SUMMARY OF SAFETY AND EFFECTIVENESS DATA (SSED)

I. GENERAL INFORMATION

Device Generic Name: Next generation sequencing oncology panel, somatic or germline variant detection system

Device Trade Name: Oncomine™ Dx Target Test

Device Procode: PQP

Applicant's Name and Address: Life Technologies Corporation
5781 Van Allen Way
Carlsbad, CA 92008

Date(s) of Panel Recommendation: None

Premarket Approval Application (PMA) Number: P160045

Date of FDA Notice of Approval: June 22, 2017

Priority Review: Granted priority review status on November 8, 2016, because the device addresses an unmet medical need, as the test is based on breakthrough technology and is in the best interest of patients.

II. INDICATIONS FOR USE

The Oncomine™ Dx Target Test is a qualitative *in vitro* diagnostic test that uses targeted high throughput, parallel-sequencing technology to detect single nucleotide variants (SNVs) and deletions in 23 genes from DNA and fusions in ROS1 from RNA isolated from formalin-fixed, paraffin-embedded (FFPE) tumor tissue samples from patients with non-small cell lung cancer (NSCLC) using the Ion PGM™ Dx System.

The test is indicated to aid in selecting NSCLC patients for treatment with the targeted therapies listed in Table 1 in accordance with the approved therapeutic product labeling.

Table 1. List of variants for therapeutic use

Gene	Variant	Targeted therapy
BRAF	BRAF V600E	TAFINLAR® (dabrafenib) in combination with MEKINIST® (trametinib)
ROS1	ROS1 fusions	XALKORI® (crizotinib)
EGFR	L858R, Exon 19 deletions	IRESSA® (gefitinib)

Safe and effective use has not been established for selecting therapies using this device for the variants in Table 1 in tissue types other than NSCLC.

Results other than those listed in Table 1 are indicated for use only in patients who have already been considered for all appropriate therapies (including those listed in Table 1).

Analytical performance using NSCLC specimens has been established for the variants listed in Table 2.

Table 2. List of variants with established analytical performance only

Gene	Variant ID	Nucleotide change
KRAS	COSM512	c.34_35delGGinsTT
KRAS	COSM516	c.34G>T
MET	COSM707	c.3029C>T
PIK3CA	COSM754	c.1035T>A

The test is not indicated to be used for standalone diagnostic purposes, screening, monitoring, risk assessment, or prognosis.

III. CONTRAINDICATIONS

There are no known contraindications.

IV. WARNINGS AND PRECAUTIONS

The warnings and precautions can be found in the Oncomine™ Dx Target Test labeling.

V. DEVICE DESCRIPTION

The Oncomine™ Dx Target Test is an *in vitro* diagnostic test that provides primer panels, assay controls and interpretative software [an Assay Definition File (ADF)] designed for use with the Ion Torrent PGM Dx System and the Ion Torrent PGM Dx Reagents for detection of alterations in DNA and RNA isolated from non-small cell lung cancer (NSCLC) formalin-fixed, paraffin-embedded (FFPE) tumor specimens.

The Oncomine™ Dx Target Test consists of the following:

Oncomine™ Dx Target Test and Controls Kit (Combo Kit):
- Oncomine™ Dx Target Test DNA and RNA Panel
- Oncomine™ Dx Target DNA Control Kit
- Oncomine™ Dx Target RNA Control Kit
- Ion Torrent™ Dx No Template Control Kit

Ion Torrent™ Dx FFPE Sample Preparation Kit:
- Ion Torrent™ Dx Total Nucleic Acid Isolation Kit
- Ion Torrent™ Dx cDNA Synthesis Kit
- Ion Torrent™ Dx DNA Quantification Kit
- Ion Torrent™ Dx RNA Quantification Kit
- Ion Torrent™ Dx Dilution Buffer Kit

Ion Torrent™ PGM™ Dx Reagents / Chips:

- Ion PGM™ Dx Library Kit
- Ion OneTouch™ Dx Template Kit
- Ion PGM™ Dx Sequencing Kit
- Ion 318™ Dx Chip Kit

Instrumentation and Software:

- The assay is run on the Ion Torrent™ PGM™ Dx System:
  - Ion OneTouch™ Dx System:
    - Ion OneTouch™ Dx Instrument
    - Ion OneTouch™ ES Dx Instrument
  - Ion PGM™ Dx Sequencer
  - Ion PGM™ Dx Chip Minifuge
  - Ion Torrent™ Server
  - Torrent Suite™ Dx Software
  - Other accessories:
    - Ion PGM™ Wireless Scanner
    - DynaMag™ 16 2mL Dx Magnet
    - DynaMag™ 96 Side Dx Magnet

The system also utilizes specified accessories. The assay's definition files are provided on a USB memory device along with the Oncomine™ Dx Target Test User Guides and ADF on a USB Memory Device:

- Oncomine™ Dx Target Assay Definition File (includes interpretive software)
- Oncomine™ Dx Target Test User Guide
- Veriti™ Dx Thermal Cycler Settings
- Electronic Document Instructions (provided to users both as a paper copy and a PDF document on the USB drive)

Nucleic Acid Extraction:

DNA and RNA extraction is performed using the proprietary Ion Torrent™ Dx FFPE Sample Preparation Kit. The deparaffinized sample is first subjected to protein digestion with Proteinase K at an elevated temperature in a guanidinium thiocyanate solution to facilitate release and protection of RNA and DNA by inhibiting nuclease activity. After a heating step to inactive the Proteinase K enzyme, the digested sample is transferred into a spin column containing a silica-based filter membrane.

The RNA is selectively eluted and separated from DNA which is retained on the filter. The eluted RNA is mixed with ethanol and captured onto a second spin column containing a silica-based membrane filter. The RNA is retained and cellular impurities are removed by a series of washes. The bound RNA is treated with DNase to reduce contaminating DNA. Following a series of washes to remove residual DNase and DNA degradation products, the purified RNA is eluted from the filter.

The DNA retained on the first filter is similarly subjected to a series of washes to remove cellular impurities and then purified DNA is eluted from the filter. The Elution Solution provided with the kit is a low ionic strength Tris-buffered solution containing EDTA that facilitates elution of nucleic acids from the silica filter. The solution provides appropriate pH for stability of RNA and DNA and inhibits nucleases by binding metal cofactors.

Quantification:
RNA and DNA quantification is performed using a fluorescence dye-binding assay and a qualified fluorometer/fluorescence reader capable of operating at the specific excitation and emission wavelengths. First, working solutions consisting of buffer and proprietary fluorophores are prepared for both DNA and RNA samples, as well as the DNA and RNA standards supplied at different concentrations in the kit (0 ng/µL to 10 ng/µL). Second, the DNA and RNA samples are incubated with their respective solutions at room temperature where the fluorophores bind to the target DNA and RNA molecules. When bound to the DNA and RNA, the fluorophores exhibit fluorescence enhancement at a specific excitation wavelength. The emitted fluorescent signals are captured and converted into signal fluorescence units. Third, the concentration (in ng/µL) of the DNA and RNA samples are determined by performing a linear regression with the values obtained from the DNA and RNA standards.

Sample Dilution Buffer is provided in the kit to dilute the DNA and RNA samples to a specific concentration required for cDNA synthesis and library preparation.

RT Step (RNA only):
RNA is enzymatically converted to cDNA using the Ion Torrent™ Dx cDNA Synthesis Kit. Ten nanograms (ng) of RNA is enzymatically converted to cDNA using an enzyme mix containing a proprietary engineered version of M-MLV reverse transcriptase (Superscript III RT), an RNase inhibitor, a proprietary helper protein, and a buffer containing random primers, dNTPs, and $MgCl_2$.

Library Preparation workflow:
The process begins with polymerase chain reaction (PCR) and uses the Oncomine™ Dx Target Test DNA and RNA Panel and the Ion PGM™ Dx Library Kit to specifically amplify target regions of interest from cDNA (including cDNA from the RNA control) and DNA (including the DNA Control and No Template Control).

Two different libraries are generated and pooled for each sample; one for DNA targets and one for RNA targets. During library preparation for each sample, one of the 16 oligonucleotide barcodes in the Library Kit is used for the DNA-derived library and another oligonucleotide barcode is used for the RNA-derived library. This ensures the correct identification of each respective portion of the assay (DNA and RNA) from each patient sample. After library preparation, the DNA and RNA libraries for all samples and controls may be blended for the templating reaction.

Data Analysis:

This process is executed by the Torrent Suite™ Dx software, which runs on the Ion Torrent™ Server. Together, these manage the complete end-to-end workflow from sample to variant call. The DNA reads are 'mapped' to the reference human genome (hg19) followed by detection of single nucleotide variants (SNV) and deletions (del) using a reference hotspot file. The RNA reads are 'mapped' to a reference containing control sequences and candidate gene fusion sequences. Gene fusions are detected as present if they map to these reference sequences and pass certain filtering criteria provided by the Oncomine™ Dx Target ADF.

VI. ALTERNATIVE PRACTICES AND PROCEDURES

There are no FDA-cleared or -approved alternatives for BRAF mutation testing of formalin-fixed, paraffin-embedded (FFPE) NSCLC tissue for the selection of patients who are eligible for treatment with TAFINLAR® (dabrafenib) in combination with MEKINIST® (trametinib). There are no FDA-cleared or -approved alternatives for ROS1 translocation testing of FFPE NSCLC tissue for the selection of patients who are eligible for treatment with XALKORI® (crizotinib). There is one FDA-approved alternative, the QIAGEN *therascreen*® RGQ PCR Test, for EGFR (Exon 19 deletions and L858R mutation) testing of FFPE NSCLC tissue for the selection of patients who are eligible for treatment with IRESSA® (gefitinib). Each alternative has its own advantages and disadvantages. A patient should fully discuss any alternative with his/her physician to select the most appropriate method.

VII. MARKETING HISTORY

The Oncomine™ Dx Target Test has not been marketed in the United States or any foreign country.

VIII. POTENTIAL ADVERSE EFFECTS OF THE DEVICE ON HEALTH

Failure of the device to perform as expected or failure to correctly interpret test results may lead to incorrect Oncomine™ Dx Target Test results and subsequently improper patient management decisions in NSCLC treatment. There is also a risk of delayed results, which may lead to delay of treatment with the appropriate targeted therapy. No adverse events were reported in connection with the clinical studies used to support this PMA as the studies were performed retrospectively using banked samples.

IX. SUMMARY OF NONCLINICAL STUDIES

A. Laboratory Studies

1. Analytical Accuracy
   Concordance of the Oncomine™ Dx Target Test was assessed for the ability to detect SNVs, deletions, and fusions with two externally validated comparator methods. Four hundred twenty-two (422) of the 436 SNVs and deletion hotspot variants that could be detected by the Oncomine™ Dx Target Test were included

in an independently developed and validated NGS assay for SNVs and deletions. In addition, a validated ROS1 FISH test, consistent with the test used in the A8081001 clinical ROS1 study, was used as the comparator method to detect ROS1 fusions. Two additional comparator methods were included in the clinical concordance studies for BRAF V600E and EGFR for Exon 19 deletions (Ex. 19del) and L858R variants.

A bank of 566 uncharacterized FFPE tumor samples were procured and sent to the reference testing labs to be screened and enrolled into the study on a rolling basis until a minimum of 275 samples were identified per the study protocol.

A total of 461 samples were tested by the validated NGS comparator method with 291 samples (63.1%) yielding valid results. Eight (8) of the 291 positive samples were invalid for the ROS1 FISH test but were included in the analysis for the other valid variants and 1 of the 291 sample was not tested by the ROS1 FISH test. Among the 290 samples, 178 (61.4%) were identified as being positive for at least one alteration. The samples included the following variant types: simple SNVs, complex SNVs [SNVs in di-/tri-nucleotide repeat regions and multi-nucleotide variant (MNV)], and deletions that are targeted by the Oncomine™ Dx Target Test.

For ROS1, a total of 469 samples were tested with the validated ROS1 FISH comparator method and 392/469 (83.6%) samples generated valid results, while 77/469 (16.4%) samples failed the comparator test due to poor hybridization of the probes.

All 290 samples were then tested with the Oncomine™ Dx Target Test. One of the 290 samples (positive by comparator method) failed both DNA and RNA sample QC metrics. In addition, 22 of the 290 RNA samples failed to meet the RNA sample QC metrics and were considered invalid for fusion variant analysis.

The results at the variant, bin and sample levels are shown in the tables below.

Table 3. Overall agreement results at bin level.

Level	Agree-ment	No Calls Included			No Calls Excluded		
		# Comp[1]	# ODx[2]	% (95% CI)	# Comp	# ODx	% (95% CI)
Variant	PPA[3]	198	195	98.50% (95.6%, 99.7%)	198	195	98.50% (95.6%, 99.7%)
	NPA[4]	122,012	118,155	96.80% (96.7%, 96.9%)	118,159	118,155	99.997% (99.991%, 99.999%)
Bin	PPA	181	176	97.20% (93.7%, 99.1%)	181	176	97.20% (93.7%, 99.1%)
	NPA	939	657	70.00% (66.9%, 72.9%)	944	942	99.80% (99.2%, 100%)
Sample	PPA	163	158	96.90%	163	158	96.90%

Level	Agree-ment	No Calls Included			No Calls Excluded		
		# Comp[1]	# ODx[2]	% (95% CI)	# Comp	# ODx	% (95% CI)
				(93.0%, 99.0%)			(93.0%, 99.0%)
	NPA	124	29	23.40% (16.3%, 31.8%)	126	124	98.40% (94.4%, 99.8%)

[1] # Comp = number of comparator results
[2] # ODx = number of Oncomine Dx Target Test results
[3] PPA = positive percent agreement = [(# ODx positive calls) ÷ (# Comp positive calls)] x 100
[4] NPA = negative percent agreement = [(# ODx negative calls) ÷ (# Comp negative calls)] x 100

Table 4. Agreement results by bin type.

Bin	Agree-ment	No Calls Included			No Calls Excluded		
		# Comp	# ODx	% (95% CI)	# Comp	# ODx	% (95% CI)
Simple SNV	PPA	83	82	98.80% (93.47%, 99.97%)	83	82	98.80% (93.47%, 99.97%)
	NPA	200	65	32.50% (26.06%, 39.47%)	206	206	100.00% (98.23%, 100.00%)
Complex SNV	PPA	85	83	97.65% (91.76%, 99.71%)	85	83	97.65% (91.76%, 99.71%)
	NPA	203	82	40.39% (33.58%, 47.49%)	204	202	99.02% (96.50%, 99.88%)
Deletion	PPA	11	11	100.00% (71.51%, 100.0%)	11	11	100.00% (71.51%, 100.0%)
	NPA	278	252	90.65% (86.60%, 93.80%)	276	276	100.00% (98.67%, 100.0%)
Fusion	PPA	2	0	0.00% (0%, 84.2%)	2	0	0.00% (0%, 84.2%)
	NPA	258	258	100.00% (98.6%, 100.0%)	258	258	100.00% (98.6%, 100.0%)

Table 5. Overall PPA results, at variant level, by gene.

Gene	No Calls Included			No Calls Excluded		
	# Comp (+)	# ODx (+)	PPA (95% CI)	# Comp (+)	# ODx (+)	PPA (95% CI)
AKT1	2	2	100.00% (15.8%, 100%)	2	2	100.00% (15.8%, 100%)
ALK	0	0	NA	0	0	NA
BRAF	26	25	96.20% (80.4%, 99.9%)	26	25	96.20% (80.4%, 99.9%)
CDK4	0	0	NA	0	0	NA
DDR2	0	0	NA	0	0	NA
EGFR	16	16	100.00% (79.4%, 100%)	16	16	100.00% (79.4%, 100%)

Gene	No Calls Included			No Calls Excluded		
	# Comp (+)	# ODx (+)	PPA (95% CI)	# Comp (+)	# ODx (+)	PPA (95% CI)
ERBB2	4	4	100.00% (39.8%, 100%)	4	4	100.00% (39.8%, 100%)
ERBB3	1	1	100.00% (2.5%, 100%)	1	1	100.00% (2.5%, 100%)
FGFR2	0	0	NA	0	0	NA
FGFR3	2	2	100.00% (15.8%, 100%)	2	2	100.00% (15.8%, 100%)
HRAS	4	4	100.00% (39.8%, 100%)	4	4	100.00% (39.8%, 100%)
KIT	0	0	NA	0	0	NA
KRAS	80	80	100.00% (95.5%, 100%)	80	80	100.00% (95.5%, 100%)
MAP2K1	1	1	100.00% (2.5%, 100%)	1	1	100.00% (2.5%, 100%)
MAP2K2	0	0	NA	0	0	NA
MET	7	7	100.00% (59%, 100%)	7	7	100.00% (59%, 100%)
MTOR	2	2	100.00% (15.8%, 100%)	2	2	100.00% (15.8%, 100%)
NRAS	9	9	100.00% (66.4%, 100%)	9	9	100.00% (66.4%, 100%)
PDGFRA	0	0	NA	0	0	NA
PIK3CA	38	38	100.00% (90.7%, 100%)	38	38	100.00% (90.7%, 100%)
ROS1 (fusion)	2	0	0.00% (0%, 84.2%)	2	0	0.00% (0%, 84.2%)
RET	0	0	NA	0	0	NA
SMO	0	0	NA	0	0	NA

Table 6. Overall NPA results, at variant level, by gene.

Gene	No Calls Included			No Calls Excluded		
	# Comp (-)	# ODx (-)	NPA (95% CI)	# Comp (-)	# ODx (-)	NPA (95% CI)
AKT1	287	238	82.90% (78.1%, 87.1%)	238	238	100.00% (98.46%, 100%)
ALK	7225	7005	97.00% (96.5%, 97.3%)	7005	7005	100.00% (99.9%, 100%)
BRAF	3730	3621	97.10% (96.5%, 97.6%)	3621	3621	100.00% (99.9%, 100%)

Gene	No Calls Included			No Calls Excluded		
	# Comp (-)	# ODx (-)	NPA (95% CI)	# Comp (-)	# ODx (-)	NPA (95% CI)
CDK4	2023	1950	96.40% (95.5%, 97.2%)	1950	1950	100.00% (99.81%, 100%)
DDR2	578	539	93.30% (90.9%, 95.2%)	539	539	100.00% (99.32%, 100%)
EGFR	12700	12497	98.40% (98.2%, 98.6%)	12498	12497	99.99% (99.96%, 100%)
ERBB2	3753	3685	98.20% (97.7%, 98.6%)	3685	3685	100.00% (99.9%, 100%)
ERBB3	3756	3731	99.30% (99%, 99.6%)	3731	3731	100.00% (99.9%, 100%)
FGFR2	4624	4482	96.90% (96.4%, 97.4%)	4482	4482	100.00% (99.92%, 100%)
FGFR3	1732	1663	96.00% (95%, 96.9%)	1663	1663	100.00% (99.78%, 100%)
HRAS	4909	4687	95.50% (94.9%, 96%)	4688	4687	99.98% (99.88%, 100%)
KIT	6647	6504	97.80% (97.5%, 98.2%)	6504	6504	100.00% (99.94%, 100%)
KRAS	8590	8318	96.80% (96.4%, 97.2%)	8319	8318	99.99% (99.93%, 100%)
MAP2K1	4045	3938	97.40% (96.8%, 97.8%)	3938	3938	100.00% (99.91%, 100%)
MAP2K2	1445	1408	97.40% (96.5%, 98.2%)	1408	1408	100.00% (99.74%, 100%)
MET	4039	3952	97.80% (97.3%, 98.3%)	3952	3952	100.00% (99.91%, 100%)
MTOR	6067	5773	95.20% (94.6%, 95.7%)	5773	5773	100.00% (99.94%, 100%)
NRAS	6637	6528	98.40% (98%, 98.6%)	6528	6528	100.00% (99.94%, 100%)
PDGFRA	2890	2860	99.00% (98.5%, 99.3%)	2860	2860	100.00% (99.87%, 100%)
PIK3CA	15857	15183	95.70% (95.4%, 96.1%)	15184	15183	99.99% (99.96%, 100%)
RET	2890	2767	95.70% (94.9%, 96.5%)	2767	2767	100.00% (99.9%, 100%)
ROS1	547	544	99.50% (98.4%, 99.9%)	544	544	100.00% (99.32%, 100%)

Gene	No Calls Included			No Calls Excluded		
	# Comp (-)	# ODx (-)	NPA (95% CI)	# Comp (-)	# ODx (-)	NPA (95% CI)
SMO	578	570	98.60% (97.3%, 99.4%)	570	570	100.00% (99.35%, 100%)

2. Analytical Sensitivity:

   a. Limit of Blank:
   To assess the performance of the Oncomine™ Dx Target Test in the absence of template and to ensure that a variant-free ("blank") sample does not generate an analytical signal that might be classified as a mutation, wild-type (WT) samples were evaluated. Three (3) FFPE clinical samples that were WT for the targeted DNA variants and RNA fusion locations and five FFPE cell line samples that were WT at all locations, were included in this study. The samples were tested using two different lots of the Oncomine™ Dx Target Test. Each sample was extracted once. The clinical samples were then prepared into twelve independent DNA and RNA libraries; and six independent DNA and RNA libraries for the cell line samples. Sequencing was carried out in duplicate for each of the libraries. For all 8 samples, there were no positive calls at any of the variant locations analyzed by the test. The false positive rate was therefore 0%, and the limit of blank (LoB) of the test is zero.

   b. Limit of Detection:
   The limit of detection (LoD) based on positive calls for the Oncomine™ Dx Target Test was estimated to determine the lowest allele frequency (AF) of SNV (simple and complex), MNV or deletion variants, and the lowest number of RNA fusion reads, at which 95% of the test replicates produced correct calls. A total of 26 specimens (19 FFPE tissue samples and 7 plasmid constructs) were evaluated and included samples with deletions ranging up to 18 nucleotides, MNVs [two base pair (bp) substitution) and SNVs, including variants in di- or trinucleotide repeat regions and in regions with 38% to 73% GC content. Samples with gene fusions in ROS1 were also included in this study. At least 6 titration levels were tested and each level was tested with 10 replicates per sample for each of the two reagent lots.

   The LoD is based on the highest %AF (DNA-based variants) or number of reads (RNA-based variants) with 95% correct calls observed for each representative variant. The claimed LoD for the variants for which clinical claims will be made are noted in Table 7 below, which were confirmed in the external panel reproducibility study. The LoDs for DNA variants were determined to have AFs of 6-13%, and the LoD for the ROS1 RNA fusion was measured at 732 fusion reads. Data to confirm the estimated LoDs for both DNA and RNA variants will be provided in an additional reproducibility study (see IX.A.5.a, below).

Table 7. Estimated LoD for clinically relevant variants.

Gene	Variant	Variant Category	LoD[1] (%AF or # Reads)
BRAF	V600E	SNV	12% AF
EGFR	L858R	SNV	8% AF
EGFR	Ex. 19del	Deletion	6% AF
ROS1	ROS1 Fusion	RNA Fusion	732 Reads

[1]Clinical specimens were tested for all variants for which clinical claims are being sought.

c. DNA/RNA Input:
This study was conducted in two parts using samples which represented the SNV (simple and complex), deletions, and fusion variant types targeted by the Oncomine™ Dx Target Test. The initial study utilized 7 variant positive and WT cell lines containing the BRAF V600E, EGFR L858R, and EGFR Ex. 19del DNA variants and a ROS1 RNA fusion. DNA and RNA isolated from multiple 10 µm FFPE cell line specimens. The DNA and RNA for the individual variants were pooled, quantified, and blended with the WT samples to the target AF (15%) or fusion target reads (300-600 fusion reads). Fifteen (15) input level combinations were tested for each sample consisting of 5 DNA input levels and 5 RNA input levels ranging from three levels below the standard of 10 ng (5 ng, 6.5 ng, and 8.5 ng), the standard input (10 ng) and one level (15 ng) above the standard input were tested in combination with 3 levels (5 ng, 10 ng, and 15 ng) of RNA or DNA respectively. The variants present in all FFPE DNA and RNA sample blends tested were called correctly 100% of the time across all DNA/RNA test combinations, including the 10 ng required input

Based on the results from the initial study, a second study using FFPE clinical specimens was conducted to confirm that the Oncomine™ Dx Target Test was able to detect variants accurately at the DNA and RNA input amount of 10 ng as specified in the Oncomine™ Dx Target Test User Guide. Four (4) pre-characterized FFPE clinical samples containing the EGFR L858R variant, the NRAS Q61L variant, and two with a ROS1 fusion variant were tested in this study. Five (5) DNA input levels and 5 RNA input levels ranging from three levels below the standard of 10 ng (5 ng, 6.5 ng and 8.5 ng), the standard input and one level (15 ng) above the standard input were tested. For each DNA and RNA test condition, 6 replicates were tested per sample. For each sample, results from the expected variant location were examined and the data generated from the study were analyzed using an analysis of variance (ANOVA) to determine the relationship of input level to allele frequency (for DNA variants) or the number of fusion reads (for RNA variants).

For samples with DNA variants, all tested DNA/RNA input combinations yielded similar allele frequencies, including input combination of 10 ng for

both DNA and RNA. Additionally, the positive and negative call rates were greater than 95% for all DNA/RNA input combinations under investigation.

For one of the ROS1 RNA fusion samples, there was no significant differences in log-fusion reads across all RNA and DNA input combinations under investigation, and the RNA fusions were called correctly 100% of the time across all DNA/RNA input combinations. However, for the second ROS1 RNA fusion sample, the input combination of 15 ng RNA/15 ng DNA yielded ROS1 fusion reads which were significantly lower than the reads yielded by the input combination of 10 ng for both RNA and DNA. In addition, the positive call rates were less than 95% at the input combination of 15 ng RNA/15 ng DNA, and at the input combination of 8.5 ng RNA/15 ng DNA.

The study confirmed the input of 10 ng for both DNA and RNA as specified in the Oncomine™ Dx Target Test User Guide.

d. Tissue Input:
Sixty (60) slide-mounted FFPE samples were analyzed to determine if samples extracted using the Ion Torrent™ Dx Total Nucleic Acid Isolation Kit yield DNA and RNA at the concentrations required by the Oncomine™ Dx Target Test when tissue input requirements are met. The test requires DNA at a concentration ≥ 0.83 ng/μL and RNA at a concentration ≥ 1.43 ng/μL. Thirty (30) resection samples with ≥ 20% tumor content were prepared without macrodissection, 15 resection samples with < 20% to ≥ 10% tumor cell content were macrodissected, and 15 samples were collected by core needle biopsy (CNB). For the resection samples, 2 × 5 μm sections were used per extraction. For CNBs, 9 × 5 μm sections were used per extraction. DNA and RNA concentrations were determined using the Ion Torrent™ Dx DNA and RNA Quantification Kits, respectively. No sequencing was performed on the extracted samples. Of the 60 samples tested, 98.3% (59/60) had a DNA concentration ≥ 0.83 ng/μL and an RNA concentration ≥ 1.43 ng/μL. One CNB sample failed the minimum DNA and RNA concentration specifications, with values of 0.52 ng/μL and 1.23 ng/μL, respectively. The low concentrations were likely caused by the small tissue size. The results demonstrated that sufficient DNA and RNA could be isolated from tissue sections which meet the specified tumor cell content requirement of ≥ 20%.

e. Tumor Content:
A range of tumor contents was tested in this study to determine the impact of varied tumor content on the Oncomine™ Dx Target Test performance. Fifty-five (55) pre-characterized clinical samples with different percentages of tumor cell content (3 to 58%) were analyzed using the Oncomine™ Dx Target Test. The samples included the clinically indicated variants BRAF V600E, EGFR (Ex. 19del and L858R), and ROS1 fusions, as well as multiple other variants to represent the different variant categories (SNVs, MNVs, and

deletions) were tested with and without macrodissection. The tumor cell contents of each specimen and region of interest (ROI) were estimated before the study by an external pathology lab. For samples analyzed without macrodissection, all variants were called positive by the Oncomine™ Dx Target Test, including all the DNA and RNA variants. For those samples that were analyzed with macrodissection, all DNA and RNA variants, except 1 DNA variant were called positive by the Oncomine™ Dx Target Test. The missed call was a no call in a BRAF V600E variant sample with a tumor content of 16%. The data supports Oncomine™ Dx Target Test's requirement of 20% tumor content, and the claim that if the tumor content in the region of interest is $\geq$ 10%, the tissue samples can be macrodissected and enriched for tumor content.

3. Analytical Specificity:

    a. Inclusivity/Cross-Reactivity:
    An *in silico* cross-reactivity analysis was performed to evaluate the specificity of the primers in the Oncomine™ Dx Target Test Kit DNA and RNA panels. The primers were checked for specificity to the human genome, human transcriptome, and representative bacterial, fungal, and viral genomes. Any unintended amplification products were required to have a minimum of 2 bp mismatches to the intended amplification product sequences generated by the panels, because mismatches of $\geq$ 2 bp prevent mapping to the same location on the genome due to a low mapping score. For the DNA panel primers, *in silico* analysis predicted 20 unintended potential amplicon generating primer pairings against the human genome. Of these, 19 unintended amplification products had mismatches of 2-122 bp to intended products, and therefore would not cause false results. The remaining unintended primer pairing would only detect the existing WT or variant location and would not cause false results. For the RNA panel primers, unintended primer pairings against the human genome and the human transcriptome were predicted; however, due to the level of mismatches none were predicted to cause false results. The results demonstrated that the primers are specific for the intended targeted sequences.

4. Interference:
    To evaluate the potential impact of endogenous (necrotic tissue and hemoglobin) and exogenous interferents (paraffin, xylene, ethanol, proteinase K, and wash buffer),this study evaluated 8 clinical FFPE samples in 6 replicates for both DNA and RNA for every combination of sample and condition taken through the entire test workflow. The samples included all clinically indicated variants and represented SNV (simple and complex), deletions, and fusion variant types, targeted by the Oncomine™ Dx Target Test. This included the sample preparation and processing steps (extraction, DNA and RNA quantification), steps prior to taking the purified nucleic acid through to library construction, templating, PGM sequencing and variant calling. The samples included WT and those with representative SNV, deletion, and fusion variants. The impact of potentially

interfering substances on assay performance was evaluated, and the results were compared to the control (no interferents) condition.

a. Endogenous Interference:
A review of the clinical study data indicated that the presence of necrotic tissue up to approximately 20% did not appear to interfere with the assay. Results from an interference study using variant positive clinical samples will be provided in order to confirm that the presence of necrotic tissue does not interfere with the performance of the Oncomine™ Dx Target Test.

Hemoglobin was evaluated at 4 mg/mL. The allelic frequencies for SNV and deletion variants were ~10-20% and the fusion reads for the RNA variants were ~500-1000 reads. The positive concordance with the control condition (with no calls being excluded) across all samples, and the overall concordance with the control condition (no calls excluded) across all samples were calculated. One false positive result and one false negative result were observed across all study samples and all Oncomine™ Dx Target Test variant locations. There were 15 invalid results for two samples both for DNA (N = 1) and RNA (N = 14) samples across both control and test conditions. The overall concordance (no calls excluded) was 99.99%, and the overall data demonstrate that hemoglobin does not adversely impact the performance of the assay.

b. Exogenous Interference:
For the study with exogenous interferents, both the positive concordance (no calls excluded) and the overall concordance (no calls excluded) for all samples exceeded the study acceptance criteria of > 95%, with respect to each interferent under investigation. The data support that these interfering substances can be tolerated by the assay at the levels tested.

c. Anti-microbial Testing
Testing was performed to determine the effectiveness of the antimicrobial preservatives in 23 reagents used in the Oncomine™ Dx Target Test with five organisms (*i.e., S. aureus, P. aeruginosa, C. albicans, E. coli, and A. brasiliensis*). Two additional reagents (Templating (TMPL) Water and TMPL Tween Solution) which do not include antimicrobial preservatives were also included in the study. These reagents - which are included as part of the Ion Torrent Dx FFPE Sample Preparation Kit, the Ion PGM Dx Library Kit, Ion PGM Dx Templating Kit and Ion PGM Dx Sequencing Kit - are designed for storage at refrigerated or room temperature (15-30°C) conditions. Testing was performed at 20-25°C and tested at 5 time points post-inoculation (T_0 days, T_1 day, T_7 days, T_{14} days and T_{28} days). In addition to the initial inoculation and testing, aliquots of un-inoculated products were stored for future inoculation and testing again at the same 5 time points after 12 and 24 months storage at the recommended storage conditions. Twenty-three (23) of the 25 reagents (92%) met acceptance criteria for bacteria of $\geq$ 2 log reduction from T_0 to T_{14}

days; no increase from T_{14} to T_{28} days; and for yeast and mold no increase from initial (T_0) to T_{14} and T_{28} days. The 2 reagents which do not include antimicrobial preservatives did not demonstrate a reduction in colony counts for *E. coli* and *P. aeruginosa*. The product instructions for use includes a warning for users to visually inspect the TMPL Water and TMPL Tween Solution for potential bacterial or fungal contamination prior to use, and if evident, the user should discard and use new vials.

5. Precision and Reproducibility:
   Two reproducibility studies, a multi-site assay reproducibility study and a separate sample processing reproducibility study, were performed to assess precision (within-run) and reproducibility (across different sample preparation reagent lots, sites, operators, and instrument systems).

   a. External Panel Reproducibility Study (Assay Reproducibility):
      The external reproducibility study was conducted across 4 US sites for the purposes of demonstrating within-run precision performance (repeatability) and variability across sites, operators and instrument platforms (reproducibility). At each site, the 4 operators were grouped into 2 pairs with each pair assigned to 2 instrument systems and were responsible for testing each of the pre-extracted DNA and RNA specimens representing all of the clinically indicated variants and each of the variant types (simple and complex SNVs, MNVs, deletions, and fusions) targeted by the Oncomine Dx Target Test. The 18 DNA samples consisting of of 16 DNA samples with two or more DNA variants (blends of clinical samples and plasmids including BRAF V600E, EGFR L858R, and EGFR Ex.19del) and 2 were WT samples, and the 9 RNA FFPE clinical samples included 3 ROS1 fusions. Each specimen in the DNA and RNA panels were run in duplicate for 5 non-consecutive days using 2 different reagent lots at 3 of the study sites and all three reagent lots at the 4th study site for a total of 72 test determinations per DNA specimen and 144 test determinations per RNA specimen.

      The positive call rates, with two-sided 95% confidence intervals using exact method, with and without no calls were calculated. When including no calls, the positive call rate for DNA variants (at the variant location) ranged from 87.1% (77%, 93.9%) to 100% (95%, 100%) and when no calls were excluded the positive call rate ranged from 97.1% (89.9%, 99.6%) to 100% (95%, 100%). Within-run precision ranged from 85.3% (68.9%, 95%) to 100% (90.3%, 100%) with no calls included and 93.9% (79.8%, 99.3%) to 100% (90.3%, 100%) when no calls are excluded. When including or excluding no calls, the positive call rate for the ROS1 fusion-containing samples (at the variant location) ranged from 86.1% (79.4%, 91.3%) to 100% (97.5%, 100%). Within-run precision ranged from 75% (63.4%, 84.5%) to 100% (95.0%, 100%) with no calls included or excluded.

The average positive agreement (APA) and average negative agreement (ANA) at the variant-level were defined for a given pair of operators, calculated by comparing each possible set of sequencing results from the first operator with each possible set of sequencing results from the second operator at a given site. The two-sided 95% confidence intervals for APA and ANA were calculated using bootstrap method when APA or ANA was not 100%, or exact method when APA or ANA was 100%. For each DNA sample that carries at least one variant (including no calls), the APAs ranged from 78.1% (56.3%, 100%) to 100% (97.2%, 100%) and the ANAs ranged from 86.2% (73.7%, 94.7%) to 99.8% (99.6%, 99.8%). For the 2 WT DNA samples, the APAs (including no calls) were either not applicable or 8.3% (0%, 20.0%) due to false positive calls and ANAs ranged from 90.5% (80.4%, 99.0%) to 99.8% (97.4%, 99.8%). Excluding no calls, the APAs ranged from 78.1% (56.3%, 100%) to 100% (97.2%, 100%) and ANAs ranged from 99.8% (99.7%, 100%) to 100% (100%, 100%) comparing two operators.

For each RNA sample that carried at least one variant, the APAs for each specimen on the variant level from 75.0% (52.5%, 91.4%) to 100% (97.2%, 100%) and ANAs ranged from 99.7% (99.6%, 99.9%) to 100% (100%, 100%) with no call included or excluded.

In this study, the AFs ranged from 0.9x - 2.2x and 1.5x - 3.2x of the determined LoD values, which for the most part exceeded the LoD established in the LoD study. As a result, the reproducibility of the test near 1x LoD could not be determined, and an additional reproducibility study is to confirm the established LoD.

b. Precision:
Precision was estimated with respect to positive variant locations for within-run, between-system, between-operator, between-site, between-lot and total variability. When excluding No Calls from the assay reproducibility study data, the estimate of repeatability was at least 99.3%, with a lower limit of the corresponding 95% CI of $\geq$ 98.3%, at each of the clinical DNA variant locations. When No Calls were included, the estimate of repeatability was at least 95.2%, with a lower limit of the corresponding 95% CI of $\geq$ 93.2%,at each of the clinical DNA variant locations assessed. The estimate of repeatability for ROS1 was 94.4% with a lower limit of the corresponding 95% CI of 92.3%.

When the variance components were assessed, the between-run component had the greatest contribution to the total variability (up to 25.3% CV) for DNA samples.

c. External Sample Processing Reproducibility Study:
Two (2) WT samples and 10 variant-positive samples, which included simple and complex SNVs, MNVs, deletions, and fusions represented by BRAF

V600E, EGFR L858R, 2 common EGFR Ex. 19del, ROS1 fusion transcripts, and other representative variants, were evaluated at 4 US testing sites, each with 4 Ion PGM™ Dx Systems and 4 operators, to determine the reproducibility and repeatability of sample processing. Each sample was tested 8 times at each site, for a total of 32 replicates per sample, or 768 sample sequencing results [12 samples × 32 replicates × 2 library types (RNA and DNA)]. The call rate, no call rate, positive call rate, negative call rate, and within-run repeatability were computed at each variant location of interest. Three DNA samples generated at least one no call, and the ROS1 RNA fusion sample generated 2 negative results out of the 32 replicates. Including or excluding no calls, all positive call rates from positive variant locations were > 93%, with a lower bound of 79.2%.

6. Tissue Heterogeneity:
   Tissue heterogeneity was assessed as part of the sample processing reproducibility study. Results from a comparison of unstained sections from across multiple sections of the same tissue sample demonstrated that the Oncomine™ Dx Target Test was able to produce repeatable and reproducible results across different measured tumor content levels and AF% or log (fusion reads).

7. Extraction Method Equivalency Studies:
   Data were provided to demonstrate the equivalency of data generated from previously extracted DNA and RNA from clinical samples included in two of the clinical studies intended to support this PMA.

   a. DNA:
      This study assessed whether the Oncomine™ Dx Target Test produces comparable DNA variant from FFPE samples prepared with the Ion Torrent™ Dx FFPE Sample Preparation Kit and the QIAamp® DSP DNA FFPE Tissue Kit™ (IVD). Twenty (20) NSCLC FFPE tissue blocks with a mix of SNVs and deletions (including 10 samples with EGFR mutations) were evaluated. Among the 20 samples, 19 met the minimum DNA concentration requirement and produced equivalent variant calling using the two different sample preparation kits. One (1) sample was discordant due to significantly lower concentration obtained using the QIAamp protocol (QIAamp protocol requires elution in 120 µL, while the Ion Torrent Dx extraction elutes in 30 µL). The valid pairs of sample results demonstrated that all concordance statistics at the variant and sample levels were 100% when excluding no calls. The results from this study support that the Oncomine™ Dx Target Test system calls at DNA variant locations are equivalent when using either of the extraction methods.

   b. RNA:
      This study was performed to determine the comparability of the Ion Torrent Dx FFPE Sample Preparation Kit and a different commercially available RNA/DNA extraction system to prepare RNA to be used with the

Oncomine™ Dx Target Test. Twenty (20) clinical FFPE specimens and FFPE cell lines, representing WT and RNA fusion positive specimens including ROS1, were evaluated. Positive concordance statistics at the variant level exceeded 85%, and negative concordance and overall concordance statistics at the variant level exceeded 95%. At the sample level, the concordance for the fusion-positive samples was greater than 85%. The negative and overall concordance were both 90% at the sample level. The relatively reduced negative concordance was due to a sample which had a low level of fusion expression, irrespective of the extraction method; and likely contamination from the assay control in another sample. Overall, the results support that results from the Oncomine™ Dx Target Test can be considered comparable when using either extraction system.

8. Contrived Sample Functional Characterization Study:
   To support the use of plasmids and cell lines as an alternative for clinical samples for certain variants, which are difficult to acquire, a study was conducted to evaluate the functional behavior of the contrived samples to that of clinical specimens using the Oncomine™ Dx Target Test. Five (5) representative variants (for SNVs and deletions), which included all DNA clinically indicated variants, were tested at 6 AF levels (2%, 4%, 5%, 6%, 8%, and 12%) for each of the 3 sample types (FFPE clinical sample, FFPE cell line, and plasmid). AFs were verified by an analytically validated comparator method. Eight operators and multiple reagent lots were used to test 20 replicates for each of the 6 AF levels.

   For each variant, the average AF associated with each sample level was computed, and the corresponding proportion of correct calls (hit rate) was determined and converted to probits (i.e., units from the cumulative standard normal distribution function). A generalized linear model was fitted, with the probit modeled as a function of AF level, utilizing the Firth penalized likelihood method. The goodness of fit of each resulting probit model was evaluated using Pearson's Chi-square test, and the fit of each model was found to be acceptable. Estimates of C25, C50, C75, and C95 (corresponding to hit rates of 25%, 50%, 75% and 95%, respectively) were computed for each sample type, along with the corresponding 95% confidence intervals. In addition, the differences of the predicted estimates of C25, C50, C75 or C95 - as well as the corresponding two-sided 95% confidence intervals using bootstrap method - between the plasmid blends or cell lines and the clinical samples were also calculated. The results show that differences in the predicted estimates of C25, C50, C75 or C95 between the plasmid blends or cell lines and the clinical samples were considered small and acceptable for all tested variants,demonstrating equivalent performance between sample types.

9. Guard Band Studies:

   a. Workflow Tolerances:

The tolerances encompassing the workflow steps, library preparation, template preparation and sequencing were assessed across 20 separate studies corresponding to the test's most critical workflow steps which could lead to assay failure. Each study included 3 test points, which included testing in low condition, nominal condition as defined by the instructions for use, and high condition. The guard banding range for each experiment was designed such that the maximum and minimum test points challenged the system, while still being within operational error range. The study was conducted across multiple runs, utilizing multiple operators and instrument systems. For each study, 6 independent libraries were prepared per condition and pooled into three sets of pools with one pool tested at the low condition, one at the high condition and the remaining samples tested at the nominal condition. Each pool was tested in a single system run, resulting in a total of 3 pools tested in 3 runs, with 3 or 6 replicates per condition.

Of the 20 studies, one study, Thermal Cycling Temperature Offset, demonstrated a significant difference in performance when the temperature deviation in the PCR thermocycler during all thermocycling steps were increased by either +0.8°C to +1°C, which resulted in the samples failing the test QC parameters. The acceptable tolerance was therefore defined as -1°C to +0.5°C of the specified temperature. Of the remaining studies, 8 demonstrated no significant difference in results while 11 did show a statistically significant difference; however, it is recommended that each step in assay preparation and sequencing be followed according to the instructions for use. An additional study using fusion-positive clinical samples is ongoing to verify the tolerance ranges of the critical elements of the RNA workflow for the Oncomine™ Dx Target Test.

b. Tissue Fixation Study:
The impact of tissue fixation time on the performance of the Oncomine™ Dx Target Test was assessed by fixing WT cell line pellets in 10% neutral buffered formalin (NBF) for 12, 24, 48, 72, and 84 hours at room temperature. The results from the fixed specimens were compared to those from the same cell line that had not undergone any fixation with 10% NBF. One (1) replicate of each fixation time condition and the non-FFPE condition were tested on three different chips, for a total of three replicates per condition. The average AF observed at each of the 103 cytosine deamination-susceptible hotspots was determined for each condition tested. The results showed two cytosine deamination events as a result of the fixation process; however, neither event resulted in a false-positive call. DNA and RNA sequencing quality was evaluated by measuring percent reads, no call rate, and total mappable reads (TMR) for each condition tested. All DNA and RNA sequencing results met the DNA and RNA sample QC metrics. The results of this study support the claim that specimens may be fixed for up to 84 hours in 10% NBF prior to testing with the Oncomine™ Dx Target Test.

c. Contamination Study:
Contamination events throughout the workflow from extraction to sequencing were assessed. Eight FFPE cell line samples, representing simple and complex SNVs, deletions, and RNA fusion variant categories, were used to ensure adequate sample quality and to allow testing the more challenging condition of high AFs (> 50%). Each FFPE cell line carried a unique variant, but was WT for all other variants evaluated in this study and WT RNA from two of the samples was also tested. The AFs ranged from 67% - 100% for the DNA variants (including EGFR Ex. 19del, KRAS G12D, NRAS, EGFR L858R), a WT sample, and for the RNA variants 14.7K fusion reads, which is much higher than the 40 read cutoff. Alternating a variant sample run and a run with samples that are WT at these variant locations replicated a situation where cross contamination could be observed. To evaluate cross contamination, each of the four operators tested all cell line samples side-by-side throughout the entire workflow. To evaluate carryover contamination, on each of the 4 systems, 5 rounds of the entire test protocol (extraction to variant calling) with the same set of samples were conducted consecutively. Samples were assigned alternating barcodes in consecutive runs conducted on the same instrument, per User Guide instruction.

The false positive (FP) rate at each of the DNA variant location examined in this study was 0% (0/100) and the FP rate for each of the RNA variants was no more than 1.25% (1/80). One FP result just above the fusion detection threshold of > 40 fusion target reads was observed in the study. The contamination event was likely caused by cross-contamination from a sample that was located in an adjacent well. The observed FP rate met the Oncomine™ Dx Target Test requirement of less than or equal to 3%.

10. Stability Studies:

a. Shelf-Life Stability:
Three (3) separately manufactured kit lots including all components of the Oncomine™ Dx Target Test and Controls Kit were stored according to the storage conditions specified in the product labeling. Stability of the reagents is being evaluated by testing 5 FFPE samples at specified time points from baseline, 2-4, and 7 months. For each sample each of the assay QC metrics was evaluated in addition to the final calls. The data demonstrate that the kit components of the Oncomine™ Dx Target Test and Controls Kit are stable for at least 6 months.

b. In-Use Stability:
The in-use stability study incorporated both open vial stability and freeze/thaw (FT) stability. Five (5) separately manufactured kit lots were tested at 6 functional testing time points and 2 mock workflow time points to cover 8 weeks for a total of 8 time points. A total of 72 samples were processed and DNA and RNA were tested across a total of 12 system runs. All runs and all

samples except for one RNA sample passed the specifications and the results were used for analysis. In place of the failed RNA sample, the RNA sample using the same barcode from the back up run (Run 2) was used. The data supported in-use stability for 7 weeks and a maximum of 8 freeze/thaws.

c. Designated Hold Times:
The workflow for the Oncomine™ Dx Target Test incorporates several optional stopping points to hold assay intermediates. The stability of the intermediate products was evaluated by incorporating all of the 13 optional extended hold times specified in the User Guide. A total of 3 samples (2 FFPE clinical samples and 1 FFPE cell line sample) were included in this study. The SNV, deletions, and fusion variant types were respresented by samples which contained EGFR L858R, EGFR Ex. 19del, BRAF V600E, a ROS1 fusion, and other representative variants. Each sample was tested under 3 different test conditions (Table 8 below).

Table 8. Designated hold time test conditions.

Condition	**Eluted Library Hold Time**
A) Nominal Hold[1]	No hold
B) Library Hold	30 day hold 3 month hold
C) Combo Hold[2]	No hold
[1]Libraries prepared for condition A were used to test condition B [2]Includes 12 of the 13 optional hold steps, excluding the eluted library hold tested in condition B. Each step was held at the maximum recommended time to approximate a "worst-case scenario".	

For DNA, AF and the log-transformed median absolute pairwise difference [log(MAPD)] were used as metrics to evaluate stability. For RNA, the log-transformed fusion reads and the log-transformed normalized read ratio [i.e., log(fusion reads/total mapped reads)] were used as metrics to evaluate stability. In all of the evaluations, the results of the test conditions with the incorporated hold times were compared to the samples tested without the hold times. The study results support the conclusion that the 30-day library hold and combo hold conditions did not result in a decrease in Oncomine™ Dx Target Test performance relative to the nominal test condition.

d. Kit Lot Interchangeability:
An initial study were provided using control samples to demonstrate that different lots of the Oncomine™ Dx Target Test Kit components (Oncomine™ Dx Target Test Control Kit DNA and Oncomine™ Dx Target Test Control Kit RNA, and the Oncomine™ Dx Target Test RNA and DNA panels) are interchangeable. A study using clinical specimens to confirm that different lots of each of the components of the Oncomine™ Dx Target Test and Controls Kit may be used interchangeably will be conducted.

e. Sample Stability - Extracted RNA and DNA:
Storage and freeze-thaw (FT) stability of DNA and RNA extracted from FFPE clinical samples and FFPE cell line samples were assessed at baseline, 7 weeks, 3 months + 1 week, 6 months + 1 week, and 9 months + 1 week to support the stability of the extracted DNA and RNA at 9 months using the Ion Torrent™ Dx FFPE Sample Preparation Kit. DNA and RNA extracted from FFPE cell lines and clinical FFPE samples, representing SNVs, deletions, and RNA fusions by the clinically indicated variants, were aliquoted and stored at -30°C to -10°C and -90°C to -60°C, respectively. The DNA and RNA samples contained a SNV and a deletion variant and RNA fusions, respectively in both the respective clinical and cell line FFPE specimens to support stability across the variant categories and represented 3 of the clinically indicated variants.

For baseline, the samples were sequenced within one week of aliquoting. For the purpose of this study, the date of library preparation target amplification is considered to be start of the time point. At each time point, DNA and RNA aliquots were frozen and thawed once or 3 times and then sequenced using the Oncomine™ Dx Target Test. The data supported stability of extracted DNA stored at -30°C to -10°C and RNA stored at -90°C to -60°C for 9 months after 3 FT cycles.

f. Stored Slide Stability:
Eight (8) clinical sample FFPE blocks and 1 cell line FFPE block representing each of the variant categories (SNVs, deletions, and fusions) with each containing a known variant, including each of the clinically indicated variants plus one additional variant detected by the test, were sectioned to provide at least 30 sections per block and every other slide was dipped in paraffin. All slides were stored at 15°C to 30°C. The slides were tested at baseline plus three additional time points [7 week (49 days), 4 months+1 week (129 days), and 6 months+1 week (190 days)] to initially demonstrate stability at 8 months. At each time point, DNA and RNA were extracted from 2 paraffin dipped and 2 un-dipped slides per sample and then sequenced according to the approved stability protocol. Based on the data provided, both paraffin dipped and undipped slides are stable for at least 8 months when stored at 15°C to 30°C. Slide stability testing is intended to continue to demonstrate stability to at least 12 months when stored at 15°C to 30°C.

g. Stored Block Stability:
Seven (7) clinical FFPE tissue blocks and one FFPE cell line block comprising 6 DNA variants and 1 ROS1 fusion, representing each of the clinically indicated variants and variant categories, were tested in quadruplicate in 6 sequencing runs for each of five time points: baseline (0 days), 8 week (56 days), 4 months+2 weeks (134 days), 6 months+2 weeks (194 days), and 8 months+2 weeks (254 days). In addition, 1 FFPE cell line block and a single clinical FFPE RNA fusion positive sample was tested at

four time points [baseline (0 days), 8 week (56 days), 4 months+2 weeks (134 days), 6 months+2 weeks (194 days)]. The mean AFs were calculated at each time point for the DNA samples and the mean fusion reads and the mean log(10)-fusion reads were calculated for the RNA sample, and comparisons were made to T_0 values. The AF at baseline for the DNA variants ranged from 26.7% to 47.3% and the mean fusion reads for the RNA variants was 9410 (3.97 mean log-fusion reads). Based on the data provided, DNA extracted from DNA variant containing clinical samples is stable for 8 months and DNA extracted from cell line FFPE blocks for 6 months when the sample block is stored at 15°C to 30°C. RNA extracted from the RNA fusion clinical sample block is stable for 6 months when stored at 15°C to 30°C.

h. Transport Stability:
The transport stability study was performed to demonstrate that the shipping configurations developed for all kit components required for the Oncomine™ Dx Target Test provide adequate thermal and physical protection as packages are transported from the manufacturing site to customers. Three (3) separately manufactured kits and component reagents lots were exposed to simulated transport challenges intended to simulate the longest estimated domestic and international shipping times of 48 and 72 hours, respectively. The simulated transport conditions included both physical and temperature challenges, which included 7 packaging configurations (domestic vs. international shipping) and 4 temperature profiles (72-hour summer, 72-hour winter, 144-hour summer and 144-hour winter), respectively. The 48-hour domestic time point was assessed using the first 48-hours of the 72-hour profile and the 144-hour profile consisted of two consecutive 72-hour time periods. Each of the kits and components were functionally tested using a combination of DNA and RNA controls, synthetic, and FFPE samples to ensure reagent functionality in order to establish transport stability. Based on the results of the functional testing for each of the conditions, demonstrating that the shipping configurations provide adequate thermal and physical protection for the simulated time points.

B. Animal Studies

Not Applicable

X. SUMMARY OF PRIMARY CLINICAL STUDIES

Thermo Fisher Scientific conducted 3 separate clinical bridging studies to establish reasonable assurance of safety and effectiveness of the Oncomine™ Dx Target Test for detection of BRAF V600E and EGFR Ex. 19del and L858R mutations and ROS1 fusions in FFPE tumor specimens to select NSCLC patients for treatment with TAFINLAR® (dabrafenib) in combination with MEKINIST® (trametinib), or IRESSA® (gefitinib), or XALKORI® (crizotinib), respectively. Justifications for the acceptance of foreign data were provided. Data from these bridging studies were the basis for the PMA approval

decision. For each of the clinical claims, drug efficacy was based on clinical studies that enrolled only biomarker positive patients; therefore, a separate cohort of NSCLC patient samples from a population similar to those enrolled into the bridging studies was obtained and screened to be negative for the targeted BRAF, EGFR, and ROS1 abnormalities [acquired PCR(-) or FISH(-) samples] using the biomarker relevant comparator method assays. A summary of the clinical studies are presented below.

A. BRAF Study Design

The clinical benefit of the Oncomine™ Dx Target Test was demonstrated in the analysis of efficacy and safety data obtained from two of three cohorts from a Phase II multi-center, multi-cohort, non-randomized, open-label trial (Study BRF113928). BRAF V600E mutation NSCLC patients enrolled into the two cohorts (B and C) received the combination therapy (dabrafenib 150 mg BID and trametinib 2 mg once daily) until disease progression or unacceptable toxicity. In the BRF113928 study, prospective patients were screened for a BRAF V600E mutation in NSCLC specimens and enrolled based on results from local lab tests (LLTs) used at each of the enrollment sites. Central confirmation testing for the BRAF V600E mutation was performed with the Oncomine™ Dx Target Test after enrollment.

Study BRF113928 included the following three cohorts:

- Cohort A – To evaluate dabrafenib monotherapy in 84 subjects with BRAF V600E NSCLC, that had relapsed or progressed on at least one platinum based chemotherapy regimen prior to enrollment. (Subjects receiving and adequately tolerating dabrafenib as a single agent and who continue to meet the inclusion and exclusion criteria had the option to switch to dabrafenib and trametinib combination treatment.) Cohort A was not included in the clinical efficacy analysis portion of the clinical bridging study, but utilized to establish concordance with the Oncomine™ Dx Target Test only.
- Cohort B – To evaluate dabrafenib and trametinib combination therapy in 57 BRAF V600E positive subjects who had progressed on at least one prior treatment for advanced / metastatic disease.
- Cohort C – To evaluate dabrafenib and trametinib combination therapy in 36 BRAF V600E positive NSCLC subjects who had not received prior treatment for advanced/metastatic disease (i.e., first line subjects).

Samples from a subset of enrolled patients from the intended population were retrospectively evaluated using the Oncomine™ Dx Target Test in a clinical bridging study (Study CDRB436E2201). The two objectives for the bridging study include: 1) agreement between results from the Oncomine™ Dx Target Test and the LLTs for BRAF V600E detection, and 2) the clinical outcomes (i.e., ORR) for the NSCLC patients (Cohorts B and C) who were positive for BRAF V600E mutations, as identified by the Oncomine™ Dx Target Test. Because no FDA-approved test was available for detection of BRAF V600E in FFPE NSCLC specimens, a validated PCR assay, BRAF V600 PCR Mutation Test, served as a surrogate assay for the LLTs in the bridging study. This assay was similar to the LLTs used at more than one

enrollment testing site and the validated assay used to screen the acquired PCR(-) samples.

Subjects with locally determined BRAF V600E mutant NSCLC were screened for clinical eligibility criteria for enrollment into the bridging study along with ~130 acquired PCR(-) samples.

1. Clinical Inclusion and Exclusion Criteria:
   Enrollment in the bridging study CDRB436E2201 was limited to patients who met the following inclusion criteria:
   - Patient informed consent provided for use of clinical sample in eligibility screening for the BRF113928 clinical trial and/or commercial samples with appropriate use consistent with guidance for leftover human specimens;
   - Availability of adequate sample to generate an Oncomine™ Dx Target Test result including the following DNA requirement:
     - DNA isolation - Use 10 ng of DNA at a concentration of $\geq$ 0.83 ng/µL.

   Patients were not permitted to enroll in the bridging study CDRB436E2201 if they met any of the following exclusion criteria:
   - Lack of clear subject identification or label on banked patient sample;
   - Obvious physical damage of banked patient sample which precludes sample processing;
   - No tumor present per pathology review.

2. Follow-up Schedule:
   As the bridging study was conducted retrospectively to establish safety and effectiveness for selecting patients using the Oncomine™ Dx Target Test, there was no follow-up conducted.

3. Clinical Endpoints:
   The objective response rate (ORR) by investigator assessment (IA) observed in Cohorts B and C were submitted to support the approval of dabrafenib and trametinib combination therapy in patients with BRAF V600E mutation positive NSCLC under NDAs 202806/S006 and 204114/S005.

   Effectiveness of the Oncomine™ Dx Target Test was determined by measuring the objective ORR by investigator assessment for patients with stage IV NSCLC who tested positive for the BRAF V600E mutation by both the LLTs and the Oncomine™ Dx Target Test. The ORR was calculated for patients in two cohorts (B and C) who were selected for treatment with dabrafenib administered in combination with trametinib.

B. Accountability of PMA Cohort

At the time of the analysis cut-off date, August 8, 2016, for Study BRF113928, 84 patients were enrolled in Cohort A, 59 patients were enrolled in Cohort B, and 34

patients were enrolled in Cohort C. For Cohort A, 6 patients were excluded because they did not meet the enrollment criteria, an additional 11 samples were excluded due lack of sample availability and 22 were not tested due to insufficient DNA, RNA, lack of tumor, etc.

For Cohort B, 2 patients received first line treatment via protocol deviation, and therefore were included as part of the intended use population in Cohort C; samples from 2 subjects were not sent for central confirmation testing by the Oncomine™ Dx Target Test; and an additional 17 subjects were excluded due to either insufficient tumor tissue to process (n = 11), or insufficient DNA yield to proceed with testing (n = 6). As a result, 38 samples were available for testing with the Oncomine™ Dx Target Test. Of those, 14 samples were excluded due to not meeting the sequencing quality control (QC) metrics (n = 9) or insufficient coverage to generate a valid result (n = 5). The final number of samples available with a valid Oncomine™ Dx Target Test result was 24 samples (24/57 = 42.11%).

For Cohort C, there were a total of 36 patients, including 34 patients enrolled, and the 2 first line patients initially enrolled into Cohort B, who actually received first line treatment. Three (3) samples were not shipped for central confirmatory testing in time for inclusion into the study, 5 samples were excluded due to insufficient tissue or insufficient tumor to process, and therefore, did not meet the minimum DNA input requirement. An additional 5 samples were excluded due to not meeting the sequencing QC metrics (n = 3) or insufficient coverage to generate an valid result (n = 2). The final number of samples available with a valid Oncomine™ Dx Target Test result was 23 samples (23/36 = 63.89%).

C. Study Population Demographics and Baseline Parameters

Baseline characteristics were compared between those patients with and without evaluable Oncomine™ Dx Target Test results. In general, for both cohorts B and C, the demographics for patients with and without a Oncomine™ Dx Target Test result are similar, apart from the specimen type for which there was a higher proportion of patients with resection specimens. Comparisons between the clinically relevant patient demographics of the study populations are shown for cohorts B and C in the tables below.

Table 9. Baseline characteristics between the patients included and excluded from the bridging study for Cohort B.

Baseline characteristics	Patients in bridging study N=38	Patients not in bridging study N=19	All N=57
Age (Years)			
Mean	64.7	65.7	65.1
SD	10.40	9.85	10.14
Median	63.5	65.0	64.0
Min	41	50	41
Max	85	88	88

Baseline characteristics	Patients in bridging study N=38	Patients not in bridging study N=19	All N=57
Gender - n (%)			
Female	18 (47.4)	10 (52.6)	28 (49.1)
Male	20 (52.6)	9 (47.4)	29 (50.9)
Race - n (%)			
American Indian Or Alaskan Native	0 (0)	0 (0)	0 (0)
Asian	3 (7.9)	1 (5.3)	4 (7.0)
Black or African American	2 (5.3)	0 (0)	2 (3.5)
Native Hawaiian Or Other Pacific Islander	0 (0)	0 (0)	0 (0)
White	32 (84.2)	17 (89.5)	49 (86.0)
Multiple	1 (2.6)	0 (0)	1 (1.8)
Missing	0 (0)	1 (5.3)	1 (1.8)
ECOG baseline - n (%)			
0	13 (34.2)	4 (21.1)	17 (29.8)
1	21 (55.3)	14 (73.7)	35 (61.4)
2	4 (10.5)	1 (5.3)	5 (8.8)
Histology - n (%)			
Adenocarcinoma	36 (94.7)	17 (89.5)	53 (93.0)
Adenosquamous Carcinoma - Predominantly Adenocarcinoma	1 (2.6)	0 (0)	1 (1.8)
Bronchioloalveolar	1 (2.6)	1 (5.3)	2 (3.5)
Larges Cells Lung Cancer	0 (0)	1 (5.3)	1 (1.8)
AJCC tumor stage - n (%)			
IIIA	0 (0)	0 (0)	0 (0)
IV	38 (100)	19 (100)	57 (100)
Smoking history - n (%)			
Never Smoked	11 (28.9)	5 (26.3)	16 (28.1)
Current Smoker	4 (10.5)	2 (10.5)	6 (10.5)
Former Smoker	23 (60.5)	12 (63.2)	35 (61.4)
Specimen type - n (%)			
Resection	15 (39.5)	0 (0)	15 (26.3)
Core needle biopsy	21 (55.3)	8 (42.1)	29 (50.9)
Fine needle aspirate	2 (5.3)	0 (0)	2 (3.5)
Not recorded	0 (0)	9 (47.4)	9 (15.8)
Other	0 (0)	2 (10.5)	2 (3.5)

All percentages calculated using N as denominator

Table 10. Baseline characteristics between the patients included and excluded from the bridging study for Cohort C.

Baseline characteristics	Patients in bridging study N=28	Patients not in bridging study N= 8	All N=36
Age (Years)			
Mean	67.0	70.6	67.8

Baseline characteristics	Patients in bridging study N=28	Patients not in bridging study N= 8	All N=36
SD	8.56	17.63	11.00
Median	66.5	71.0	67.0
Min	53	44	44
Max	86	91	91
Gender - n (%)			
Female	18 (64.3)	4 (50.0)	22 (61.1)
Male	10 (35.7)	4 (50.0)	14 (38.9)
Race - n (%)			
American Indian Or Alaskan Native	0 (0)	0 (0)	0 (0)
Asian	2 (7.1)	1 (12.5)	3 (8.3)
Black or African American	1 (3.6)	0 (0)	1 (2.8)
Native Hawaiian Or Other Pacific Islander	1 (3.6)	0 (0)	1 (2.8)
White	23 (82.1)	7 (87.5)	30 (83.3)
Multiple	0 (0)	0 (0)	0 (0)
Missing	1 (3.6)	0 (0)	1 (2.8)
ECOG baseline - n (%)			
0	12 (42.9)	1 (12.5)	13 (36.1)
1	15 (53.6)	7 (87.5)	22 (61.1)
2	1 (3.6)	0 (0)	1 (2.8)
Histology - n (%)			
Adenocarcinoma	25 (89.3)	7 (87.5)	32 (88.9)
Adenosquamous Carcinoma - Predominantly adenocarcinoma	1 (3.6)	0 (0)	1 (2.8)
Adenosquamous Carcinoma - Predominantly Squamous Cell Carcinoma	1 (3.6)	0 (0)	1 (2.8)
Large Cell Carcinoma	0 (0)	1 (12.5)	1 (2.8)
Non-Small Cell Carcinoma Without Other	1 (3.6)	0 (0)	1 (2.8)
AJCC tumor stage - n (%)			
IIIA	1 (3.6)	0 (0)	1 (2.8)
IV	27 (96.4)	8 (100)	35 (97.2)
Smoking history - n (%)			
Never Smoked	9 (32.1)	1 (12.5)	10 (27.8)
Current Smoker	3 (10.7)	2 (25.0)	5 (13.9)
Former Smoker	16 (57.1)	5 (62.5)	21 (58.3)
Specimen type - n (%)			
Resection	10 (35.7)	0 (0)	10 (27.8)
Core needle biopsy	17 (60.7)	1 (12.5)	18 (50.0)
Fine needle aspirate	1 (3.6)	2 (25.0)	3 (8.3)
Not recorded	0 (0)	2 (25.0)	2 (5.6)
Other	0 (0)	3 (37.5)	3 (8.3)

All percentages calculated using N as denominator

Clinically important demographic and prognostic features including ECOG performance status and tumor stage were well balanced between the Oncomine™ Dx Target Test-evaluable and the Oncomine™ Dx Target Test-unevaluable populations.

D. Safety and Effectiveness Results

1. Safety Results

The safety with respect to treatment with TAFINLAR® (dabrafenib) in combination with MEKINIST® (trametinib) will not be addressed in detail in the SSED for the Oncomine™ Dx Target Test. Briefly, in the drug study, the most commonly occurring adverse reactions (≥ 20%) in these patients were: pyrexia, fatigue, nausea, vomiting, diarrhea, dry skin, decreased appetite, edema, rash, chills, hemorrhage, cough, and dyspnea. Adverse reactions resulting in discontinuation of TAFINLAR occurred in 18% of patients; ; the most common were pyrexia (2.2%), ejection fraction decreased (2.2%), and respiratory distress (2.2%). Adverse reactions leading to dose reductions of TAFINLAR occurred in 35% of patients; the most common were pyrexia (10%), diarrhea (4.3%), nausea (4.3%), vomiting (4.3%), and neutropenia (3.2%). Adverse reactions leading to dose interruptions of TAFINLAR occurred in 62% of patients; the most common were pyrexia (27%), vomiting (11%), neutropenia (8%), and chills (6%). Adverse reactions resulting in discontinuation of MEKINIST occurred in 19% of patients; the most common were pyrexia (2.2%), ejection fraction decreased (2.2%), and respiratory distress (2.2%). Adverse reactions leading to dose reductions of MEKINIST occurred in 30% of patients receiving MEKINIST plus dabrafenib; the most common were pyrexia (5%), nausea (4.3%), vomiting (4.3%), diarrhea (3.2%), and neutropenia (3.2%). Adverse reactions leading to dose interruptions of MEKINIST occurred in 57% of patients receiving MEKINIST plus dabrafenib; the most common were pyrexia (16%), vomiting (10%), neutropenia (8%), nausea (5%), and ejection fraction decreased (5%). Please refer to the drug labels for more information.

In the PMA clinical study, no adverse events were reported in connection with the studies used to support this PMA as the studies were performed retrospectively using banked samples.

2. Effectiveness Results

a. Concordance Study:
The concordance between the Oncomine™ Dx Target Test and BRAF V600 PCR Mutation Test (surrogate assay) were assessed in the combined Cohorts A, B and C as well as in Cohort B and C separately.

The agreements between the Oncomine™ Dx Target Test and the BRAF V600 surrogate assay are shown in the table below for the combined cohorts.

Table 11. Agreements between Oncomine™ Dx Target Test and PCR comparator method results in combined cohorts A, B and C.

Measure of agreement	Including No Calls		Excluding No Calls	
	% agreement (N)	95% CI[1]	% agreement (N)	95% CI[1]
PPA	100.0% (67/67)	(94.6%, 100.0%)	91.8%(67/73)	(83.0%, 96.9%)
NPA	100.0% (114/114)	(96.7%, 100.0%)	97.4%(114/117)	(92.7%, 99.5%)
OPA[2]	100.0% (181/181)	(97.9%, 100.0%)	95.3%(181/190)	(91.2%, 97.8%)

[1] 95%CI calculated using Pearson-Clopper Exact method
[2] Overall Percent Agreement

A subset of LLT positive [LLT(+)] specimens were retrospectively tested using the Oncomine™ Dx Target Test (CDx). Since LLT-negative [LLT(-)] specimens were not available for retesting, the acquired PCR(-) cohort samples were used to derive the NPA. The acquired PCR(-) cohort samples were randomly assigned to each study cohort in proportion to the size of the enrolled population of each cohort. The numbers of Acquired PCR(-) samples assigned to each cohort were as follows: 59 for Cohort A, 43 for Cohort B, and 27 for Cohort C.

In the combined cohorts, the agreements between the Oncomine™ Dx Target Test with LLT are shown in the tables below.

Table 12. Agreement between the Oncomine™ Dx Target Test and LLT/Acquired PCR(-) samples tested in combined Cohorts A, B and C.

Oncomine™ Dx Target Test	Available enrolled LLT(+) samples	Acquired PCR(-) samples	Total
Positive	72	1	73
Negative	8	114	122
No call	8	2	10
Invalid	23	7	30
Total	111	124	235

Table 13. Agreement between Oncomine™ Dx Test and LLT/Acquired PCR(-) sample results in combined Cohorts A, B and C.

Agreement Measure	Including No Calls		Excluding No Calls	
	% agreement (N)	95% CI[1]	% agreement (N)	95% CI[1]
PPA	90.0% (72/80)	(81.2%, 95.6%)	64.9% (72/111)	(55.2%, 73.7%)
NPA	99.1% (114/115)	(95.3%, 100.0%)	91.9% (114/124)	(85.7%, 96.1%)
OPA	95.4% (186/195)	(91.4%, 97.9%)	79.1% (186/235)	(73.4%, 84.2%)

[1]The 95%CI calculated using Pearson-Clopper Exact method

In Cohort B, the agreements between the Oncomine™ Dx Target Test with LLT are shown in the tables below.

Table 14. Agreement between the Oncomine™ Dx Target Test and LLT/Acquired PCR(-) samples from Cohort B.

Oncomine™ Dx Target Test	Available enrolled LLT(+) samples	Acquired PCR(-) samples	Total
Positive	22	1	23
Negative	2	36	38
No Call	5	1	6
Invalid	9	3	12
Total	38	41	79

Table 15. Agreement between Oncomine™ Dx Test and LLT/Acquired PCR(-) samples based on LLT/Acquired PCR(-) samples results in Cohort B.

Agreement Measure	Including No Calls		Excluding No Calls	
	% agreement (N)	95% CI[1]	% agreement (N)	95% CI[1]
PPA	91.7% (22/24)	(73.0%, 99.0%)	75.9% (22/29)	(56.5%, 89.7%)
NPA	97.3% (36/37)	(85.8%, 99.9%)	94.7% (36/38)	(82.3%, 99.4%)
OPA	95.1% (58/61)	(86.3%, 99.0%)	86.6% (58/67)	(76.0%, 93.7%)

[1] 95% CI calculated using Pearson-Clopper Exact method

In Cohort C, the agreements between the Oncomine™ Dx Target Test with LLT are shown in the tables below.

Table 16. Agreement between the Oncomine™ Dx Target Test and LLT/Acquired PCR(-) samples from Cohort C.

Oncomine™ Dx Target Test	Available enrolled LLT(+) samples	Acquired PCR(-) Samples	Total
Positive	23	0	23
Negative	0	22	22
No call	2	1	3
Invalid	3	2	5
Total	28	25	53

Table 17. Agreements between Oncomine™ Dx Test and LLT/Acquired PCR(-) samples in Cohort C.

Agreement Measure	Including No Calls		Excluding No Calls	
	% agreement (N)	95% CI[1]	% agreement (N)	95% CI[1]
PPA	100.0% (23/23)	(85.2%, 100.0%)	82.1% (23/28)	(63.1%, 93.9%)
NPA	100.0% (22/22)	(84.6%, 100.0%)	88.0% (22/25)	(68.8%, 97.5%)
OPA	100.0% (45/45)	(92.1%, 100.0%)	84.9% (45/53)	(72.4%, 93.3%)

[1] 95% CI calculated using Pearson-Clopper Exact method

b. Bridging Study:
The analysis of effectiveness was based on a bridging study conducted using Cohorts B and C of the clinical study which included 87 evaluable patients at

the time of database lock and samples from the acquired PCR(-) cohort which were randomized as part of the retrospective bridging study.

The analyses of primary efficacies were based on ORR by investigator assessment for Cohorts B and C separately. The efficacies were calculated in the LLT(+) and the Oncomine™ Dx Target Test positive [CDx(+)] population [denoted as "LLT(+), CDx(+)"] and the LLT(+) population using Cohorts B and C at the analysis cut-off date of August 8, 2016. Key effectiveness outcomes are presented in the tables below.

Table 18. Cohort B ORR by IA in [LLT(+), CDx(+)] and LLT(+) populations.

Clinical outcome	[LLT(+), CDx(+)] N=22 (%)	LLT(+) N=57 (%)
Best response		
Complete response (%)	2 (9.1)	3 (5.3)
Partial response (%)	14 (63.6)	35 (61.4)
Stable disease (%)	4 (18.2)	8 (14.0)
Progressive disease (%)	1 (4.5)	7 (12.3)
Non-PD/Non-CR (%)	0 (0)	0 (0)
Not evaluable (%)	1 (4.5)	4 (7.0)
Response rate		
CR + PR (%)	16 (72.7)	38 (66.7)
95% CI [(1)]	(49.8, 89.3)	(52.9, 78.6)

[1]95% CI calculated using Pearson-Clopper Exact method

Table 19. Cohort C ORR by IA in [LLT(+), CDx(+)] and LLT(+) populations.

Clinical outcome	[LLT(+), CDx(+)] N=23 (%)	LLT(+) N=36 (%)
Best Response		
Complete response (%)	2 (8.7)	2 (5.6)
Partial response (%)	12 (52.2)	20 (55.6)
Stable disease (%)	1 (4.3)	5 (13.9)
Progressive disease (%)	5 (21.7)	5 (13.9)
Non-PD/Non-CR (%)	0 (0)	0 (0)
Not evaluable (%)	3 (13.0)	4 (11.1)
Response Rate		
CR + PR (%)	14 (60.9)	22 (61.1)
95% CI [(1)]	(38.5, 80.3)	(43.5, 76.9)

[1]The 95% CI calculated using Pearson-Clopper Exact method

The ORR based on [LLT(+), CDx(+)] was 72.7% (49.8%, 89.3%) in Cohort B and 60.9% (38.5%, 80.3%) in Cohort C. The ORR estimate in the [LLT(+), CDx(+)] population was comparable to that in the LLT(+) population for Cohorts B and C, respectively, as determined investigator assessment in the therapeutic efficacy study.

c. Sensitivity Analysis:
The effectiveness for the CDx(+) population was estimated by considering the potential impact of missing data arising from patients with a positive CDx test result, but who may have been negative by the LLT. Patients with such test results are part of the intended use population of the CDx assay; however, they were excluded from the clinical trial due to negative results upon local test screening. To account for this missing data, the efficacy of the treatment of dabrafenib in combination with trametinib in patients with positive results from the CDx was estimated assuming different combinations for the following parameters:

- The ORR among patients with positive results with both the CDx and LLT for Cohorts B and C were fixed based on the observed data for each cohort respectively.
- The missing ORR among patients with positive CDx and negative LLT results was assumed to be c-value times of that observed in the CDx test positive and LLT positive with c ranging from 0 (no efficacy) to 1.0 (having the same efficacy). For example, c=0.3 refers to the assumed efficacy in the CDx(+) and LLT(-) population equal to 30% of the observed efficacy in the CDx(+) and LLT(+) population.
- The proportion of cases with positive LLT are assumed to be 2%, 4%, 10%, or 25%.
- The NPAs of the two tests (i.e., probability of CDx negative conditional on LLT negative) were assumed to vary from 80% to 97.3% for Cohort B and 80% to 100% for Cohort C. The upper ends of those NPAs were selected from the comparison of CDx and the validated BRAF V600 Mutation Comparator Test result [probability of CDx(-)] conditional on Comparator Test negative is 97.3% in Cohort B and 100% in Cohort C).

Combining all of the above assumed parameter values, the ORR modeled for the CDx positive population, including patients who may have tested negative by LLTs, was calculated. The smallest ORR value estimated for the CDx positive population, including those who may have tested negative by local lab tests, is 31.8% (23.1%, 39.5%) for Cohort B and 30.6% (20.6%, 40.2%) for Cohort C. The results are listed in the tables below.

Table 20. Sensitivity analysis for ORR by IA in CDx(+) population in Cohort B.

Pr(LLT+) (%)	Pr[CDx(-) |LLT(-)] (%)	c-value = 0.3	c-value = 0.7	c-value = 1.0
2.0	92.3	31.8 (23.1, 39.5)	55.2 (39.9, 68.7)	72.7 (52.6, 90.5)
	95.0	35.7 (25.9, 44.5)	56.8 (41.1, 70.8)	72.7 (52.6, 90.5)
	97.3	42.7 (31.0, 53.2)	59.8 (43.3, 74.4)	72.7 (52.6, 90.5)
4.0	92.3	38.7 (28.1, 48.2)	58.1 (42.0, 72.4)	72.7 (52.6, 90.5)
	95.0	43.9 (31.9, 54.7)	60.4 (43.6, 75.1)	72.7 (52.6, 90.5)
	97.3	51.6 (37.5, 64.3)	63.7 (46.0, 79.2)	72.7 (52.6, 90.5)
10.0	92.3	50.8 (36.9, 63.2)	63.3 (45.8, 78.8)	72.7 (52.6, 90.5)

Pr(LLT+) (%)	Pr[CDx(-) |LLT(-)] (%)	c-value = 0.3	c-value = 0.7	c-value = 1.0
	95.0	56.0 (40.6, 69.6)	65.5 (47.4, 81.6)	72.7 (52.6, 90.5)
	97.3	62.1 (44.9, 77.2)	68.2 (49.3, 84.8)	72.7 (52.6, 90.5)
25.0	92.3	62.5 (45.2, 77.7)	68.3 (49.4, 85.0)	72.7 (52.6, 90.5)
	95.0	65.6 (47.4, 81.5)	69.7 (50.4, 86.6)	72.7 (52.6, 90.5)
	97.3	68.6 (49.6, 85.3)	71.0 (51.4, 88.3)	72.7 (52.6, 90.5)

Table 21. Sensitivity analysis for ORR by IA in CDx(+) population in Cohort C.

Pr(LLT+) (%)	Pr(CDx-|LLT-) (%)	c-value = 0.3	c-value = 0.7	c-value = 1.0
2.0	95.0	30.6 (20.6, 40.2)	47.9 (32.2, 63.0)	60.9 (40.9, 80.0)
	98.0	39.8 (26.7, 52.3)	51.8 (34.8, 68.1)	60.9 (40.9, 80.0)
	100.0	60.9 (40.9, 80.0)	60.9 (40.9, 80.0)	60.9 (40.9, 80.0)
4.0	95.0	37.6 (25.3, 49.5)	50.9 (34.2, 66.9)	60.9 (40.9, 80.0)
	98.0	47.1 (31.6, 61.8)	54.9 (36.9, 72.2)	60.9 (40.9, 80.0)
	100.0	60.9 (40.9, 80.0)	60.9 (40.9, 80.0)	60.9 (40.9, 80.0)
10.0	95.0	47.6 (32.0, 62.6)	55.2 (37.1, 72.6)	60.9 (40.9, 80.0)
	98.0	54.4 (36.5, 71.5)	58.1 (39.0, 76.3)	60.9 (40.9, 80.0)
	100.0	60.9 (40.9, 80.0)	60.9 (40.9, 80.0)	60.9 (40.9, 80.0)
25.0	95.0	55.3 (37.2, 72.7)	58.5 (39.3, 76.9)	60.9 (40.9, 80.0)
	98.0	58.5 (39.3, 76.8)	59.8 (40.2, 78.6)	60.9 (40.9, 80.0)
	100.0	60.9 (40.9, 80.0)	60.9 (40.9, 80.0)	60.9 (40.9, 80.0)

Clinical relevant covariates were identified as either predictive for the clinical outcome (drug response) or the CDx results using a significant threshold of 20% for each Cohorts B and C. Covariates age, gender, sample type and smoking history were identified as clinical relevant covariates for Cohort B, and covariates gender, sample type and smoking history for Cohort C. Imputation model was used to impute the missing CDx results for the 33 patients in cohort B and 13 patients in cohort C.

For Cohort B, all clinical relevant covariates identified as well as the clinical outcome (e.g. drug response) were included in the imputation models. Missing CDx results were imputed 20 times to create the "complete" datasets. Finally the complete datasets were used to estimate the primary efficacy in the CDx(+) population assuming various levels of LLT(+) prevalence, negative percent agreement (Pr(CDx-|LLT-), and c values. For Cohort C, statistical models (logistic regression) including clinical relevant covariates cannot be applied to impute the missing CDx results as there is no CDx negative result in Cohort C. Missing CDx results were then imputed in patients with clinical outcome in Cohort C. The missing data in both Cohort B and C had minimal impact on the efficacy results.

3. Subgroup Analyses:

No subgroup analyses were performed.

4. Pediatric Extrapolation:
   In this premarket application, existing clinical data was not leveraged to support approval of a pediatric patient population.

E. ROS1 Study Design

The safety and effectiveness of the Oncomine™ Dx Target Test for the detection of ROS1 fusions was evaluated by retrospective analysis of FFPE NSCLC specimens were obtained from patients enrolled in the ROS1 cohort of Study A8081001 (Study 1001). Study 1001 is an ongoing single-arm, Phase I safety, pharmacokinetic, and pharmacodynamics study of crizotinib in patients with advanced cancer. The ROS1 fusion status of NSCLC tissue samples was determined by laboratory-developed break apart FISH (96%) or RT-PCR (4%) clinical trial assays. In Study 1001, ROS1 positivity by FISH required that ≥ 15% of a minimum of 50 evaluated nuclei contained a ROS1 gene rearrangement.

To support the ROS1 indication for the PMA, a bridging study was performed utilizing available ROS1-positive [ROS1(+)] tumor specimens from patients enrolled in Study 1001, together with additional ROS1(+) NSCLC tumor specimens identified from screening archival NSCLC samples, not associated with a crizotinib clinical study. The samples were tested with a validated central laboratory ROS1 FISH assay, consistent with a FISH assay used in Study 1001. The FISH assay served as a validated comparator method. A separate set of archival FFPE NSCLC specimens were determined to be ROS1-negative [ROS1(-)] based on a similar validated ROS1 FISH assay.

1. Clinical Inclusion and Exclusion Criteria:
   Enrollment in Study 1001 was limited to patients who met the following inclusion criteria:
   1. Histologically confirmed NSCLC positive for chromosomal translocations at ROS gene.
   2. Solid tumors must have measurable disease as per Response Evaluation Criteria in Solid Tumors (RECIST v. 1.0). However, patients whose tumors are not measurable may enter the study upon approval by the Sponsor. Target lesions that have been previously irradiated will not be considered measurable (lesion) unless increase in size is observed following completion of radiation therapy.
   3. Able, in the investigator's opinion, to receive at least 2 cycles of treatment.
   4. Female or male, 18 years of age or older.
   5. ECOG performance status 0 or 1. However, patients with an ECOG performance status of 2 may enter the study upon agreement between the investigator and sponsor.
   6. Resolution of all acute toxic effects of prior therapy or surgical procedures to Grade ≤ 1 (except alopecia).

7. Adequate organ function as defined by specified criteria.
8. Signed and dated informed consent document indicating that the patient (or legally acceptable representative) has been informed of all the pertinent aspects of the trial prior to enrollment.
9. Willingness and ability to comply with scheduled visits, treatment plans, laboratory tests, and other study procedures.

Patients were not permitted to enroll into Study1001 if they met any of the following exclusion criteria:

1. Major surgery, radiation therapy, or systemic anti-cancer therapy within 2 weeks of starting study treatment.
2. Prior high-dose chemotherapy requiring hematopoietic stem cell rescue.
3. Current treatment on another clinical trial.
4. Brain metastases, spinal cord compression, carcinomatous meningitis, or leptomeningeal disease unless appropriately treated and neurologically stable for at least 2 weeks and not taking medications contraindicated to Exclusion Criteria #10-12.
5. Any of the following within the 6 months prior to starting study treatment: myocardial infarction, severe/unstable angina, coronary/peripheral artery bypass graft, congestive heart failure, cerebrovascular accident including transient ischemic attack, or pulmonary embolus. However, upon agreement between the investigator and sponsor, the 6 month post-event-free period for a patient with a pulmonary embolus can be waived if due to advanced cancer. Appropriate treatment with anticoagulants is permitted.
6. Ongoing cardiac dysrhythmias of NCI CTCAE Grade ≥2, uncontrolled atrial fibrillation of any grade, or QTc interval >470 msec.
7. Hypertension that cannot be controlled by medications (>150/100 mmHg despite optimal medical therapy).
8. Pregnancy or breastfeeding. Female patients must be surgically sterile or be postmenopausal, or must agree to the use of effective contraception during the period of therapy. All female patients with reproductive potential must have a negative pregnancy test (serum or urine) prior to enrollment. Male patients must be surgically sterile or must agree to use effective contraception during the period of therapy. The definition of effective contraception will be based on the judgment of the principal investigator or a designated associate.
9. Other severe acute or chronic medical or psychiatric condition or laboratory abnormality that would impart, in the judgment of the investigator and/or sponsor, excess risk associated with study participation or study drug administration, which would make the patient inappropriate for entry into this study.
10. Use of drugs that are known strong CYP3A4 inhibitors within 7 days prior to the first dose of PF-02341066, including but not limited to atazanavir, clarithromycin, ketoconazole, itraconazole, telithromycin, troleandomycin, ritonavir, indinavir, nelfinavir, saquinavir, nefazodone, and voriconazole. Grapefruit or grapefruit juice should also be avoided. The topical use of these

medications (if applicable), such as 2% ketoconazole cream, may be allowed. All concomitant medication must be approved by the Sponsor.

11. Use of drugs that are known strong CYP3A4 inducers within 12 days prior to the first dose of PF-02341066, including but not limited to carbamazepine, phenobarbital, phenytoin, rifabutin, rifampin, and St. John's wort. All concomitant medication must be approved by the Sponsor.
12. Concurrent use of drugs that are CYP3A4 substrates with narrow therapeutic indices, including but not limited to dihydroergotamine, ergotamine, pimozide, astemizole*, cisapride*, and terfenadine* (*withdrawn from U.S. market). All concomitant medication must be approved by the Sponsor.
13. Patients with known interstitial fibrosis or interstitial lung disease.

Inclusion and exclusion criteria for enrollment into the bridging study consisted of:

Inclusion criteria:

1. All available samples enrolled into original A8081001 clinical study.
2. Additional ROS1 positive samples identified from archival NSCLC samples not associated with a crizotinib clinical study.

Exclusion criteria:

1. Samples with insufficient tumor to meet the minimum sample requirements.
2. Samples with insufficient isolated nucleic acids to meet the minimum sample quality and quantity requirements.

2. Follow-up Schedule:

As the bridging study was conducted retrospectively to establish safety and effectiveness for selecting patients using the Oncomine™ Dx Target Test there was no follow-up conducted.

3. Clinical Endpoints:

The primary endpoint of the original Study 1001 was to assess the efficacy of crizotinib as measured by ORR. Response was assessed according to Response Evaluation Criteria in Solid Tumors (RECIST) version 1.0 by investigator, while patients in the ROS1 NSCLC cohort were to be assessed according to RECIST version 1.0. Tumor assessments were to be performed by the investigators every second cycle [every 8 weeks in the ROS1(+) NSCLC cohort]. Clinical data from Study 1001 is based on a data cutoff date of November 30, 2014.

Evaluation of the safety and effectiveness of the Oncomine™ Dx Target Test included determination of concordance between the Oncomine™ Dx Target Test and the validated ROS1 FISH comparator test and an assessment of clinical outcomes, based on ORR and duration of response (DoR), for those patients from Study 1001 whose tumors were designated as ROS1(+) by the Oncomine™ Dx Target Test. The primary evaluation of concordance was based on PPA as determined by analysis of ROS1(+) specimens obtained from patients enrolled in the ROS1 Cohort of Study 1001 and also obtained from screening of archival

non-clinical trial specimens. NPA was determined by analysis of a separate set of ROS1(-) specimens derived from clinical trial screening. The efficacy outcome measures for Study 1001 were ORR and DoR, according to RECIST version 1.0 as assessed by IRR and investigator.

F. Accountability of PMA Cohort

At a data cutoff date of November 30, 2014, there were 50 NSCLC patients enrolled in the ROS1 cohort of Study 1001. Of those, data from 31 patients were excluded due to lack of specimen availability for testing, resulting in specimens from 19 patients available for testing by the Oncomine™ Dx Target Test. An additional 28 ROS1(+) samples were identified from screening of non-clinical study specimens, yielding a total of 47 specimens available for the bridging study. Of the 19 samples from Study 1001, 6 were not tested by the Oncomine™ Dx Target Test, leaving 13 from the trial available for testing. Two samples generated invalid results, and 11 samples had valid results. Nine (9) of the 28 non-clinical study samples were excluded due to insufficient RNA obtained from the samples. Nineteen (19) of the 28 non-clinical study samples were tested with the Oncomine™ Dx Target Test. Together with the Study 1001 specimens, a total of 32 samples were tested with the Oncomine™ Dx Target Test.

Among the 47 NSCLC specimens in the bridging study, 42 were tested with the ROS1 FISH comparator test. Five (5) specimens were not tested with the ROS1 FISH comparator test as they were provided as previously extracted RNA samples. Out of the 32 ROS1(+) samples that were tested with the Oncomine™ Dx Target Test, a ROS1 FISH comparator test result could not be obtained for 2 samples due to insufficient material for analysis and 1 was ROS1(-) [original false positive by local test confirmed to be ALK positive was also excluded (see below)], for a total of 29 ROS1(+) samples.

A total of 130 FFPE NSCLC specimens determined to be negative for the ROS1 biomarker [Acquired FISH(-) samples] were included and 5 specimens were excluded due to not meeting input criteria. Seven (7) ROS1 negative samples generated invalid results. Therefore, a total of 125 ROS1 negative specimens were tested by the Oncomine™ Dx Target Test. One additional sample, described above, which was falsely classified as ROS1(+) by local testing, and subsequently demonstrated to be negative by the ROS1 FISH comparator test, was included as a negative sample to yield a total of 126 ROS1(-) specimens with valid ROS1 FISH comparator test results.

G. Study Population Demographics and Baseline Parameters

The demographics of the study population are for the Study 1001 subjects included and excluded from the bridging study are described in the table below.

Table 22. Summary of patients with ROS1(+) NSCLC enrolled in Study 1001: All patients and by CDx evaluable status.

Characteristics	Summary Statistics	All Patients	CDx Test Non-Evaluable	CDx Test Evaluable[1]	p-value[2]
Age (years)	n Mean ± std Median Range	50 53.2 ± 13.2 53 25.0, 77.0	39 53.7 ± 13.7 54.0 25.0, 77.0	11 51.5 ± 11.4 52.0 35.0, 77.0	0.6271
Age <65 vs. ≥65	**% (n/N)**				
≥65		28.0% (14/50)	33.3% (13/39)	9.1% (1/11)	0.1477
< 65		72.0% (36/50)	66.7% (26/39)	90.9% (10/11)	
Sex	**% (n/N)**				
Female		61.5% (24/39)	36.4% (4/11)	61.5% (24/39)	0.1781
Male		38.5% (15/39)	63.6% (7/11)	38.5% (15/39)	
Race	**% (n/N)**				
Asian[3]		42.0% (21/50)	41.0% (16/39)	45.5% (5/11)	1.0000
Other		58.0% (29/50)	59.0% (23/39)	54.5% (6/11)	
Smoking classification	**% (n/N)**				
Ex-Smoker		22.0% (11/50)	25.6% (10/39)	9.1% (1/11)	0.4162
Never Smoked		78.0% (39/50)	74.4% (29/39)	90.9% (10/11)	
ECOG performance status	**% (n/N)**				
0		44.0% (22/50)	41.0% (16/39)	54.5% (6/11)	0.6174
1		54.0% (27/50)	56.4% (22/39)	45.5% (5/11)	
2		2.0% (1/50)	2.6% (1/39)		
Histology	**% (n/N)**				
Adenocarcinoma		96.0% (48/50)	97.4% (38/39)	90.9% (10/11)	0.3951
Non-Adenocarcinoma		2.0% (1/50)	2.6% (1/39)	0.0% (0/11)	
Other		2.0% (1/50)	0.0% (0/39)	9.1% (1/11)	
Percent FISH Positivity – Comparator method	n Mean ± std Median Range	13 78.0 ± 23.1 84.0 8.0, 96.0	3 77.3 ± 15.0 78.0 62.0, 92.0	10 78.2 ± 25.7 85.0 8.0, 96.0	0.1013
ROS1 FISH Comparator Result[4]	**% (n/N)**				
Negative		5.6% (1/18)	0.0% (0/7)	9.1% (1/11)	
No Result		27.8% (5/18)	57.1% (4/7)	9.1% (1/11)	
Positive		66.7% (12/18)	42.9% (3/7)	81.8% (9/11)	
ORR	**% (n/N)**				
0		28.0% (14/50)[5]	33.3% (13/39)	9.1% (1/11)	0.1477
1		72.0% (36/50)	66.7% (26/39)	90.9% (10/11)	
DoR (months)	n[6] Mean ± std Median Range	36 17.2 ± 9.8 15.4 2.8, 46.2	26 16.9 ± 10.4 14.3 2.8, 46.2	10 18.1 ± 8.4 17.5 7.3, 36.0	0.5022

[1] Of the 19 specimen available for analysis, 6 were not tested due to sample insufficiency (did not meet tumor content or RNA concentration criteria) and analysis of 2 specimens yielded invalid results.
[2] p-value for categorical variables is based on a two-sided Fisher's Exact test and for continuous variables it is based on an unpaired t- test or Wilcoxon rank sum test, as appropriate.
[3] Korean ethnicity
[4] 1 of the 19 NGS evaluable clinical study specimens was not tested using ROS1 FISH Comparator Test
[5] Number of patients with an objective response of CR or PR
[6] In DoR n = number of patients exhibiting a response

H. Safety and Effectiveness Results

1. Safety Results

   The safety with respect to treatment with XALKORI® (crizotinib) will not be addressed in the SSED for the Oncomine™ Dx Target Test. Please refer to the drug label for more information.

   In the PMA clinical study, no adverse events were reported in connection with the studies used to support this PMA as the studies were performed retrospectively using banked samples.

2. Effectiveness Results

   A single-arm, Phase I safety, pharmacokinetic, and pharmacodynamics study of crizotinib that enrolled a cohort of patients with ROS1-positive NSCLC selected by local laboratory testing served as the basis for the approval of ROS1(+) NSCLC patients with XALKORI® (crizotinib) in NDA 202570/S016 (Study 1001). As part of a bridging study intended to support the clinical performance of the Oncomine™ Dx Target Test for selection of ROS1(+) patients for treatment with crizotinib, available FFPE specimens from Study 1001 and additional non-clinical study specimens were retrospectively tested using the Oncomine™ Dx Target Test. Effectiveness of the Oncomine™ Dx Target Test was determined based on a combination of data from the concordance study, bridging, and sensitivity analyses.

   a. Concordance Study:
      The concordances between Oncomine™ Dx Target Test and ROS1 FISH comparator test were assessed based on a total of 157 specimens (Table 23). The PPAs, NPAs, and OPAs of the Oncomine™ Dx Target Test with ROS1 FISH comparator test, including and excluding invalid results are shown in the tables below.

Table 23. Agreement table with ROS1 concordance results.

		ROS1 FISH Comparator Test Result			Total
		ROS1(+)	ROS1(-)	No Result	
Oncomine™ Dx	**ROS1(+)**	20	0	0	20

		ROS1 FISH Comparator Test Result			Total
		ROS1(+)	ROS1(-)	No Result	
Target Test	ROS1(-)	5	119[1]	2	126
	Invalid	4	7	0	11
Total[2]		29	126	2	157

[1] Includes 1 FISH compartor negative result that was originally classified as local test FISH positive (original false positive as noted in [2])

[2] Data exclusion was based on Oncomine DxTarget Test NGS data. As noted in above, of the 32 ROS1-positive specimens by local lab test, 2 were QNS (quantity not sufficient) by the FISH comparator result and 1 was negative (original false positive by local test confirmed to be ALK positive and reported in Shaw et al, NEJM 2014), leading to 29 ROS1-positive samples and 126 ROS1-negative samples by the FISH comparator.

Table 24. Agreements between the Oncomine™ Dx Target Test and ROS1 FISH comparator.

Agreement Measure	% agreement (N)	95% CI[1]
PPA	80.0% (20/25)	(59.3%, 93.2%)
NPA	100% (119/119)	(96.9%, 100%)
OPA	96.5% (139/144)	(92.1%, 98.9%)

Table 25. Agreement table with ROS1 concordance results based on FFPE specimens.

		ROS1 FISH Comparator Test Result			Total
		ROS1(+)	ROS1(-)	No Result	
Oncomine™ Dx Target Test	ROS1(+)	17	0	0	17
	ROS1(-)	3	119[1]	2	124
	Invalid	4	7	0	11
Total[2]		24	126	2	152

[1] Data exclusion was based on Oncomine Dx Target Test NGS data.

[2] Inclusion of 1 FISH comparator negative result that was originally classified as local test FISH positive

Table 26. Agreements between the Oncomine™ Dx Target Test and ROS1 FISH comparator for FFPE specimens.

Agreement Measure	% agreement (N)	95% CI[3]
PPA	85.0% (17/20)	(62.1%, 96.8%)
NPA	100% (119/119)	(96.9%, 100%)
OPA	97.8% (136/139)	(93.8%, 99.6%)

b. Bridging Study:
The analyses of primary efficacy were based on ORR. The efficacy was calculated for (a) all ROS1(+) as determined for enrollment into the ROS1 cohort of Study 1001; (b) all ROS1(+) as determined for enrollment into the ROS1 cohort of Study 1001 and included in this PMA; (c) ROS1(+) by both ROS1 FISH comparator test and Oncomine™ Dx Target Test; and (d)

ROS1(+) by Oncomine™ Dx Target Test. The ORR estimate in population (a) was 72.0% (57.5%, 83.8%); in population (b) was 78.9% (54.4%, 94.0%); and in population (c) was 83.3% (35.9%, 99.6%). As the NPA of Oncomine™ Dx Target Test conditional on the ROS1 FISH comparator test results is 100%, no bridging study was required, and the efficacy in the population (d) is equivalent to that in the population (c). The results are summarized in Tables 27 – 28 below.

Table 27. Objective Response Rate.

Analysis Population		**Summary Statistics (n/N) [Proportion (Exact 95% CI)]**
a	ROS1(+) as determined for enrollment into the ROS1 cohort of Study 1001: All patients included in Study 1001	36/50 [72.0% (57.5%, 83.8%)]
b	ROS1(+) as determined for enrollment into the ROS1 cohort of Study 1001 and included in this PMA	15/19 [78.9% (54.4%, 94.0%)]
c	ROS1(+) by both ROS1 FISH Comparator Test and by Oncomine™ Dx Target Test	5/6 [83.3% (35.9%, 99.6%)]
d	ROS1(+) by Oncomine™ Dx Target Test	5/6 [83.3% (35.9%, 99.6%)]

Table 28. Duration of Response (Months).

Analysis Population		**Summary Statistics** **n** **Mean ± std** **Median** **Range** **(95% CI)**	**p-value***
a	ROS1(+) as determined for enrollment into the ROS1 cohort of Study 1001: All patients included in Study 1001	36 17.2 ± 9.80 15.4 2.76, 46.2 (13.9, 20.6)	NA
b	ROS1(+) as determined for enrollment into the ROS1 cohort of Study 1001 and included into this PMA	15 19.3 ± 8.20 17.6 7.29, 36.0 (14.8, 23.9)	0.30
c	ROS1(+) by both ROS1 FISH Comparator Test and by Oncomine™ Dx Target Test	5 17.5 ± 5.34 17.5 10.2, 24.8 (10.9, 24.1)	0.65

NA=Not applicable

* Based on Wilcoxon rank sum test for differences between patients in Study 1001 and other Analysis Populations

3. Sensitivity Study:
   Sensitivity analyses were conducted to assess the robustness of the efficacy results to the missing data of the Oncomine™ Dx Target Test. Among a total of 50 specimens determined to be ROS1(+) by the ROS1 FISH comparator test from enrolled patients in Study 1001, 11 specimens had valid results for the Oncomine™ Dx Target Test and 39 specimens were non-evaluable or invalid for the Oncomine™ Dx Target Test.

   Missing imputations were conducted by including different sets of variables as follows:
   a. Variables age, gender, race, smoking classification, ECOG PS (0 vs 1 or 2), histological classification and objective response were used to impute missing values.
   b. Variables identified as potentially predicting Oncomine™ Dx Target Test status (sex) or outcome (smoking classification and ECOG PS) using a significant threshold of 20%. Objective response and DoR were not included in this model as it was not considered appropriate to model Oncomine™ Dx Target Test positive or negative status as a function of outcome.
   c. Variables identified as potentially predicting Oncomine™ Dx Target Test status (sex) or outcome (smoking classification and ECOG PS) using a significant threshold of 20%, plus ROS1 FISH comparator test result and objective response.
   d. A stepwise selection model yielded 0 variables remained in the model at significant level of 20%. Therefore the imputation model included only objective response.

   Results from the sensitivity analyses for all 4 imputation approaches above for the ROS1(+) subgroup by the Oncomine™ Dx Target Test (including imputed ROS1-positives by the Oncomine™ Dx Target Test) are provided in Table 29, below.

Table 29. Imputed estimates for ORR by variables included in imputation model.

Imputation	Estimate (95% CI)
1	80.5% (51.5%, 100.0%)*
2	81.5% (52.9%, 100.0%)*
3	68.2% (47.2%, 89.2%)
4	74.7% (43.8%, 100.0%)*

* Lower and upper limit truncated

   The missing data had minimal impact on the conclusions based on observed data.

3. Subgroup Analyses
   No subgroup analyses were performed.

4. Pediatric Extrapolation

In this premarket application, existing clinical data was not leveraged to support approval of a pediatric patient population.

I. EGFR Study Design

The safety and effectiveness of the Oncomine™ Dx Target Test were evaluated by retrospective analysis of FFPE NSCLC specimens consisting of a convenience sample obtained from a subset of patients who were enrolled in an ongoing, multi-national, multi-center, randomized, open-label, Phase III clinical study of patients with newly diagnosed stage IIIB/IV or recurrent NSCLC. Patients enrolled in this study were determined to have tumors positive for mutations in exons 19 (deletions) or 21 (L858R) of the EGFR gene by the QIAGEN *therascreen*® EGFR RGQ PCR Kit. Available FFPE NSCLC tumor specimens from patients enrolled in the clinical study included EGFR-positive and EGFR-negative tissue sections and extracted nucleic acid samples from FFPE tissue sections. A separate set of archival FFPE NSCLC specimens was included and determined to be EGFR-negative based on testing using the QIAGEN *therascreen*® EGFR RGQ PCR Kit. Evaluation of the safety and effectiveness of the Oncomine™ Dx Target Test included determination of concordance of valid results from the Oncomine™ Dx Target Test and the QIAGEN *therascreen*® EGFR RGQ PCR Kit (comparator method). The primary evaluation of concordance was based on PPA as determined by analysis of EGFR-positive specimens obtained from patients enrolled in the clinical study, and NPA as determined by analysis of EGFR-negative specimens from the clinical study, as well as a separate set of EGFR-negative specimens derived from clinical trial screening.

1. Clinical Inclusion and Exclusion Criteria:
   Specimens included in the study were limited to a subset of patient samples that met the following criterion:
   a. The results from the specimens must have been centrally confirmed.
   b. Samples that were not permitted for the concordance study were specimens that did not meet sample input criteria.

2. Follow-up Schedule:
   As the bridging study was conducted retrospectively to establish safety and effectiveness for selecting patients using the Oncomine™ Dx Target Test there was no follow-up conducted.

3. Clinical Endpoints:
   The primary endpoint of this concordance study was the establishment of the PPA and NPA between the QIAGEN *therascreen*® EGFR RGQ PCR Kit and the Oncomine™ Dx Target Test.

J. Accountability of PMA Cohort

A total of 153 EGFR mutation positive specimens as determined by the QIAGEN *therascreen*® EGFR RGQ PCR Kit from the clinical study were available to support

this PMA. Eighty (80) of those specimens were slides with tissue sections from FFPE NSCLC tumors and 73 specimens were DNA extracts from NSCLC tumors. In addition, there were 142 specimens that tested negative using the QIAGEN *therascreen*® EGFR RGQ PCR Kit including 12 clinical study specimens (3 DNA extracts and 9 tissue specimens) and 130 archival EGFR-negative FFPE NSCLC specimens sourced through commercial vendors.

A total of 69 specimens were excluded from analysis due to not meeting the tumor content requirement or not passing the DNA concentration threshold. Of the 69 excluded, 64 EGFR positive clinical study specimens (10 FFPE samples and 54 DNA extracts) and 5 archival EGFR-negative FFPE NSCLC specimens sourced through commercial vendors. In addition, sample slides for one patient who was EGFR-negative by the QIAGEN *therascreen*® EGFR RGQ PCR Kit were not shipped inadvertently to the lab for testing with the Oncomine™ Dx Target Test. In summary, a total of 225 samples (92 EGFR-positive and 133 EGFR-negative) remained available for Oncomine™ Dx Target Test.

Of the 92 EGFR-positive samples, 20 samples generated invalid or no call Oncomine™ Dx Target Test results, and 72 generated valid results both from the QIAGEN *therascreen*® EGFR RGQ PCR Kit and the Oncomine™ Dx Target Test. Of the 133 EGFR-negative samples, 12 samples generated invalid or no call NGS results, resulting in 121 valid results using for both tests. A total of 72 EGFR-positive samples (42 with EGFR Ex. 19del and 30 with EGFR L858R mutations) and 121 EGFR-negative samples (i.e., 193 total samples) were included to evaluate concordance between the Oncomine™ Dx Target Test and the QIAGEN *therascreen*® EGFR RGQ PCR Kit.

K. Study Population Demographics and Baseline Parameters

Baseline characteristics were compared between those patients included and those excluded in the EGFR concordance study. Among a total of 295 specimens, 225 were available for Oncomine™ Dx Target Test.

Table 30. Baseline characteristics for patients whose samples were included vs. excluded from the EGFR concordance study analysis.

	Included (n=225)	**Excluded (n=70)**
Sex		
Male	123 (55%)	32 (46%)
Female	102 (45%)	38 (54%)
Race[1]		
Asian	43 (19%)	43 (61%)
White	77 (34%)	22 (31%)
Other	1 (1%)	1 (1%)
Missing	104 (46%)	4 (6%)
Stage		

	Included (n=225)	Excluded (n=70)
IA	5 (2%)	0
IB	4 (2%)	1 (1%)
IIA	2 (1%)	1 (1%)
IIB	1 (1%)	0
III	15 (7%)	1(1%)
IIIA	65 (29%)	2 (3%)
IIIB	26 (12%)	2 (3%)
IV	98 (44%)	59 (84%)
Metastatic Lesion consistent with NSCLC (From path report)	2 (1%)	0
Missing	7 (3%)	4(6%)

[1] Race data of the majority of negative samples is missing;
Asian: Japanese, Other East Asian;
White: Caucasian;
Other: Ethiopian, Moroccan.

L. Safety and Effectiveness Results

1. Safety Results:
   The safety with respect to treatment with IRESSA® (gefitinib) will not be addressed in the SSED for the Oncomine™ Dx Target Test. Please refer to the drug label for more information.

2. Effectiveness Results:

   a. Concordance Results:
   The concordances between the Oncomine™ Dx Target Test and the QIAGEN *therascreen*® EGFR RGQ PCR Kit were assessed based on total 225 specimens. The agreements between the Oncomine™ Dx Target Test with the QIAGEN *therascreen*® EGFR RGQ PCR Kit are shown in the tables below.

Table 31. EGFR concordance results in aggregate.

		QIAGEN *therascreen*® EGFR RGQ PCR Kit		Total
		EGFR(+)	EGFR(-)	
Oncomine™ Dx Target Test	EGFR(+)	71	1[1]	72
	EGFR(-)	1[1]	120	121
	No Call	15	3	18
	Invalid	5	9	14
Total		92	133	225

[1] Incorrect calls were both for Ex. 19del.

Table 32. Agreements between the Oncomine™ Dx Target Test and the QIAGEN *therascreen*® EGFR RGQ PCR Kit.

Agreement Measure	Including No Calls		Excluding No Calls	
	% agreement (N)	95% CI[1]	% agreement (N)	95% CI[1]
PPA	98.6% (71/72)	(92.5%, 100.0%)	81.6% (71/87)	(71.9%, 89.1%)
NPA	99.2% (120/121)	(95.5%, 100.0%)	96.8% (120/124)	(92.0%, 99.1%)
OPA	99.0% (191/193)	(96.3%, 99.9%)	90.5% (191/211)	(85.7%, 94.1%)

[1] The 95% CI calculated using Pearson-Clopper Exact method

Of the samples included in the study, the concordance between the Oncomine™ Dx Target Test and the QIAGEN *therascreen*® EGFR RGQ PCR Kit for the Exon 19del and L858R variants are described in the tables below:

Table 33. EGFR concordance results by individual variant.

		QIAGEN *therascreen*® EGFR RGQ PCR Kit					
		EGFR Exon 19del			EGFR L858R		
		EGFR(+)	EGFR(-)	Total	EGFR(+)	EGFR(-)	Total
Oncomine™ Dx Target Test	**EGFR(+)**	41	1	42	30	0	30
	EGFR(-)	1	147	148	0	167	167
	No Call	13	8	21	2	12	14
	Invalid	4	10	14	1	13	14
Total		59	166	225	33	192	225

Table 34. Agreements between the Oncomine™ Dx Target Test and the QIAGEN therascreen® EGFR RGQ PCR Kit based on individual variant.

EGFR Variant	Agreement Measure	Including No Calls		Excluding No Calls	
		% Agreement (N)	95% Exact CI	% Agreement (N)	95% Exact CI
Exon 19del	**PPA**	74.6% (41/55)	(61.00%, 85.33%)	97.6% (41/42)	(87.43%, 99.94%)
	NPA	94.2% (147/156)	(89.33%, 97.33%)	99.3% (147/148)	(96.29%, 99.98%)
	OA	89.1% (188/211)	(84.09%, 92.96%)	99.0% (188/190)	(96.25%, 99.87%)
L858R	**PPA**	93.8% (30/32)	(79.19%, 99.23%)	100% (30/30)	(88.43%, 100%)
	NPA	93.3% (167/179)	(88.58%, 96.49%)	100% (167/167)	(97.82%, 100%)
	OA	93.4% (197/211)	(89.12%, 96.33%)	100% (197/197)	(98.14%, 100%)

The reproducibility results observed between the Oncomine™ Dx Target Test and the QIAGEN therascreen® EGFR RGQ PCR Kit were comparable.

A confirmatory study will be conducted to support the effectiveness results from this study.

b. Sensitivity Results:
Sensitivity analyses were conducted to assess the robustness of the concordance results to the missing data of Oncomine™ Dx Target Test. The missing data had minimal impact on the conclusions based on observed data.

3. Subgroup Analyses:
   No subgroup analyses were performed.

4. Pediatric Extrapolation:
   In this premarket application, existing clinical data was not leveraged to support approval of a pediatric patient population.

M. Financial Disclosure

The Financial Disclosure by Clinical Investigators regulation (21 CFR 54) requires applicants who submit a marketing application to include certain information concerning the compensation to, and financial interests and arrangement of, any clinical investigator conducting clinical studies covered by the regulation. None of the clinical investigators had disclosable financial interests/arrangements as defined in sections 54.2(a), (b), (c), and (f). The information provided does not raise any questions about the reliability of the data.

XI. PANEL MEETING RECOMMENDATION AND FDA'S POST-PANEL ACTION

In accordance with the provisions of section 515(c)(3) of the act as amended by the Safe Medical Devices Act of 1990, this PMA was not referred to the Molecular and Clinical Genetics Panel of Medical Devices, an FDA advisory committee, for review and recommendation because the information in the PMA substantially duplicates information previously reviewed by this panel.

XII. CONCLUSIONS DRAWN FROM PRECLINICAL AND CLINICAL STUDIES

A. Effectiveness Conclusions

The clinical benefit of the Oncomine™ Dx Target Test in the detection of V600E and EGFR Ex. 19del and L858R mutations in DNA, and ROS1 fusions in RNA, isolated from FFPE tumor tissue was demonstrated in retrospective analyses of patients enrolled in two clinical studies (BRF113928 for BRAF V600E mutations and A8081001 for ROS1) and one concordance study (for EGFR Ex. 19del and L858R mutations). The clinical outcomes, based on ORR, observed for both clinical studies (BRF113928 for BRAF V600E and Study A8081001 for ROS1) were maintained based on the ORR estimated from the respective bridging studies supporting the effectiveness of the Oncomine™ Dx Target Test to select NSCLC patients whose tumors are positive for BRAF V600E or ROS1 fusions for treatment with TAFINLAR® (dabrafenib) in combination with MEKINIST® (trametinib) and XALKORI® (crizotinib), respectively. In addition, the high concordance observed between the Oncomine™ Dx Target Test and the QIAGEN *therascreen*® EGFR RGQ PCR Kit and the comparable reproducibility performance observed between the two tests support the effectiveness of the Oncomine™ Dx Target Test to identify NSCLC patients whose tumors are positive for the EGFR Ex. 19del or L858R mutations for treatment with IRESSA (gefitinib).

Analytical performance studies were conducted with the Oncomine™ Dx Target Test using DNA and RNA extracted from FFPE tissue of NSCLC patients. When the test is used according to the directions provided, the sensitivity for detecting the tested variants is shown in Table 7, above.

B. Safety Conclusions

The Oncomine™ Dx Target Test involves testing on FFPE human NSCLC cancer tissue sections. The risks of the Oncomine™ Dx Target Test are associated with the potential mismanagement of patients resulting from false test results. Failure of the device to perform as expected or failure to correctly interpret test results may lead to incorrect BRAF, EGFR, or ROS1 test results, and consequently improper patient management decisions in NSCLC treatment. A patient with a false positive result may undergo treatment with TAFINLAR® (dabrafenib) in combination with MEKINIST® (trametinib), IRESSA® (gefitinib), or XALKORI® (crizotinib) with inappropriate expectation of therapeutic benefit and experience side effects. A patient with a false negative result may be treated without TAFINLAR® (dabrafenib) in combination with MEKINIST® (trametinib), IRESSA® (gefitinib), or XALKORI® (crizotinib) and not experience the potential therapeutic benefit.

C. Benefit-Risk Determination

The probable benefits of the device are based on data collected in the BRF113928 (BRAF) and A8081001 (ROS1) clinical studies and the EGFR concordance study, which were conducted to support PMA approval.

The clinical benefit of the Oncomine™ Dx Target Test for the selection of NSCLC patients with a BRAF V600E mutation was demonstrated in the retrospective analyses of efficacy and safety data obtained from a Phase II multi-center, multi-cohort, non-randomized, open-label study in which patients with a BRAF V600E variant who had progressed on at least on prior treatment or were treatment naïve were treated with dabrafenib in combination with trametinib. The ORR based on [LLT(+), CDx(+)] was 72.7% (49.8% - 89.3%) in Cohort B and 60.9% (38.5% - 80.3%) in Cohort C. The ORR estimate in the [LLT(+), CDx(+)] population was comparable to that of the LLT(+) population for Cohort B and C, respectively.

The clinical benefit of the Oncomine™ Dx Target Test for the selection of NSCLC patients with a ROS1 fusion was demonstrated in a retrospective analyses of efficacy and safety data obtained from a Phase I single-arm, safety, pharmacokinetic, and pharmacodynamics study. The ORR based on investigator assessment for the ROS1(+) population identified by both the ROS1 FISH comparator test and the Oncomine™ Dx Target Test was 83.3% (35.88%, 99.58%) and the DoR was 17.5 ± 5.34 months. Both estimates are similar to those observed for the enrolled ROS1 cohort from Study 1001.

The clinical benefit of the Oncomine™ Dx Target Test for the selection of NSCLC patients with an EGFR (Ex. 19del or L858R variant) mutation was demonstrated in a retrospective analysis of concordance between the Oncomine™ Dx Target Test and the FDA-approved QIAGEN *therascreen*® EGFR RGQ PCR Kit. The PPA was 98.6% (92.5%, 100%); NPA was 99.2% (95.5%, 100%); and OA was 99.0% (96.3%, 99.9%) when invalids and no calls are excluded. Comparable performance was observed between the reproducibility studies for the Oncomine™ Dx Target Test and the QIAGEN *therascreen*® EGFR RGQ PCR Kit. A confirmatory study, however, will be conducted to support the effectiveness results for this indication.

The risks of the Oncomine™ Dx Target Test device are associated with the potential mismanagement of patients resulting from erroneous test results. The device is a key part of diagnostic evaluation for NSCLC patients in decisions regarding treatment with TAFINLAR® (dabrafenib) in combination with MEKINIST® (trametinib) for BRAF, XALKORI® (crizotinib) for ROS1, and IRESSA® (gefitinib) for EGFR.

1. Patient Perspectives
   This submission did not include specific information on patient perspectives for this device.

In conclusion, given the available information above, the data support that for the selection of NSCLC patients with BRAF V600E, EGFR Ex. 19del or L858R mutations, or ROS1 fusions by the Oncomine™ Dx Target Test for treatment with TAFINLAR® (dabrafenib) in combination with MEKINIST® (trametinib), IRESSA® (gefitinib), or XALKORI® (crizotinib), respectively, the probable benefits outweigh the probable risks.

D. Overall Conclusions

The data in this application support the reasonable assurance of safety and effectiveness of this device when used in accordance with the indications for use. Data from the clinical studies [Phase II study BRF113928 (BRAF), Phase I study A8081001 (ROS1)] and the concordance study (for EGFR) support the performance of the Oncomine™ Dx Target Test as an aid in selecting patients with NSCLC for whom TAFINLAR®(dabrafenib) in combination with MEKINIST® (trametinib), IRESSA® (gefitinib), or XALKORI® (crizotinib) are indicated.

XIII. CDRH DECISION

CDRH issued an approval order on June 22, 2017. The final conditions of approval cited in the approval order are described below.

The applicant will provide the following in a post-approval report:

- Final study data, study conclusions, and labeling revisions within 9 months of the PMA approval date for:

- Results from a three site reproducibility study using clinical samples with allelic frequencies near the established limit of detection (i.e., 1x LoD) to confirm the values from the limit of detection study.

- Results from an interference study using variant positive clinical samples in order to confirm that the presence of necrotic tissue does not interfere with the performance of the Oncomine™ Dx Target Test.

- Results from a study using clinical specimens to demonstrate that 3 different lots of each of the components of the Oncomine™ Dx Target Test and Controls Kit may be used interchangeably, in order to confirm that the interchangeability demonstrated using control samples reflects performance with clinical samples as well.

- Results from an additional guard-banding study using fusion positive clinical samples to verify the tolerance ranges of the critical elements of the RNA workflow for the Oncomine™ Dx Target Test.

- Validation testing, results, and associated software documentation within 3 months of the PMA approval date from regression testing on the commercial release configuration (Torrent Suite Dx Software v5.6.4 and ADF v1.8) to confirm there are no defects for ADF and no new defects other than those listed in Torrent Suite Dx v5.6.4 Software Release Note (Rev. D).

- Validation testing, results, and associated software documentation within 6 months of the PMA approval date to confirm the resolution of existing unresolved anomalies 2519 and 2520.

The applicant's manufacturing facilities have been inspected and found to be in compliance with the device Quality System (QS) regulation (21 CFR 820).

XIV. APPROVAL SPECIFICATIONS

Directions for use: See device labeling.

Hazards to Health from Use of the Device: See Indications, Contraindications, Warnings, Precautions, and Adverse Events in the device labeling. Refer to the drug labels for TAFINLAR® (dabrafenib), MEKINIST® (trametinib), IRESSA® (gefitinib), and XALKORI® (crizotinib) for additional information related to the use of the drugs.

Post-approval Requirements and Restrictions: See approval order.