CNB samples, and 98.9% for the surgical resection samples. These success rates decreased between the clinical single-gene testing success rates for 3 and 4 biomarkers on FNA samples (75.0% and 40.0%, respectively), 4 and 5 biomarkers on CNB samples (77.9% and 70.8%, respectively), and 1 and 2 biomarkers on surgical resection samples (100.0% and 98.5%, respectively).

Further subgroup analyses of the CNB samples stratified by tumor content found similar numbers of slides consumed to run

Figure 1 Probability of Test Order Stratified by Number of Genes

Test type	Total number of single-gene tests ordered							
	≥1	≥2	≥3	≥4	≥5	≥6	≥7	≥8
N samples	1,402	1,111	682	205	120	104	30	5
EGFR therascreen	95.1%	97.9%	98.7%	98.5%	99.2%	100.0%	100.0%	100.0%
ALK Vysis	75.4%	92.3%	97.9%	98.5%	98.3%	100.0%	100.0%	100.0%
ROS1 LDT	47.3%	58.5%	90.3%	95.1%	100.0%	100.0%	100.0%	100.0%
BRAF cobas or LDT	7.1%	8.9%	14.4%	45.4%	36.7%	39.4%	26.7%	100.0%
KRAS LDT	14.9%	18.8%	23.8%	50.7%	61.7%	67.3%	100.0%	100.0%
MET LDT	9.2%	11.6%	18.8%	61.0%	98.3%	100.0%	100.0%	100.0%
RET LDT	8.9%	11.2%	18.0%	59.0%	96.7%	99.0%	96.7%	100.0%
FGFR1 LDT	2.7%	3.4%	5.4%	15.6%	22.5%	26.0%	90.0%	100.0%
ERBB2 LDT	0.4%	0.5%	0.7%	2.4%	2.5%	1.9%	3.3%	0.0%

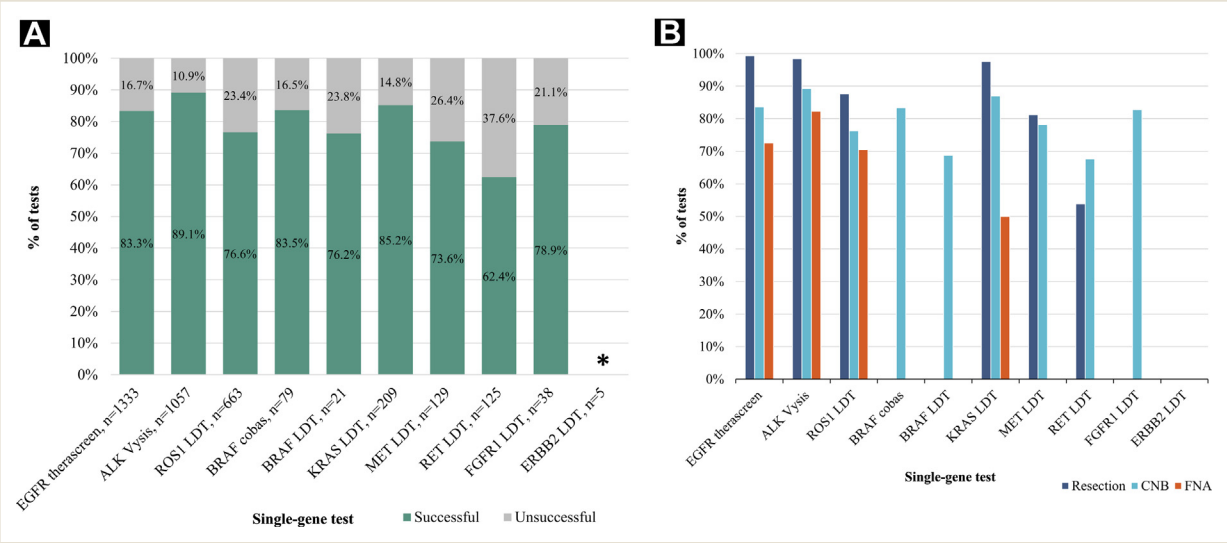
Abbreviation: LDT = laboratory developed test.

multiple single-gene tests on samples with < 25% tumor content compared with samples with ≥ 25% tumor content (Figure 5A). When multiple single-gene tests were ordered, the rates of successfully reporting the mutation status for multiple biomarkers were consistently greater among the samples with ≥ 25% tumor content than among the samples with < 25% tumor content (Figure 5B). The tumor content was not reported for 9.5% of the clinical CNB samples (n = 94 of 988). The success rates were very low among these CNB samples with unknown tumor content. The success rates with the investigational use Oncomine Dx Target Test were 70.7% for CNB samples with < 25% tumor content and 82.1% for CNB samples with ≥ 25% tumor content. Just as with the overall analysis of the success rates on CNB samples, these success rates were between the clinical single-gene testing success rates for 4 and 5 biomarkers.

Discussion

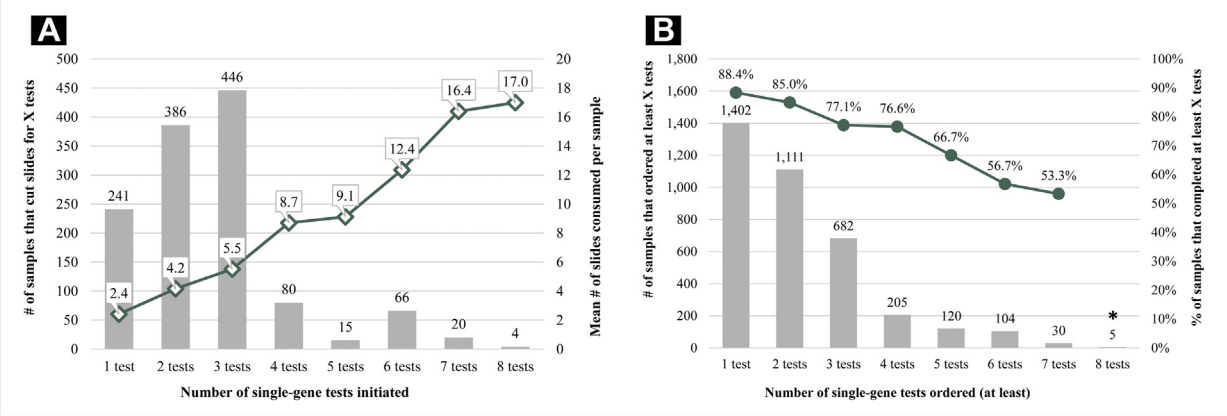
Most of the 1402 lung cancer patients' tumor tissue samples submitted by physicians for clinical testing at this large US-based reference laboratory were small samples. More than three fourths were CNB samples and another one tenth were FNA samples. Among the CNB samples, more than one fifth had < 25% tumor

Figure 2 Clinical Testing Success Rates Across Single-Gene Tests: (A) Overall and (B) Stratified by Sample Type. **ERBB2* Was Not Evaluated Owing to the Small Number of Samples. Success Rates Were Evaluated Only if ≥ 10 Tests Were Available



Abbreviation: LDT = laboratory developed test

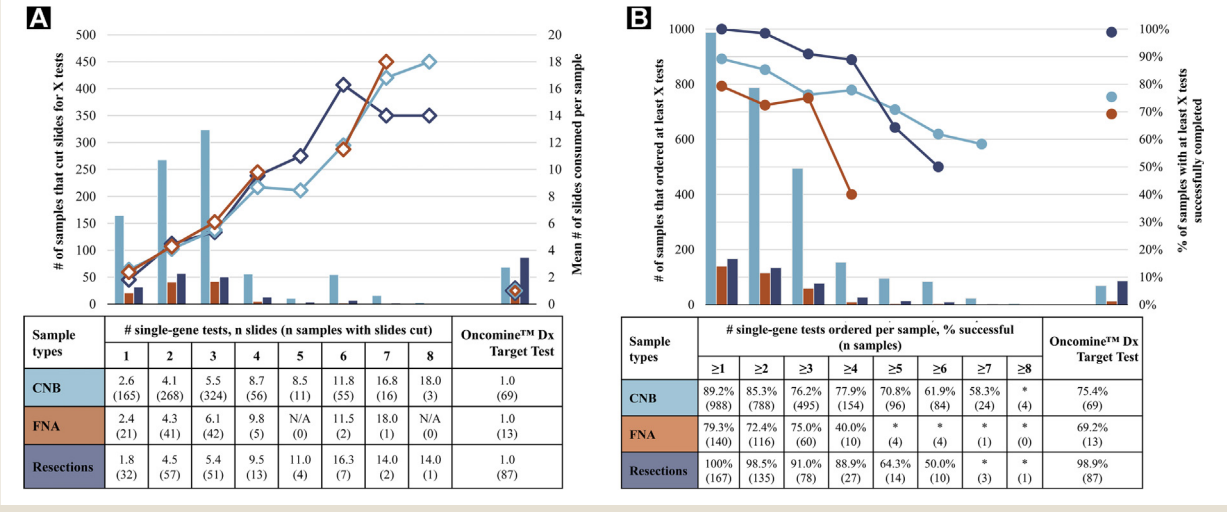
Figure 3 Multiple Biomarker Testing on Clinical Samples Stratified by Number of Single-Gene Tests. (A) Tissue Consumption Stratified by Number of Tests Run. (B) Testing Success Rates Stratified by Number of Single-Gene Tests Ordered. Lines Show (A) the Number of Tissue Slides Cut to Attempt Exactly Each Number of Single-Gene Tests or (B) the Testing Success Rates for at Least Each Number of Single-Gene Tests. Columns Show the Number of Samples Available for Each Evaluation. *Success Rates Were Evaluated Only if ≥ 10 Samples Were Available



content. These findings demonstrate the small amounts of tissue available for most aNSCLC patients who attempt to use genetic testing to inform treatment decisions.

The amount of tissue required to attempt a single-gene test for these patients varied widely across test types. Although the *BRAF* LDT had the greatest average tissue consumption among the single-gene tests (7.1 slides), the laboratory switched to the FDA-approved *BRAF* cobas test (1.0 slide) during the study period, reducing the tissue consumption necessary to test for *BRAF*-activating mutations. *KRAS* has been acknowledged by the recommended guidelines as having prognostic value in lung cancer. However, running the *KRAS* LDT required an average of 6.8 slides per test. The *EGFR* therascreen required 2.7 slides per test on average to run, and the other single-gene tests required < 2 slides per test on average. For some samples in this real-world analysis, some of the physician-ordered single-gene tests were not attempted and no slides were cut, likely owing to insufficient or depleted tumor tissue.

Figure 4 Multiple Biomarker Testing Stratified by Sample Type for Clinical Single-Gene Testing and Investigational Use of the Oncomine Dx Target Test. (A) Tissue Consumption and (B) Testing Success Rates. Lines Show (A) the Number of Tissue Slides Cut to Attempt Exactly Each Number of Single-Gene Tests and (B) the Testing Success Rates for At Least Each Number of Single-Gene Tests. The Numbers of Samples Available for Each Evaluation Are Graphed as Columns and Shown in Parentheses in the Table. *Success Rates Were Evaluated Only if ≥ 10 Samples Were Available

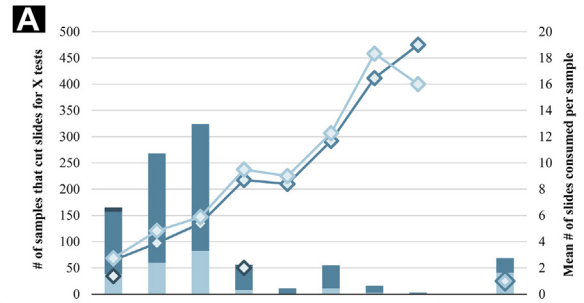


Sample types	# single-gene tests, n slides (n samples with slides cut)								Oncomine™ Dx Target Test
	1	2	3	4	5	6	7	8	
CNB	2.6 (165)	4.1 (268)	5.5 (324)	8.7 (56)	8.5 (11)	11.8 (55)	16.8 (16)	18.0 (3)	1.0 (69)
FNA	2.4 (21)	4.3 (41)	6.1 (42)	9.8 (5)	N/A (0)	11.5 (2)	18.0 (1)	N/A (0)	1.0 (13)
Resections	1.8 (32)	4.5 (57)	5.4 (51)	9.5 (13)	11.0 (4)	16.3 (7)	14.0 (2)	14.0 (1)	1.0 (87)

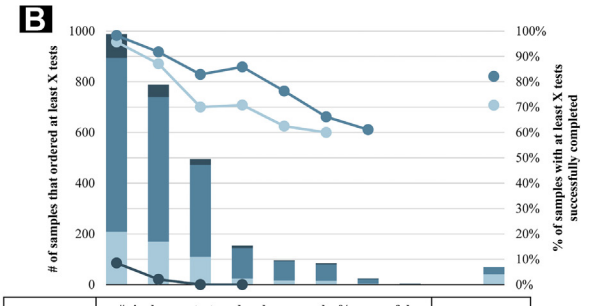
Sample types	# single-gene tests ordered per sample, % successful (n samples)								Oncomine™ Dx Target Test
	≥1	≥2	≥3	≥4	≥5	≥6	≥7	≥8	
CNB	89.2% (988)	85.3% (788)	76.2% (495)	77.9% (154)	70.8% (96)	61.9% (84)	58.3% (24)	* (4)	75.4% (69)
FNA	79.3% (140)	72.4% (116)	75.0% (60)	40.0% (10)	* (4)	* (4)	* (1)	* (0)	69.2% (13)
Resections	100% (167)	98.5% (135)	91.0% (78)	88.9% (27)	64.3% (14)	50.0% (10)	* (3)	* (1)	98.9% (87)

Abbreviations: CNB = core needle biopsy; FNA = fine needle aspiration.

Figure 5 Multiple Biomarker Testing on Core Needle Biopsy (CNB) Samples Stratified by Tumor Content: Clinical Single-Gene Testing and Investigational Use of the Oncomine Dx Target Test: (A) Tissue Consumption and (B) Testing Success Rates. Lines Show (A) the Number of Tissue Slides Cut to Attempt Exactly Each Number of Single-Gene Tests, and (B) the Testing Success Rates for at Least Each Number of Single-Gene Tests. The Numbers of Samples Available for Each Evaluation Are Graphed as Columns and Are In Parentheses in the Table. *Success Rates Were Evaluated Only if ≥ 10 Samples Were Available



Sample types	# single-gene tests, n slides (n samples with slides cut)								Oncomine™ Dx Target Test
	1	2	3	4	5	6	7	8	
CNB, <25% tumor	2.7 (39)	4.8 (60)	5.9 (83)	9.5 (8)	9.0 (1)	12.4 (11)	18.3 (3)	16.0 (1)	1.0 (41)
CNB, ≥25% tumor	2.6 (118)	3.9 (208)	5.4 (241)	8.7 (47)	8.4 (10)	11.7 (44)	16.5 (13)	19.0 (2)	1.0 (28)
CNB unknown	1.4 (8)	N/A (0)	N/A (0)	2.0 (1)	N/A (0)	N/A (0)	N/A (0)	N/A (0)	N/A (0)
All CNB	2.6 (165)	4.1 (268)	5.5 (324)	8.7 (56)	8.5 (11)	11.8 (55)	16.8 (16)	18.0 (3)	1.0 (69)



Sample types	# single-gene tests ordered per sample, % successful (n samples)								Oncomine™ Dx Target Test
	≥1	≥2	≥3	≥4	≥5	≥6	≥7	≥8	
CNB, <25% tumor	95.7% (209)	87.1% (170)	70.0% (110)	70.8% (24)	62.5% (16)	60.0% (15)	* (4)	* (1)	70.7% (41)
CNB, ≥25% tumor	98.2% (685)	91.8% (570)	82.9% (362)	85.8% (120)	76.3% (76)	66.2% (65)	61.1% (18)	* (2)	82.1% (28)
CNB unknown	8.5% (94)	2.1% (48)	0.0 % (23)	0.0% (10)	* (4)	* (4)	* (2)	* (1)	* (0)
Total	89.2% (988)	85.3% (788)	70.2% (495)	77.9% (154)	70.8% (96)	61.9% (84)	58.3% (24)	* (4)	75.4% (69)

Across all physician-ordered tests in the data set, the success rates for different types of single-gene tests were all < 90%, including those for activating mutations addressed by FDA-approved targeted therapies. The *ALK* Vysis testing success rate was greatest at 89.1%. The success rates for *EGFR* therascreen or *BRAF* cobas were both ~83%, and the success rate for the *ROS1* LDT was only 76.6%. The subanalyses showed that the success rates were greatest with the surgical resection samples but lowest for the FNA samples, showing that lung cancer patients with smaller samples have greater challenges in accessing the genetic information needed to guide targeted treatment decisions.

Physicians ordered > 1 single-gene test for nearly 80% of the submitted clinical samples. The number of slides needed to attempt multiple single-gene tests increased steadily with the number of biomarkers to be tested. At the same time, the probability of successfully reporting the mutation status results from the additional single-gene tests decreased. Only 88.4% of the clinical samples submitted for single-gene testing had ≥ 1 biomarker successfully reported, suggesting that > 1 in 10 of these patients (11.6%) had no genetic information available to inform their treatment decisions. The success rates for determining the mutation status decreased significantly for 2 biomarkers and 3 biomarkers and continued to show a trend downward after that. Currently, FDA-approved targeted therapies are available for activating mutations in 4 genes in aNSCLC. The success rate for reporting the mutation status for 4 biomarkers in the present study was 76.6%, suggesting that the necessary genetic information to guide appropriate selection for current targeted therapies might be unavailable for 23.4% of aNSCLC patients in the current single-gene testing paradigm.

The subanalyses stratified by sample type and tumor content included both clinical single-gene testing and investigational use of the Oncomine Dx Target Test. Although tissue consumption for multiple single-gene tests did not vary widely by sample type or tumor content, the testing success rates showed more variation. The success rates were consistently lower among CNB than among surgical resection samples and were lower still among FNA samples. Among the CNB samples, the success rates were consistently lower among those samples with < 25% tumor content compared with those with ≥ 25% tumor content. The lower success rates underscore the difficulties in molecular assessment for patients with aNSCLC whose samples are mostly small and for whom the number of therapeutically relevant biomarkers is only increasing.

Investigational use of the Oncomine Dx Target Test was artificially restricted to a single tissue slide based on a previous study's protocol but still showed testing success rates that were at least comparable to the clinical success rates for ≥ 2 single-gene tests on surgical resection samples, ≥ 4 single-gene tests on FNA samples, and ≥ 5 single-gene tests on CNB samples. Running that number of single-gene tests required a mean of 4.5 slides, 9.8 slides, and 8.5 slides per surgical resection, FNA, and CNB sample, respectively. Although only 1 slide was used for each investigational Oncomine Dx Target Test, the FDA-approved protocol has recommended 2 slides when testing a surgical resection sample and 9 when testing a CNB sample. Therefore, in the real-world clinical testing setting, in which the number of tissue slides is limited only by the amount of sample available, the success rates and tissue consumption with the Oncomine Dx Target Test might be greater than that reported in the present retrospective study.

A previously reported prospective study conducted from July 2011 to June 2013 at the University of Pittsburgh Medical Center (UPMC) conducted multiple biomarker testing on CNB and FNA lung tumor tissue samples for *EGFR*, *ALK*, and *KRAS*, specifically.

The mutation status for all three genes could be successfully reported for 67% of the CNB samples and 46% of FNA samples.[9] The present retrospective study found testing success rates for 3 biomarkers of 76.2% among CNB samples and 75.0% among FNA samples. The differences in testing success rates for the 3 biomarkers between these 2 studies might be attributable to the specific genes tested. The study at UPMC specifically aimed to test *EGFR*, *ALK*, and *KRAS* on all their samples. In contrast, although the orders in the present study for $\geq$ 3 single-gene tests on a single sample overwhelmingly included *EGFR* and/or *ALK* testing (98.7% and 97.9%, respectively), only 23.8% included *KRAS* testing. The present study showed that evaluating *KRAS* mutation status required the largest number of tissue slides of all the included biomarkers. The rate of success for reporting the mutation status of 3 biomarkers might have been greater in the present study than in the UPMC study owing to the inclusion of less tissue-intensive single-gene tests. This suggests that the success rates for single-gene testing for multiple biomarkers might be even lower than reported in the present study for patients and physicians interested in the prognostic information provided by *KRAS*.

Study Limitations

The retrospective clinical testing data contained some clinical samples for which data on the number of slides cut for single-gene tests or the tumor content of the sample were missing. Analyses of tissue consumption excluded the single-gene tests in which the number of slides cut was missing. However, these analyses included 90% of all single-gene tests and samples in the data set, suggesting that the results are representative of single-gene testing at this large, US-based, Clinical Laboratory Improvement Amendments–certified, commercial, reference laboratory.

Samples for which tumor content was unknown were included and evaluated separately in the subanalyses of CNB samples stratified by tumor content. The subanalyses found that the tissue consumption and success rates among these samples were very low compared with that from the samples for which the tumor content was known. This suggests that these samples might have been smaller or might have had lower tumor content. Therefore, the true success rates for CNB samples with low tumor content ($<$ 25%) might be lower than reported in the present study.

The clinical samples included in the present analysis were submitted from September 2015 to October 2016. At that time, the only FDA-approved, National Comprehensive Cancer Network guideline-recommended, first-line targeted therapies for aNSCLC patients were for *EGFR* or *ALK* activating mutations.[15] As expected, the *EGFR* therascreen and *ALK* Vysis were consistently the most commonly ordered single-gene tests in the present study, and $\geq$ 3 single-gene tests were ordered for only 14.6% (n = 205 of 1402) of the clinical samples. Since then, targeted therapies for aNSCLC patients with *ROS1* or *BRAF* activating mutations have been approved by the FDA and recommended by the National Comprehensive Cancer Network guidelines.[6] With these developments, the genetic testing ordering patterns in clinical practice are likely to include single-gene tests for *ROS1* and *BRAF* more frequently than was described in the present study. Furthermore, with more genes identified as therapeutically relevant to aNSCLC, it is more likely that additional biomarkers will be ordered on a single sample. Therefore, the distribution of genetic testing order patterns described in the present study might no longer be representative of the current single-gene testing paradigm, and the results among samples with larger numbers of single-gene tests ordered might be more applicable to the molecular assessment needs for targeted treatment selection for patients with aNSCLC in current clinical practice.

The sample characteristics available for both sets of samples were limited to sample type and tumor content. Other potentially relevant characteristics were unknown and could limit the comparability of these findings on clinical single-gene testing and the investigational Oncomine Dx Target Test. The clinical single-gene testing was run on a large number of real patient samples submitted for testing and is therefore likely to be reflective of clinical practice and real-world genetic testing. In comparison, relatively fewer Oncomine Dx Target Tests were included in the present study, and the archival tissue samples were purchased commercially, were more likely from surgical resections, and had lower tumor content regardless of sample type. Therefore, the results with the Oncomine Dx Target Test were presented by sample type and, for CNBs, by tumor content group. However, other unobserved characteristics could have also influenced the success rates. Future prospective studies might benefit from evaluating more runs of the FDA-approved Oncomine Dx Target Test ordered by physicians as part of real-world clinical testing and collecting and controlling for more sample characteristics.

Finally, the retrospective data on the investigational use of the Oncomine Dx Target Test were from tests run using a previous study's protocol which limited each test to using only a single slide. This differs from the FDA-approved protocol, which has recommended 2 tissue slides to test surgical resection samples and 9 tissue slides to test CNB samples. Therefore, the potential mean tissue consumption of the newly FDA-approved Oncomine Dx Target Test for clinical use cannot be estimated from our results. Both tissue consumption and success rates might be greater in clinical settings in which additional slides can be used.

Conclusion

The large majority of lung cancer samples submitted for genetic testing were small tissue samples. Sequential single-gene testing can require large numbers of tissue slides and might not be able to determine the mutation status for all relevant biomarkers for aNSCLC, especially for patients with smaller sample types or lower tumor content samples. This highlights the need for tissue stewardship and efficient molecular assessment methods for lung cancer, especially as new targeted therapies are developed for activating mutations in additional genes. The present preliminary assessment of the investigational use of the Oncomine Dx Target Test suggests that it could provide a viable option for testing multiple biomarkers with fewer slides required for patients with aNSCLC. Additional studies of this test in the clinical setting would be useful to inform aNSCLC genetic testing decisions and strategies to inform treatment selection.

Clinical Practice Points

- Several targeted aNSCLC treatments have shown the potential to improve outcomes by delaying disease progression for patients with activating mutations.

- Directing patients to appropriate treatment rests on the ability to complete multiple biomarker genetic testing using the small tissue samples available for lung cancer patients.
- Tissue stewardship is essential as more treatments are developed and more genes need to be tested.
- The present retrospective analysis of clinical testing for lung cancer patients showed that sequential single-gene testing required increasing tissue consumption and had decreasing rates of successful tests completion as the number of genes tested increased.
- Investigational use of the Oncomine Dx Target Test on 1 tissue slide per test demonstrated success rates comparable to, or better than, those with single-gene testing for 2 genes on a surgical resection sample, 4 genes on an FNA sample, and 5 genes on a CNB sample, suggesting that it might facilitate multiple biomarker assessments on small tissue samples to inform treatment decisions for aNSCLC patients.

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Disclosure

T.M.Y., A.T., and A.J.L. are employees of Navigant Consulting, Inc. C.M. is an OmniSeq employee and stockholder and an employee of Roswell Park Cancer Institute. The remaining author declares that he has no competing interests.

Supplemental Data

Supplemental table accompanying this article can be found in the online version at https://doi.org/10.1016/j.cllc.2018.08.010.

References

1. American Cancer Society. Cancer Facts & Figures 2017, Available at: https://www.cancer.org/research/cancer-facts-statistics/all-cancer-facts-figures/cancer-facts-figures-2017.html. Accessed June 5, 2018.
2. American Cancer Society. Lung Cancer (Non-Small Cell) 2016, Available at: https://www.cancer.org/cancer/non-small-cell-lung-cancer.html. Accessed June 5, 2018.
3. Morgensztern D, Ng SH, Gao F, Govindan R. Trends in stage distribution for patients with non-small cell lung cancer: a National Cancer Database survey. *J Thorac Oncol* 2010; 5:29-33.
4. Abdel-Rahman O. Impact of the staging method on the prognostic utility of the 8th AJCC staging system for non-small-cell lung cancer. *Future Oncol* 2017; 13:2277-84.
5. American Cancer Society. Non-Small Cell Lung Cancer Survival Rates, by Stage 2017, Available at: https://www.cancer.org/cancer/non-small-cell-lung-cancer/detection-diagnosis-staging/survival-rates.html. Accessed July 17, 2018.
6. National Comprehensive Cancer Network®. NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) Non-Small Cell Lung Cancer. Version 8.2017, Available at: https://www.nccn.org/professionals/physician_gls/default.aspx. Accessed August 29, 2017.
7. Rosell R, Carcereny E, Gervais R, et al. Erlotinib versus standard chemotherapy as first-line treatment for European patients with advanced EGFR mutation-positive non-small-cell lung cancer (EURTAC): a multicentre, open-label, randomised phase 3 trial. *Lancet Oncol* 2012; 13:239-46.
8. Solomon BJ, Mok T, Kim D-W, et al. First-line crizotinib versus chemotherapy in ALK-positive lung cancer. *N Engl J Med* 2014; 371:2167-77.
9. Schneider F, Smith MA, Lane MC, Pantanowitz L, Dacic S, Ohori NP. Adequacy of core needle biopsy specimens and fine-needle aspirates for molecular testing of lung adenocarcinomas. *Am J Clin Pathol* 2015; 143:193-200, quiz 306.
10. Doroshow JH. Update: NCI Formulary, NCI-MATCH Trial, NCI Patient-Derived Models Repository. National Cancer Institutes of Health 2017, Available at: https://deainfo.nci.nih.gov/advisory/joint/0617/Doroshow.pdf. Accessed September 6, 2017.
11. Flaherty KT, Chen AP, O'Dwyer PJ, et al. *NCI-Molecular Analysis for Therapy Choice (NCI-MATCH or EAY131): Interim Analysis Results*. National Cancer Institutes of Health; 2016.
12. Food and Drug Administration. Summary of Safety and Effectiveness Data 2017, Available at: https://www.accessdata.fda.gov/cdrh_docs/pdf16/P160045B.pdf. Accessed April 8, 2018.
13. US Department of Health and Human Services. Protection of Human Subjects. US Department of Health and Human Services. Vol 45, part 46. HHS.gov 2009, Available at: https://www.hhs.gov/ohrp/regulations-and-policy/regulations/45-cfr-46/index.html. Accessed September 28, 2017.
14. Food and Drug Administration. Oncomine™ Dx Target Test Part I: Sample Preparation and Quantification User Guide. Revision C.0 2017, Available at: https://www.accessdata.fda.gov/cdrh_docs/pdf16/P160045C.pdf. Accessed March 22, 2018.
15. National Comprehensive Cancer Network®. NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) Non-Small Cell Lung Cancer. Version 4.2016, Available at: https://www.nccn.org/professionals/physician_gls/default.aspx. Accessed June 17, 2016.

Supplemental Table 1 Gene Variants Included in the Oncomine™ Dx Target Test

Gene	Display Name	Amino Acid Change	Nucleotide Change	Hotspot ID
AKT1		p.Glu17Lys	c.49G>A	COSM33765
ALK		p.Arg1275Gln	c.3824G>A	COSM28056
ALK		p.Arg1275Leu	c.3824G>T	COSM28060
ALK		p.Cys1156Tyr	c.3467G>A	COSM99136
ALK		p.Gly1128Ala	c.3383G>C	COSM98475
ALK		p.Gly1202Arg	c.3604G>A	COSM144250
ALK		p.Ile1171Asn	c.3512T>A	COSM28498
ALK		p.Ile1171Thr	c.3512T>C	COSM4381100
ALK		p.Leu1152Arg	c.3455T>G	COSM97185
ALK		p.Leu1152Pro	c.3455T>C	COSM1407659
ALK		p.Leu1196Gln	c.3587T>A	COSM1169447
ALK		p.Leu1196Met	c.3586C>A	COSM99137
ALK		p.Phe1174Cys	c.3521T>G	COSM28059
ALK		p.Phe1174Ile	c.3520T>A	COSM28491
ALK		p.Phe1174Leu	c.3522C>G	COSM28061
ALK		p.Phe1174Leu	c.3522C>A	COSM28055
ALK		p.Phe1174Leu	c.3520T>C	COSM28057
ALK		p.Phe1174Ser	c.3521T>C	COSM53063
ALK		p.Phe1174Val	c.3520T>G	COSM28054
ALK		p.Phe1245Cys	c.3734T>G	COSM28500
ALK		p.Phe1245Ile	c.3733T>A	COSM28492
ALK		p.Phe1245Leu	c.3735C>G	COSM28062
ALK		p.Phe1245Leu	c.3735C>A	COSM28493
ALK		p.Phe1245Val	c.3733T>G	COSM28499
ALK		p.Ser1206Tyr	c.3617C>A	COSM144251
ALK		p.Val1180Leu	c.3538G>C	COSM4381101
BRAF	BRAF V600E	p.Val600Glu	c.1799T>A	COSM476
BRAF	BRAF V600E	p.Val600Glu	c.1799_1800delTGin sAA	COSM475
BRAF		p.Asp594Asn	c.1780G>A	COSM27639
BRAF		p.Asp594Gly	c.1781A>G	COSM467
BRAF		p.Gly466Glu	c.1397G>A	COSM453
BRAF		p.Gly466Val	c.1397G>T	COSM451
BRAF		p.Gly469Ala	c.1406G>C	COSM460
BRAF		p.Gly469Arg	c.1405G>A	COSM457
BRAF		p.Gly469Val	c.1406G>T	COSM459
BRAF		p.Lys601Glu	c.1801A>G	COSM478
BRAF		p.Val600Arg	c.1798_1799delGTinsAG	COSM474
BRAF		p.Val600Lys	c.1798_1799delGTinsAA	COSM473
BRAF		p.Val600_Lys601delinsGlu	c.1799_1801delTGA	COSM1133
CDK4		p.Arg24Cys	c.70C>T	COSM1677139
CDK4		p.Arg24His	c.71G>A	COSM1989836
CDK4		p.Arg24Leu	c.71G>T	COSM363684
CDK4		p.Arg24Ser	c.70C>A	COSM3463914
CDK4		p.Lys22Arg	c.65A>G	COSM232013
CDK4		p.Lys22Gln	c.64A>C	OM3153
CDK4		p.Lys22Met	c.65A>T	COSM3463915
DDR2		p.Arg124Leu	c.371G>T	COSM400880
DDR2		p.Arg124Trp	c.370C>T	COSM4024594
EGFR	EGFR Exon 19 deletion	p.Glu746_Ala750del	c.2235_2249delGGAA TTAAGAGAAGC	COSM6223
EGFR	EGFR Exon 19 deletion	p.Glu746_Ala750del	c.2236_2250delGAAT TAAGAGAAGCA	COSM6225
EGFR	EGFR Exon 19 deletion	p.Glu746_Arg748del	c.2239_2247delTTAA GAGAA	COSM6218
EGFR	EGFR Exon 19 deletion	p.Glu746_Glu749del	c.2235_2246delGGAA TTAAGAGA	COSM28517

Supplemental Table 1 Continued

Gene	Display Name	Amino Acid Change	Nucleotide Change	Hotspot ID
EGFR	EGFR Exon 19 deletion	p.Glu746_Ser752delinsAsp	c.2238_2255delATTA AGAGAAGCAACATC	COSM6220
EGFR	EGFR Exon 19 deletion	p.Glu746_Ser752delinsVal	c.2237_2255delAATT AAGAGAAGCAACATCi T	COSM12384
EGFR	EGFR Exon 19 deletion	p.Glu746_Thr751del	c.2236_2253delGAAT TAAGAGAAGCAACA	COSM12728
EGFR	EGFR Exon 19 deletion	p.Glu746_Thr751delinsAla	c.2237_2251delAATT AAGAGAAGCAA	COSM12678
EGFR	EGFR Exon 19 deletion	p.Glu746_Thr751delinsIle	c.2235_2252delGGAA TTAAGAGAAGCAACins AT	COSM13551
EGFR	EGFR Exon 19 deletion	p.Glu746_Thr751delinsValA la	c.2237_2253delAATT AAGAGAAGCAACAinsT GCT	COSM12416
EGFR	EGFR Exon 19 deletion	p.Leu747_Ala750delinsPro	c.2239_2248delTTAA GAGAAGinsC	COSM12382
EGFR	EGFR Exon 19 deletion	p.Leu747_Ala750delinsPro	c.2238_2248delATTA AGAGAAGinsGC	COSM12422
EGFR	EGFR Exon 19 deletion	p.Leu747_Pro753delinsGln	c.2239_2258delTTAA GAGAAGCAACATCTCC sCA	COSM12387
EGFR	EGFR Exon 19 deletion	p.Leu747_Pro753delinsSer	c.2240_2257delTAAG AGAAGCAACATCTC	COSM12370
EGFR	EGFR Exon 19 deletion	p.Leu747_Ser752del	c.2239_2256delTTAA GAGAAGCAACATCT	COSM6255
EGFR	EGFR Exon 19 deletion	p.Leu747_Thr751del	c.2240_2254delTAAG AGAAGCAACAT	COSM12369
EGFR	EGFR Exon 19 deletion	p.Leu747_Thr751delinsGln	c.2238_2252delATTA AGAGAAGCAACinsGCA	COSM12419
EGFR	EGFR Exon 19 deletion	p.Leu747_Thr751delinsPro	c.2239_2251delTTAA GAGAAGCAAinsC	COSM12383
EGFR	EGFR Exon 19 deletion	p.Leu747_Thr751delinsSer	c.2240_2251delTAAG AGAAGCAA	COSM6210
EGFR	EGFR L858R	p.Leu858Arg	c.2573T>G	COSM6224
EGFR	EGFR Exon 19 deletion	p.Lys745_Ala750delinsThr	c.2234_2248delAGGA ATTAAGAGAAG	COSM1190791
EGFR	EGFR Exon 19 deletion	p.Lys745_Glu749del	c.2233_2247delAAGG AATTAAGAGAA	COSM26038
EGFR		p.Arg108Gly	c.322A>G	COSM1451536
EGFR		p.Leu861Arg	c.2582T>G	COSM12374
EGFR		p.Ala289Asp	c.866C>A	COSM21685
EGFR		p.Ala289Thr	c.865G>A	COSM21686
EGFR		p.Ala289Val	c.866C>T	COSM21687
EGFR		p.Arg108Lys	c.323G>A	COSM21683
EGFR		p.Glu709Ala	c.2126A>C	COSM13427
EGFR		p.Glu709Gly	c.2126A>G	COSM13009
EGFR		p.Glu709Lys	c.2125G>A	COSM12988
EGFR		p.Glu709Val	c.2126A>T	COSM12371
EGFR		p.Gly598Ala	c.1793G>C	COSM3412196
EGFR		p.Gly598Val	c.1793G>T	COSM21690
EGFR		p.Gly719Ala	c.2156G>C	COSM6239
EGFR		p.Gly719Asp	c.2156G>A	COSM18425
EGFR		p.Gly719Cys	c.2155G>T	COSM6253
EGFR		p.Gly719Ser	c.2155G>A	COSM6252
EGFR		p.Leu858Met	c.2572C>A	COSM12366
EGFR		p.Leu861Gln	c.2582T>A	COSM6213
EGFR		p.Ser492Arg	c.1474A>C	COSM236671
EGFR		p.Ser492Arg	c.1476C>A	COSM236670
EGFR		p.Ser768Ile	c.2303G>T	COSM6241
ERBB2		p.Arg678Gln	c.2033G>A	COSM436498
ERBB2		p.Arg896Cys	c.2686C>T	COSM14066
ERBB2		p.Arg896His	c.2687G>A	COSM119971
ERBB2		p.Asp769His	c.2305G>C	COSM13170
ERBB2		p.Asp769Tyr	c.2305G>T	COSM1251412
ERBB2		p.Gly776Val	c.2327G>T	COSM18609
ERBB2		p.Leu755Met	c.2263T>A	COSM1205571
ERBB2		p.Leu755Pro	c.2263_2264delTTinsCC	COSM683
ERBB2		p.Ser310Phe	c.929C>T	COSM48358
ERBB2		p.Ser310Tyr	c.929C>A	COSM94225
ERBB2		p.Thr733Ile	c.2198C>T	COSM14059
ERBB2		p.Val777Leu	c.2329G>T	COSM14062
ERBB2		p.Val842Ile	c.2524G>A	COSM14065

Supplemental Table 1 Continued

Gene	Display Name	Amino Acid Change	Nucleotide Change	Hotspot ID
ERBB3		p.Ala232Thr	c.694G>A	COSM4043440
ERBB3		p.Ala232Val	c.695C>T	COSM1242239
ERBB3		p.Asp297Tyr	c.889G>T	COSM160822
ERBB3		p.Asp297Val	c.890A>T	COSM941490
ERBB3		p.Glu332Lys	c.994G>A	COSM254677
ERBB3		p.Met60Arg	c.179T>G	COSM941484
ERBB3		p.Met60Leu	c.178A>T	COSM1606366
ERBB3		p.Met60Lys	c.179T>A	COSM254678
ERBB3		p.Met91Ile	c.273G>A	COSM122890
ERBB3		p.Met91Ile	c.273G>C	COSM1299636
ERBB3		p.Val104Leu	c.310G>C	COSM160824
ERBB3		p.Val104Leu	c.310G>T	COSM191840
ERBB3		p.Val104Met	c.310G>A	COSM172423
FGFR2		p.Ala314Asp	c.941C>A	COSM49171
FGFR2		p.Asn549His	c.1645A>C	COSM250083
FGFR2		p.Asn549Lys	c.1647T>G	COSM36902
FGFR2		p.Asn549Lys	c.1647T>A	COSM36912
FGFR2		p.Asn549Ser	c.1646A>G	COSM3665553
FGFR2		p.Cys382Arg	c.1144T>C	COSM36906
FGFR2		p.Cys382Tyr	c.1145G>A	COSM915493
FGFR2		p.Lys659Asn	c.1977G>T	COSM49173
FGFR2		p.Lys659Asn	c.1977G>C	COSM683054
FGFR2		p.Lys659Glu	c.1975A>G	COSM36909
FGFR2		p.Lys659Met	c.1976A>T	COSM49175
FGFR2		p.Pro253Arg	c.758C>G	COSM49170
FGFR2		p.Pro253Leu	c.758C>T	COSM537801
FGFR2		p.Ser252Trp	c.755C>G	COSM36903
FGFR2		p.Tyr375Cys	c.1124A>G	COSM36904
FGFR2		p.Tyr375His	c.1123T>C	COSM1560916
FGFR3		p.Arg248Cys	c.742C>T	COSM714
FGFR3		p.Gly697Cys	c.2089G>T	COSM24802
FGFR3		p.Lys650Asn	c.1950G>T	COSM1428730
FGFR3		p.Lys650Gln	c.1948A>C	COSM726
FGFR3		p.Lys650Glu	c.1948A>G	COSM719
FGFR3		p.Ser249Cys	c.746C>G	COSM715
HRAS		p.Gln61Arg	c.182A>G	COSM499
HRAS		p.Gln61His	c.183G>T	COSM502
HRAS		p.Gln61His	c.183G>C	COSM503
HRAS		p.Gln61Leu	c.182A>T	COSM498
HRAS		p.Gln61Lys	c.181C>A	COSM496
HRAS		p.Gln61Pro	c.182A>C	COSM500
HRAS		p.Gly12Ala	c.35G>C	COSM485
HRAS		p.Gly12Arg	c.34G>C	COSM482
HRAS		p.Gly12Asp	c.35G>A	COSM484
HRAS		p.Gly12Cys	c.34G>T	COSM481
HRAS		p.Gly12Ser	c.34G>A	COSM480
HRAS		p.Gly12Val	c.35G>T	COSM483
HRAS		p.Gly13Arg	c.37G>C	COSM486
HRAS		p.Gly13Asp	c.38G>A	COSM490
HRAS		p.Gly13Cys	c.37G>T	COSM488
HRAS		p.Gly13Ser	c.37G>A	COSM487
HRAS		p.Gly13Val	c.38G>T	COSM489

Supplemental Table 1 Continued

Gene	Display Name	Amino Acid Change	Nucleotide Change	Hotspot ID
KIT		p.Asn822Lys	c.2466T>A	COSM1321
KIT		p.Asn822Lys	c.2466T>G	COSM1322
KIT		p.Asp419_Arg420del	c.1255_1260delGACAGG	COSM1578132
KIT		p.Asp419del	c.1255_1257delGAC	COSM29014
KIT		p.Asp579del	c.1735_1737delGAT	COSM1294
KIT		p.Asp816His	c.2446G>C	COSM1311
KIT		p.Asp816Tyr	c.2446G>T	COSM1310
KIT		p.Asp816Val	c.2447A>T	COSM1314
KIT		p.Leu576Pro	c.1727T>C	COSM1290
KIT		p.Lys642Glu	c.1924A>G	COSM1304
KIT		p.Trp557Arg	c.1669T>A	COSM1216
KIT		p.Trp557Arg	c.1669T>C	COSM1219
KIT		p.Trp557Gly	c.1669T>G	COSM1221
KIT		p.Trp557_Lys558del	c.1669_1674delTGGAAG	COSM1217
KIT		p.Trp557_Val559delinsPhe	c.1670_1675delGGAAGG	COSM1226
KIT		p.Val559Ala	c.1676T>C	COSM1255
KIT		p.Val559Asp	c.1676T>A	COSM1252
KIT		p.Val559Gly	c.1676T>G	COSM1253
KIT		p.Val559del	c.1679_1681delTTG	COSM1247
KIT		p.Val560Asp	c.1679T>A	COSM1257
KIT		p.Val654Ala	c.1961T>C	COSM12706
KIT		p.Val825Ala	c.2474T>C	COSM1323
KIT		p.Arg796Lys	c.2387G>A	COSM1600411
KRAS		p.Ala146Pro	c.436G>C	COSM19905
KRAS		p.Ala146Thr	c.436G>A	COSM19404
KRAS		p.Ala146Val	c.437C>T	COSM19900
KRAS		p.Ala59Glu	c.176C>A	COSM547
KRAS		p.Ala59Gly	c.176C>G	COSM28518
KRAS		p.Ala59Thr	c.175G>A	COSM546
KRAS		p.Gln61Arg	c.182A>G	COSM552
KRAS		p.Gln61Glu	c.181C>G	COSM550
KRAS		p.Gln61His	c.183A>T	COSM555
KRAS		p.Gln61His	c.183A>C	COSM554
KRAS		p.Gln61Leu	c.182A>T	COSM553
KRAS		p.Gln61Lys	c.181C>A	COSM549
KRAS		p.Gln61Lys	c.180_181delTCinsAA	COSM87298
KRAS		p.Gln61Pro	c.182A>C	COSM551
KRAS		p.Gly12Ala	c.35G>C	COSM522
KRAS		p.Gly12Arg	c.34G>C	COSM518
KRAS		p.Gly12Asp	c.35G>A	COSM521
KRAS		p.Gly12Cys	c.34G>T	COSM516
KRAS		p.Gly12Phe	c.34_35delGGinsTT	COSM512
KRAS		p.Gly12Ser	c.34G>A	COSM517
KRAS		p.Gly12Val	c.35G>T	COSM520
KRAS		p.Gly13Ala	c.38G>C	COSM533
KRAS		p.Gly13Arg	c.37G>C	COSM529
KRAS		p.Gly13Asp	c.38_39delGCinsAT	COSM531
KRAS		p.Gly13Asp	c.38G>A	COSM532
KRAS		p.Gly13Cys	c.37G>T	COSM527
KRAS		p.Gly13Ser	c.37G>A	COSM528
KRAS		p.Gly13Val	c.38G>T	COSM534
KRAS		p.Lys117Asn	c.351A>T	COSM28519

Supplemental Table 1 Continued

Gene	Display Name	Amino Acid Change	Nucleotide Change	Hotspot ID
KRAS		p.Lys117Asn	c.351A>C	COSM19940
MAP2K1		p.Glu203Lys	c.607G>A	COSM232755
MAP2K1		p.Glu203Val	c.608A>T	COSM3386991
MAP2K1		p.Lys57Asn	c.171G>C	OM3156
MAP2K1		p.Lys57Asn	c.171G>T	OM3157
MAP2K1		p.Lys57Met	c.170A>T	COSM1235478
MAP2K1		p.Lys57Thr	c.170A>C	OM3155
MAP2K1		p.Phe53Ile	c.157T>A	COSM3503329
MAP2K1		p.Phe53Leu	c.157T>C	COSM555604
MAP2K1		p.Phe53Leu	c.159T>A	COSM1725008
MAP2K1		p.Phe53Leu	c.159T>G	OM3154
MAP2K1		p.Phe53Val	c.157T>G	COSM1562837
MAP2K1		p.Pro124Gln	c.371C>A	COSM1167912
MAP2K1		p.Pro124Leu	c.371C>T	COSM1315861
MAP2K1		p.Pro124Ser	c.370C>T	COSM235614
MAP2K2		p.Gln60Pro	c.179A>C	COSM145610
MAP2K2		p.Phe57Leu	c.171T>G	OM3158
MAP2K2		p.Phe57Leu	c.171T>A	COSM3389034
MAP2K2		p.Phe57Leu	c.169T>C	COSM1235618
MAP2K2		p.Phe57Val	c.169T>G	COSM3534171
MET		NA	NA	COSM29633
MET		NA	NA	COSM24687
MET		NA	NA	COSM35468
MET		p.His1112Arg	c.3335A>G	COSM703
MET		p.His1112Leu	c.3335A>T	COSM698
MET		p.His1112Tyr	c.3334C>T	COSM696
MET		p.Met1268Ile	c.3804G>A	COSM694
MET		p.Met1268Thr	c.3803T>C	COSM691
MET		p.Thr1010Ile	c.3029C>T	COSM707
MET		p.Tyr1021Asn	c.3061T>A	COSM48564
MET		p.Tyr1021Phe	c.3062A>T	COSM339515
MET		p.Tyr1248Cys	c.3743A>G	COSM699
MET		p.Tyr1248His	c.3742T>C	COSM690
MET		p.Tyr1253Asp	c.3757T>G	COSM700
MTOR		p.Cys1483Arg	c.4447T>C	COSM3747775
MTOR		p.Cys1483Phe	c.4448G>T	COSM462616
MTOR		p.Cys1483Trp	c.4449C>G	OM3149
MTOR		p.Cys1483Tyr	c.4448G>A	COSM462615
MTOR		p.Glu1799Lys	c.5395G>A	COSM180789
MTOR		p.Leu2427Arg	c.7280T>G	OM3148
MTOR		p.Leu2427Gln	c.7280T>A	COSM1185313
MTOR		p.Phe1888Ile	c.5662T>A	COSM3358968
MTOR		p.Phe1888Leu	c.5664C>G	COSM462604
MTOR		p.Phe1888Leu	c.5664C>A	COSM893813
MTOR		p.Phe1888Leu	c.5662T>C	COSM3358967
MTOR		p.Phe1888Val	c.5662T>G	COSM893814
MTOR		p.Ser2215Phe	c.6644C>T	COSM1686998
MTOR		p.Ser2215Pro	c.6643T>C	COSM1560108
MTOR		p.Ser2215Tyr	c.6644C>A	COSM20417
MTOR		p.Thr1977Arg	c.5930C>G	COSM462602
MTOR		p.Thr1977Lys	c.5930C>A	COSM462601
MTOR		p.Thr1977Ser	c.5929A>T	COSM1289945

Supplemental Table 1 Continued

Gene	Display Name	Amino Acid Change	Nucleotide Change	Hotspot ID
MTOR		p.Val2006Ile	c.6016G>A	COSM893804
MTOR		p.Val2006Leu	c.6016G>C	COSM1134662
MTOR		p.Val2006Phe	c.6016G>T	COSM249481
NRAS		p.Ala146Thr	c.436G>A	COSM27174
NRAS		p.Ala146Val	c.437C>T	COSM4170228
NRAS		p.Ala59Thr	c.175G>A	COSM578
NRAS		p.Gln61Arg	c.182A>G	COSM584
NRAS		p.Gln61Glu	c.181C>G	COSM581
NRAS		p.Gln61His	c.183A>T	COSM585
NRAS		p.Gln61His	c.183A>C	COSM586
NRAS		p.Gln61Leu	c.182A>T	COSM583
NRAS		p.Gln61Lys	c.181C>A	COSM580
NRAS		p.Gln61Pro	c.182A>C	COSM582
NRAS		p.Gly12Ala	c.35G>C	COSM565
NRAS		p.Gly12Arg	c.34G>C	COSM561
NRAS		p.Gly12Asp	c.35G>A	COSM564
NRAS		p.Gly12Cys	c.34G>T	COSM562
NRAS		p.Gly12Ser	c.34G>A	COSM563
NRAS		p.Gly12Val	c.35G>T	COSM566
NRAS		p.Gly13Ala	c.38G>C	COSM575
NRAS		p.Gly13Arg	c.37G>C	COSM569
NRAS		p.Gly13Asp	c.38G>A	COSM573
NRAS		p.Gly13Cys	c.37G>T	COSM570
NRAS		p.Gly13Ser	c.37G>A	COSM571
NRAS		p.Gly13Val	c.38G>T	COSM574
NRAS		p.Lys117Asn	c.351G>T	MAN13
PDGFRA		p.Asn659Lys	c.1977C>A	COSM22415
PDGFRA		p.Asn659Lys	c.1977C>G	COSM22414
PDGFRA		p.Asn659Tyr	c.1975A>T	COSM22416
PDGFRA		p.Asp842Tyr	c.2524G>T	COSM12396
PDGFRA		p.Asp842Val	c.2525A>T	COSM736
PDGFRA		p.Asp842_His845del	c.2526_2537delCATCATGCATGA	COSM737
PDGFRA		p.Asp842_Met844del	c.2524_2532delGACATCATG	COSM12401
PDGFRA		p.Ile843_Asp846del	c.2527_2538delATCATGCATGAT	COSM12400
PDGFRA		p.Ile843_Ser847delinsThr	c.2528_2539delTCATGCATGATT	COSM12407
PDGFRA		p.Val561Asp	c.1682T>A	COSM739
PIK3CA		p.Arg108His	c.323G>A	COSM27497
PIK3CA		p.Arg38Cys	c.112C>T	COSM744
PIK3CA		p.Arg38Gly	c.112C>G	COSM40945
PIK3CA		p.Arg38His	c.113G>A	COSM745
PIK3CA		p.Arg38Ser	c.112C>A	COSM87310
PIK3CA		p.Arg88Gln	c.263G>A	COSM746
PIK3CA		p.Arg93Gln	c.278G>A	COSM86041
PIK3CA		p.Arg93Trp	c.277C>T	COSM27493
PIK3CA		p.Asn1044Lys	c.3132T>A	COSM12592
PIK3CA		p.Asn345Ile	c.1034A>T	COSM94978
PIK3CA		p.Asn345Lys	c.1035T>A	COSM754
PIK3CA		p.Cys378Arg	c.1132T>C	COSM756
PIK3CA		p.Cys378Phe	c.1133G>T	COSM21450
PIK3CA		p.Cys378Tyr	c.1133G>A	COSM1041478
PIK3CA		p.Cys420Arg	c.1258T>C	COSM757
PIK3CA		p.Cys901Arg	c.2701T>C	COSM1420899

Supplemental Table 1 Continued

Gene	Display Name	Amino Acid Change	Nucleotide Change	Hotspot ID
PIK3CA		p.Cys901Phe	c.2702G>T	COSM769
PIK3CA		p.Cys901Tyr	c.2702G>A	COSM1420901
PIK3CA		p.Gln546Arg	c.1637A>G	COSM12459
PIK3CA		p.Gln546Glu	c.1636C>G	COSM6147
PIK3CA		p.Gln546Lys	c.1636C>A	COSM766
PIK3CA		p.Gln546Pro	c.1637A>C	COSM767
PIK3CA		p.Glu365Gly	c.1094A>G	COSM1420797
PIK3CA		p.Glu365Val	c.1094A>T	COSM1484860
PIK3CA		p.Glu39Lys	c.115G>A	COSM30625
PIK3CA		p.Glu542Lys	c.1624G>A	COSM760
PIK3CA		p.Glu542Val	c.1625A>T	COSM762
PIK3CA		p.Glu545Ala	c.1634A>C	COSM12458
PIK3CA		p.Glu545Asp	c.1635G>C	COSM27374
PIK3CA		p.Glu545Asp	c.1635G>T	COSM765
PIK3CA		p.Glu545Gln	c.1633G>C	COSM27133
PIK3CA		p.Glu545Gly	c.1634A>G	COSM764
PIK3CA		p.Glu545Lys	c.1633G>A	COSM763
PIK3CA		p.Glu547Lys	c.1639G>A	COSM29315
PIK3CA		p.Glu726Gly	c.2177A>G	COSM1420887
PIK3CA		p.Glu726Lys	c.2176G>A	COSM87306
PIK3CA		p.Glu81Lys	c.241G>A	COSM27502
PIK3CA		p.Gly1049Arg	c.3145G>C	COSM12597
PIK3CA		p.Gly1049Ser	c.3145G>A	COSM777
PIK3CA		p.Gly106Val	c.317G>T	COSM748
PIK3CA		p.His1047Arg	c.3140A>G	COSM775
PIK3CA		p.His1047Leu	c.3140A>T	COSM776
PIK3CA		p.His1047Tyr	c.3139C>T	COSM774
PIK3CA		p.His701Arg	c.2102A>G	COSM1420881
PIK3CA		p.His701Pro	c.2102A>C	COSM778
PIK3CA		p.Lys111Glu	c.331A>G	COSM13570
PIK3CA		p.Met1043Ile	c.3129G>A	COSM29313
PIK3CA		p.Met1043Ile	c.3129G>T	COSM773
PIK3CA		p.Met1043Val	c.3127A>G	COSM12591
PIK3CA		p.Thr1025Ala	c.3073A>G	COSM771
PIK3CA		p.Tyr1021Cys	c.3062A>G	COSM12461
PIK3CA		p.Val344Ala	c.1031T>C	COSM86951
PIK3CA		p.Val344Gly	c.1031T>G	COSM22540
PIK3CA		p.Glu365Lys	c.1093G>A	COSM86044
PIK3CA		p.Pro539Arg	c.1616C>G	COSM759
RAF1		p.Ser257Leu	c.770C>T	COSM181063
RAF1		p.Ser257Trp	c.770C>G	COSM581519
RAF1		p.Thr421Met	c.1262_1263delCCinsTG	MAN9
RET		p.Ala883Phe	c.2646_2648delAGCinsTTT	COSM981
RET		p.Ala883Ser	c.2647G>T	COSM133167
RET		p.Asp898_Glu901del	c.2694_2705delTGTTTATGAAGA	COSM962
RET		p.Cys618Arg	c.1852T>C	COSM29803
RET		p.Cys618Tyr	c.1853G>A	COSM980
RET		p.Cys620Arg	c.1858T>C	COSM29804
RET		p.Cys634Arg	c.1900T>C	COSM966
RET		p.Glu768Asp	c.2304G>C	COSM21338
RET		p.Glu768Gly	c.2303A>G	COSM1347811
RET		p.Met918Thr	c.2753T>C	COSM965

Supplemental Table 1 Continued

Gene	Display Name	Amino Acid Change	Nucleotide Change	Hotspot ID
ROS1	ROS1 Fusion			
ROS1		p.Gly2032Arg	c.6094G>C	MAN11
ROS1		p.Gly2032Arg	c.6094G>A	MAN10
ROS1		p.Leu1951Met	c.5851C>A	COSM1072521