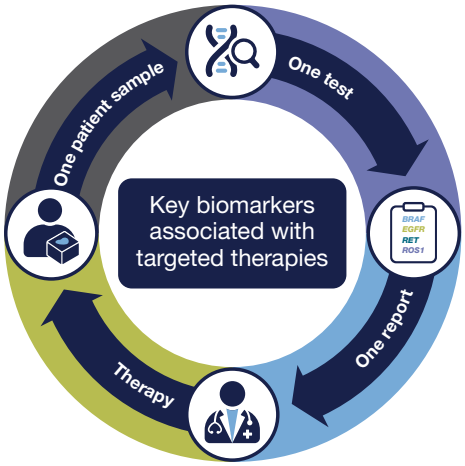
ion torrent

Oncomine Dx Target Test

A new paradigm in testing for targeted therapies in NSCLC and CC with an FDA-approved NGS CDx test

The Ion Torrent™ Oncomine™ Dx Target Test is the first targeted next-generation sequencing (NGS) *in vitro* diagnostic (IVD) test for non–small cell lung cancer (NSCLC) and cholangiocarcinoma (CC), simultaneously delivering multiple biomarker results for multiple targeted therapies from one sample within 4 days.

This test is now reimbursed by Medicare and the top 40 commercial payers, covering over 200 million US lives.



The Oncomine Dx Target Test enables:

- **Fast results**—The single streamlined sequencing workflow enables concurrent analysis of both DNA and RNA targets. From sample extraction to clinical test report, the total workflow turnaround time is 4 days.
- **Clinical performance**—Based on Ion AmpliSeq™ technology, the test is designed to deliver robust and reproducible results in 23 genes clinically associated with NSCLC and one gene in CC, all from 10 ng of DNA and RNA from formalin-fixed, paraffin-embedded (FFPE) tissue.
- **Automated clinical report**—The Oncomine Dx Target Test results are presented in a single two-part Clinical Test Report that incorporates companion diagnostic (CDx) biomarker results, with associated therapy indications, and other detected cancer-associated gene variant results.

Cancer type	Gene	Targeted therapies
NSCLC	*BRAF*	TAFINLAR® (dabrafenib) in combination with MEKINIST® (trametinib)
	EGFR L858R, exon 19 deletions	IRESSA® (gefitinib)
	EGFR exon 20 insertions	EXKIVITY™ (mobocertinib) RYBREVANT™ (amivantamab-vmjw)
	ERBB2/HER2 activating mutations (SNVs and exon 20 insertions)	ENHERTU® (fam-trastuzumab deruxtecan-nxki)
	RET	GAVRETO™ (pralsetinib)
	ROS1	XALKORI® (crizotinib)
CC	*IDH1*	TIBSOVO® (ivosidenib)

Figure 1. List of genes for therapeutic use.

ThermoFisher SCIENTIFIC

For *In Vitro* Diagnostic Use.

Complete system: from sample to actionable result, powered by proven Ion Torrent and Ion AmpliSeq NGS technology

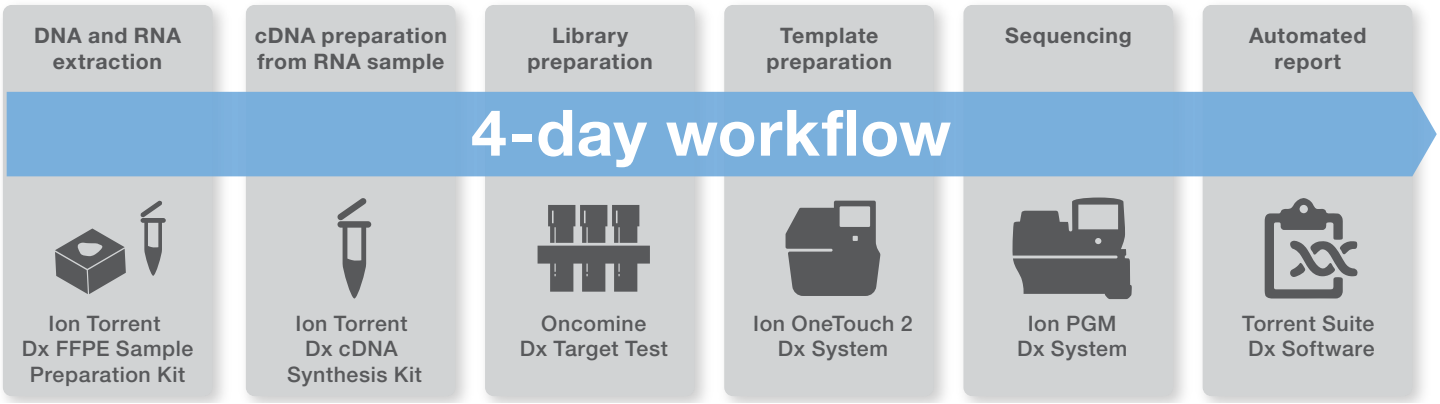


Figure 2. The Oncomine Dx Target Test utilizes a single streamlined NGS workflow for detecting cancer-associated biomarkers, incorporating reagents, instrument systems, and bioinformatics. The turnaround time, from FFPE sample to report, is 4 days.

Optimized for challenging FFPE samples

Based on Ion AmpliSeq technology, the Oncomine Dx Target Test requires as little as 10 ng of input DNA and RNA. This enables analysis of small and challenging samples. Alternative NGS methods require more FFPE slides and hundreds of nanograms of DNA and RNA, making them less practical for routine analysis of FFPE tumor samples.

Quality controls included

The Oncomine Dx Target Test incorporates DNA, RNA, and no-template controls for automatic assessment of run success.

Ion PGM Dx Sequencer

Targeted sequencing is performed on an Ion PGM™ Dx Sequencer using an Ion 318™ Dx Chip, which can accommodate up to 5.5 million reads and 6 patient samples per run (barcode adapters for multiplexing included). Run setup is fast with an easy user interface, and sequencing run time is approximately 4.5 hours. Data analysis and reporting are fully automated and streamlined using Torrent Suite™ Dx Software v5.12.5 or later.

A complete and flexible system

The Oncomine Dx Target Test is used in conjunction with the Ion PGM Dx sequencing system, which includes a complete NGS system of instruments, reagents, and software, initially validated using challenging germline variants and now validated with the Oncomine Dx Target Test for somatic mutation reporting for FFPE samples. The Ion PGM Dx sequencing system is a Class II 510K Medical Device and incorporates combined functionality, with both “IVD Mode” for molecular diagnostic tests and “Assay Development Mode” for clinical research. The system also facilitates 21 CFR Part 11 compliance, role-based workflows, sample and reagent tracking, QC metrics, and audit trails.

Oncomine Dx Target Test for NSCLC

Oncomine Dx Target Test—content

The Oncomine Dx Target Test includes targets for cancer-associated genes that all play an important role in NSCLC pathogenesis. Six biomarkers are companion diagnostics to aid in selecting patients for approved targeted therapies, while others are currently being investigated in clinical trials and are potentially actionable in the future as referenced in Figure 3. The Oncomine Dx Target Test is indicated as a companion diagnostic to aid in selecting NSCLC patients for treatment with the seven targeted therapies listed in Table 1, in accordance with the approved therapeutic product labeling. See Drugs@FDA Database.

Gene targets included for NSCLC				
Gene targets for therapeutic use				
BRAF: V600E		*EGFR:* L858R, exon 19 deletions, and exon 20 insertions		
ERBB2/HER2: activating mutations (SNVs and exon 20 insertions)			*ROS1:* fusions	*RET*: fusions
Analytically validated targets				
	KRAS	*MET**	*PIK3CA*	
Additional targets**				
AKT1	*ERBB3*	*KIT*	*NRAS*	*ROS1*
*ALK**	*FGFR2*	*MAP2K1*	*PDGFRA*	
CDK4	*FGFR3*	*MAP2K2*	*RAF1*	
DDR2	*HRAS*	*MTOR*	*RET*	

Figure 3. Complete gene list. * The test reports fusion/translocation variants for *ROS1* and *RET* only. The test only reports *ALK and MET* mutations.
** Performance for the additional gene target variants has been validated based on a representative method.

Table 1. Companion diagnostic biomarkers and therapies.

Gene	Variant status	Targeted therapies
BRAF	*BRAF* V600E	TAFINLAR® (dabrafenib) in combination with MEKINIST® (trametinib)
EGFR	*EGFR* L858R, exon 19 deletions	IRESSA® (gefitinib)
EGFR	*EGFR* exon 20 insertions	EXKIVITY™ (mobocertinib) RYBREVANT™ (amivantamab-vmjw)
ERBB2/HER2	*ERBB2/HER2* activating mutations (SNVs and exon 20 insertions)	ENHERTU® (fam-trastuzumab deruxtecan-nxki)
RET	*RET* fusions	GAVRETO™ (pralsetinib)
ROS1	*ROS1* fusions	XALKORI® (crizotinib)

Method comparison studies evaluated the accuracy of the Oncomine Dx Target Test for the detection of *BRAF* V600E, *EGFR* exon 19 deletions, L858R, and exon 20 insertions, *ERBB2/HER2* activating mutations (SNVs and exon 20 insertions), *ROS1* fusions, and *RET* fusions, using a *BRAF* V600E PCR assay, *therascreen*™ *EGFR* PCR kit, *ROS1* FISH assay, and validated NGS assays respectively. A summary of the concordance studies' results is included in Table 2. For details, see the User Guide.

Validation of performance for additional gene targets

The Oncomine Dx Target Test also detects DNA sequence variations in an additional 18 genes (approximately 343 targets) that are clinically associated with NSCLC. The variants for *KRAS*, *MET*, and *PIK3CA* have been analytically validated. Performance of all other variants identified by the test, other than clinically validated therapeutic variants and analytically validated variants, has not been directly demonstrated and has been validated based on a representative method.

Table 2. Concordance between the Oncomine Dx Target Test and reference methods for six companion diagnostic biomarkers.

Variants for therapy selection	Validated comparator methods	Excluding no-calls or unknowns*			Including no-calls or unknowns*		
		Positive percent agreement	Negative percent agreement	Overall percent agreement	Positive percent agreement	Negative percent agreement	Overall percent agreement
***BRAF* V600E**	Validated *BRAF* V600E qPCR test	100% (67/67)	100% (114/114)	100% (181/181)	91.8% (67/73)	97.4% (114/117)	95.3% (181/190)
EGFR	*therascreen*™ *EGFR* PCR kit	98.6% (71/72)	99.2% (120/121)	99.0% (191/193)	81.6% (71/87)	96.8% (120/124)	90.5% (191/211)
***EGFR* exon 19 deletions**		97.6% (41/42)	99.3% (147/148)	99.0% (188/190)	74.6% (41/55)	94.2% (147/156)	89.1% (188/211)
***EGFR* exon 21 L858R**		100% (30/30)	100% (167/167)	100% (197/197)	93.8% (30/32)	93.3% (167/179)	93.4% (197/211)
***EGFR* exon 20 insertions**	Validated NGS Assay 1	100% (54/54)	100% (95/95)	100% (149/149)	98.2% (54/55)	90.5% (95/105)	93.1% (149/160)
	Validated NGS Assay 2	100% (46/46)	100% (63/63)	100% (109/109)	97.9% (46/47)	91.3% (63/69)	94.0% (109/116)
***ERBB2/HER2* activating mutations (SNVs and exon 20 insertions)**	Validated NGS Assay	100% (38/38)	99.1% (108/109)	99.3% (146/147)	97.4% (38/39)	92.3% (108/117)	93.6% (146/156)
***ROS1* fusions**	Validated *ROS1* FISH test	100% (9/9)	100% (62/62)	100% (71/71)	90.0% (9/10)	88.6% (62/70)	88.8% (71/80)
***RET* fusions**	Validated NGS Assay	90.9% (40/44)	91.8% (101/110)	91.6% (141/154)	90.9% (40/44)	91.8% (101/110)	91.6% (141/154)

* No-calls are for DNA variants and unknowns are for RNA fusions.

Oncomine Dx Target Test performance for NSCLC

Accuracy study

To evaluate the ability of the Oncomine Dx Target Test DNA and RNA panels to identify somatic variants in human specimens, 290 FFPE tumor samples were analyzed using the Oncomine Dx Target Test to demonstrate positive percent agreement (PPA) and negative percent agreement (NPA) concordance with validated reference detection methods. The following reference detection methods were used:

- Validated NGS method to detect single-nucleotide variant (SNV) and deletion hotspot variants
- Validated *ROS1* FISH test, to detect *ROS1* fusions

The study demonstrated a variant level PPA of 98.5%, NPA of 100%, and OPA of 100%, excluding invalids and no-calls; and a PPA level of 98.5%, NPA of 96.8%, and OPA of 96.8% including no-calls. A summary of the data is included in Table 3. For details, see the User Manual.

Establishment of limit of detection

Six limit of detection (LoD) studies were performed to evaluate DNA variants, *ROS1* fusions, *RET* fusions, *EGFR* exon 20 insertions, *ERBB2/HER2* SNVs and exon 20 insertions.

Study I: The LoD was evaluated for 14 representative DNA variants representing 3 variant categories detected by the Oncomine Dx Target Test. The LoD is the lowest allele frequency of SNV, multi-nucleotide polymorphism (MNP), or deletion variants that can be detected at least 95% of the time. The study demonstrated that the Oncomine Dx Target Test can detect DNA variants with allele frequencies between 6 and 8%.

Study II: The LoD was calculated for 2 clinical *ROS1* RNA fusion isoforms using the updated RNA library preparation workflow, and determined at 516 fusion reads.

Study III: The LoD was calculated for 2 clinical *RET* fusion isoforms using the updated RNA library preparation workflow, and determined at 405 fusion reads.

Study IV: The LoD was calculated for 2 clinical *EGFR* exon 20 insertion positive samples, and determined to be 4.8-5.2% allele frequencies.

Study V: The LoD was calculated for 2 clinical *ERBB2/HER2* exon 20 insertion positive samples, and determined to be 4.8-5.0% allele frequencies.

Study VI: The LoD was calculated for 2 clinical *ERBB2/HER2* SNV positive samples, and determined to be 4.5-5.8% allele frequencies.

Assay reproducibility study

Six reproducibility studies were performed to evaluate DNA variants, *ROS1* fusions, *RET* fusions, *EGFR* exon 20 insertions, *ERBB2/HER2* SNVs and exon 20 insertions.

Study I: The reproducibility and repeatability of the Oncomine Dx Target Test was evaluated for 30 representative variants from 18 DNA samples. The study was designed to evaluate within-run precision performance (repeatability) and variability across sites, operators, and instruments (reproducibility). Due to the large number of variants detected by the test and the rarity of some of the variants, a representative variant approach was used. Variants were selected in the following categories:

- Simple SNVs
- Complex SNVs and MNPs, including SNVs in di- or tri-nucleotide repeat regions and SNVs in high-GC (>60%) or low-GC (<40%) content regions
- Deletions (including deletions of 6, 9, 15, and 18 bp)

Table 3. Variant level accuracy study results.

Variant level measure of agreement	Percent agreement excluding no-calls (N)	Percent agreement including no-calls (N)
Positive percent agreement	98.5% (195/198)	98.5% (195/198)
Negative percent agreement	100.0% (118, 155/118, 159)	96.8% (118, 155/122, 012)
Overall percent agreement	100.0% (118, 350/118, 357)	96.8% (118, 350/122, 210)

Excluding no-calls, the percent of correct calls is >96%. The estimate of repeatability at each DNA variant location across all the samples was ≥98.8% (95% CI lower limit of ≥97.5%). A summary of the results of Study I is included in Table 4. For details, see the User Manual.

Study II: An additional study was performed to evaluate the reproducibility and repeatability of the Oncomine Dx Target Test for 6 representative variants from 11 DNA samples and 4 RNA samples. One wild-type (WT) DNA sample and 4 WT RNA samples were included in the study.

The study was designed to evaluate within-run precision performance (repeatability) and variability across sites, operators, and instrument platforms (reproducibility). The updated RNA library preparation workflow was used. Due to the large number of variants detected by the test and the rarity of some variants, a representative variant approach was used. Variants were selected in the following categories:

- 15 bp deletion
- Simple SNVs
- Complex SNVs and MNPs
- Fusions

Excluding no-calls, the estimate of repeatability at each DNA variant location across all the samples was ≥94.4% (95% CI lower limit of ≥72.7%). The estimate of repeatability at each RNA clinical variant location was 100%. A summary of the results of Study II is included in Tables 5 and 6.

Study III: An additional study was performed to evaluate the reproducibility and repeatability of the Oncomine Dx Target Test for 4 *RET* fusion positive samples and 2 *RET* fusion–negative samples.

The study was designed to evaluate within-run precision performance (repeatability) and variability across sites, operators, and instrument platforms (reproducibility). The updated RNA library preparation workflow was used.

Excluding unknowns, estimates of the repeatability ranged from 98.1% to 100% for two *RET* variants. A summary of the results of Study III is included in Table 7.

Study IV: A study was performed to evaluate the reproducibility and repeatability of the Oncomine™ Dx Target Test for detection of *EGFR* exon 20 insertion variants using FFPE DNA from 2 *EGFR* variant-positive samples (blended with WT clinical samples and 2 *EGFR* variant-negative (WT samples.

The study was designed to evaluate within-run precision performance (repeatability) and variability across sites, operators, and instrument platforms (reproducibility).

Excluding no-calls, estimates of the repeatability is 100% for both *EGFR* exon 20 insertion variants. A summary of the results of Study IV is included in Table 8.

Study V: A study was performed to evaluate the reproducibility and repeatability of the Oncomine™ Dx Target Test for detection of *ERBB2/HER2* exon 20 insertion variants using FFPE DNA from 2 *ERBB2/HER2* variant-positive samples and 2 negative samples.

The study was designed to evaluate within-run precision performance (repeatability) and variability across sites, operators, and instrument platforms (reproducibility).

Excluding no-calls, estimates of the repeatability is 100% for both *ERBB2/HER2* exon 20 insertion variants. A summary of the results of Study V is included in Table 9.

Study VI: A study was performed to evaluate the reproducibility and repeatability of the Oncomine™ Dx Target Test for detection of *ERBB2/HER2* SNV variants using FFPE DNA from 3 *ERBB2/HER2* variant-positive samples and 4 negative samples.

The study was designed to evaluate within-run precision performance (repeatability) and variability across sites, operators, and instrument platforms (reproducibility).

Excluding no-calls, estimates of the repeatability is 100% for four *ERBB2/HER2* SNV variants. A summary of the results of Study VI is included in Table 10.

Table 4. Study I—assay reproducibility study results.

Description	No. of variants	Call rate excluding no-calls		Call rate including no-calls	
		Mean	Median	Mean	Median
DNA positive variants (positive calls)	46	96.60%	97.10%	94.50%	95.80%
WT DNA variant locations (negative calls)	872	96.10%	95.00%	96.10%	95.00%

Table 5. Study II—assay reproducibility study results (DNA variants).

Description	No. of variants	Call rate excluding no-calls		Call rate including no-calls	
		Mean	Median	Mean	Median
DNA positive variants (positive calls)	11	99%	100%	98%	99%
WT DNA variant locations (negative calls)	367	100%	100%	99%	100%

Table 6. Study II—assay reproducibility study results (*ROS1* fusions)

Description	No. of variants	Call rate including or excluding unknowns	
		Mean	Median
ROS1 positive variants (positive calls)	4	100%	100%
WT RNA variant locations (negative calls)	4	99%	100%

Table 7. Study III—assay reproducibility study results (*RET* fusions)

Description	No. of variants	Call rate including or excluding unknowns	
		Mean	Median
RET positive variants (positive calls)	4	99%	100%
WT RNA variant locations (negative calls)	2	100%	100%

Table 8. Study IV—assay reproducibility study results (*EGFR* exon 20 insertions).

Description	No. of variants	Call rate excluding no-calls		Call rate including no-calls	
		Mean	Median	Mean	Median
EGFR insertion positive variants (positive calls)	2	100%	100%	100%	100%
WT DNA variants (negative calls)	2	100%	100%	100%	100%

Table 9. Study V—assay reproducibility study results (*ERBB2*/*HER2* exon 20 insertions).

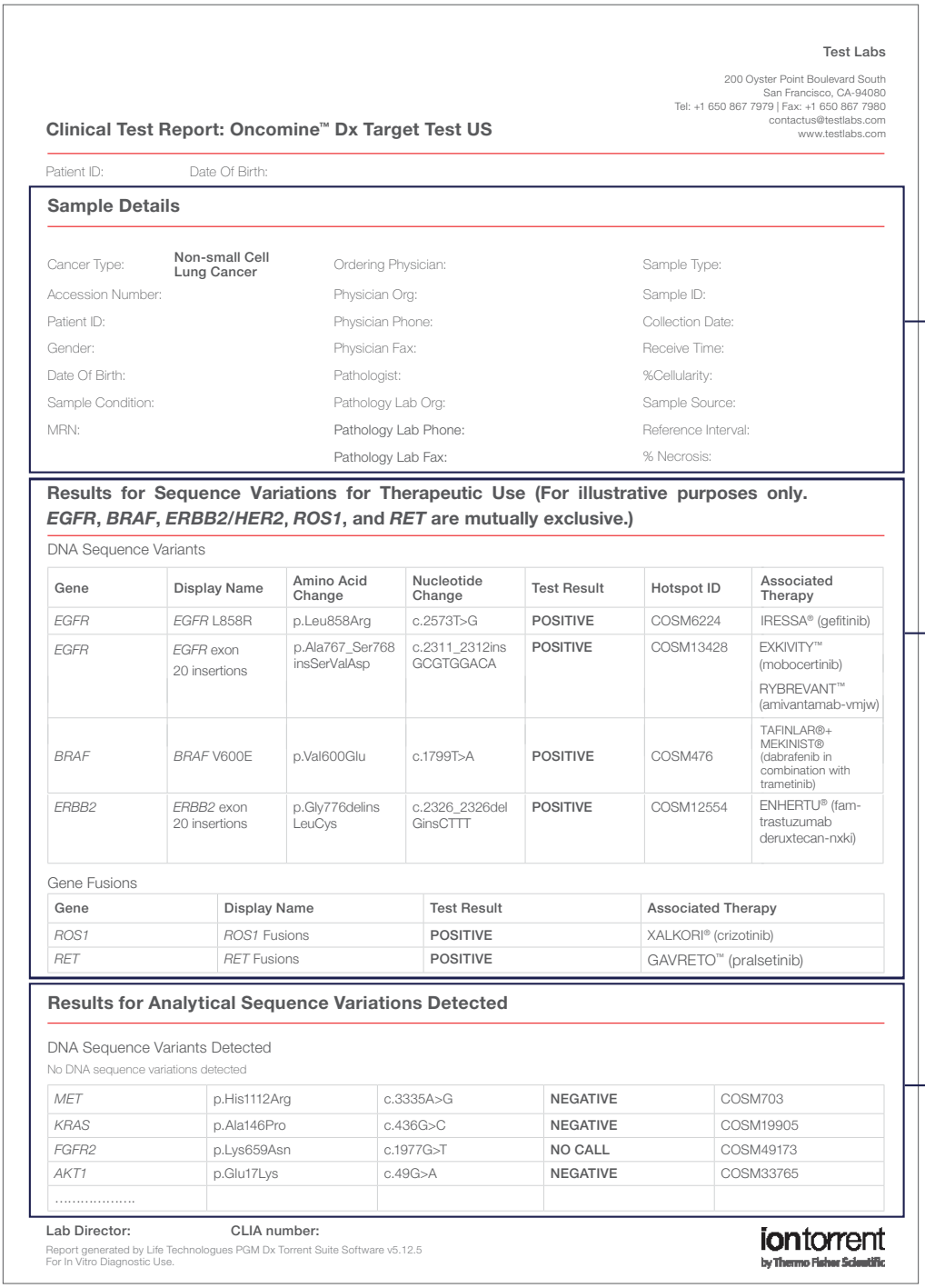
Description	No. of variants	Call rate excluding no-calls		Call rate including no-calls	
		Mean	Median	Mean	Median
ERBB2/*HER2* insertion positive variants (positive calls)	2	100%	100%	98.6%	100%
WT DNA variants (negative calls)	2	100%	100%	100%	100%

Table 10. Study VI—assay reproducibility study results (*ERBB2*/*HER2* SNVs).

Description	No. of variants	Call rate excluding no-calls		Call rate including no-calls	
		Mean	Median	Mean	Median
ERBB2/*HER2* SNV positive variants (positive calls)	4	100%	100%	100%	100%
WT DNA variants (negative calls)	4	98.3%	100%	98.3%	98.4%

Oncomine Dx Target Test report for Non-small Cell Lung Cancer

The Clinical Test Report for the Oncomine Dx Target Test is automatically generated as a PDF and incorporates relevant patient, sample, and test information required to help ensure high performance standards, and to assist with regulatory compliance and quality control. The test results are presented in two parts: companion diagnostic biomarker results with associated therapy indications, and analytically detected NSCLC-associated biomarker results in a separate section. The report is laboratory information management system (LIMS)–compatible and customizable for sample details.



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Clinical Test Report: Oncomine™ Dx Target Test US

Patient ID: Date Of Birth:

Sample Details

Cancer Type:	Non-small Cell Lung Cancer	Ordering Physician:		Sample Type:	
Accession Number:		Physician Org:		Sample ID:	
Patient ID:		Physician Phone:		Collection Date:	
Gender:		Physician Fax:		Receive Time:	
Date Of Birth:		Pathologist:		%Cellularity:	
Sample Condition:		Pathology Lab Org:		Sample Source:	
MRN:		Pathology Lab Phone:		Reference Interval:	
		Pathology Lab Fax:		% Necrosis:	

Results for Sequence Variations for Therapeutic Use (For illustrative purposes only. *EGFR*, *BRAF*, *ERBB2*/*HER2*, *ROS1*, and *RET* are mutually exclusive.)

DNA Sequence Variants

Gene	Display Name	Amino Acid Change	Nucleotide Change	Test Result	Hotspot ID	Associated Therapy
EGFR	*EGFR* L858R	p.Leu858Arg	c.2573T>G	**POSITIVE**	COSM6224	IRESSA® (gefitinib)
EGFR	*EGFR* exon 20 insertions	p.Ala767_Ser768 insSerValAsp	c.2311_2312ins GCGTGGACA	**POSITIVE**	COSM13428	EXKIVITY™ (mobocertinib) RYBREVANT™ (amivantamab-vmjw)
BRAF	*BRAF* V600E	p.Val600Glu	c.1799T>A	**POSITIVE**	COSM476	TAFINLAR®+ MEKINIST® (dabrafenib in combination with trametinib)
ERBB2	*ERBB2* exon 20 insertions	p.Gly776delins LeuCys	c.2326_2326del GinsCTTT	**POSITIVE**	COSM12554	ENHERTU® (fam-trastuzumab deruxtecan-nxki)

Gene Fusions

Gene	Display Name	Test Result	Associated Therapy
ROS1	*ROS1* Fusions	**POSITIVE**	XALKORI® (crizotinib)
RET	*RET* Fusions	**POSITIVE**	GAVRETO™ (pralsetinib)

Results for Analytical Sequence Variations Detected

DNA Sequence Variants Detected

No DNA sequence variations detected

MET	p.His1112Arg	c.3335A>G	**NEGATIVE**	COSM703
KRAS	p.Ala146Pro	c.436G>C	**NEGATIVE**	COSM19905
FGFR2	p.Lys659Asn	c.1977G>T	**NO CALL**	COSM49173
AKT1	p.Glu17Lys	c.49G>A	**NEGATIVE**	COSM33765
.....				

Lab Director: **CLIA number:**

Report generated by Life Technologues PGM Dx Torrent Suite Software v5.12.5
For In Vitro Diagnostic Use.

ion torrent by Thermo Fisher Scientific

1

Section 1. Includes the patient ID, date of birth, date of the report, and specifics such as the cancer type, sample type, and quality, source, and pathologic characteristics customizable by the lab.

2

Section 2. Includes results of the companion diagnostic markers, with associated therapy indications. For illustrative purposes only. *EGFR*, *BRAF*, *ERBB2/HER2*, *ROS1*, and *RET* are mutually exclusive.

3

Section 3. Contains results of the additional analytically detected DNA biomarkers—here, for illlustrative purposes, only a few rows are shown. The real report will, however, contain results of all remaining over 300 variants detectable by the test, and will therefore be several pages long.

Figure 4. Example of Oncomine Dx Target Test report format.

Oncomine Dx Target Test for Cholangiocarcinoma (CC)

Oncomine Dx Target Test—content

The Oncomine Dx Target Test includes *IDH1* R132 mutations as a companion diagnostics to aid in selecting cholangiocarcinoma (CC) patients for TIBSOVO® (ivosidenib), in accordance with the approved therapeutic product labeling, referenced in Table 9. See Drugs@FDA Database.

Table 9. Gene targets for CC.

	Gene Targets	Targeted Therapies
List of Genes for Therapeutic Use	*IDH1* R132C *IDH1* R132G *IDH1* R132H *IDH1* R132L *IDH1* R132S	TIBSOVO® (ivosidenib)

Establishment of limit of detection

The limit of detection (LoD) was evaluated for 5 *IDH1* R132 variants detected by the Oncomine Dx Target Test. The LoD is the lowest allele frequency of SNV that can be detected at least 95% of the time. The study demonstrated LoD of the 5 *IDH1* R132 variants ranged from 4.5-5.7% allele frequencies, including 4.5% for R132C, 5.7% for R132G, 4.9% for R132H, 5.1% for R132L, and 5.3% for R132S.

Assay reproducibility study

The reproducibility and repeatability of *IDH1* R132 variant detection using Oncomine Dx Target Test were assessed with 1 *IDH1* WT sample and 3 *IDH1* R132 variant positive samples at two allelic frequency (AF) levels. Testing was performed at 4 testing sites, each site had 2 PGM Dx instrument systems, 2 operators, and using 4 lots of reagents. The overall positive call rate for *IDH1* R132 variants was 92.6% when including no calls and 97.1% when excluding no calls. The negative call rate for *IDH1* WT sample were 100% at all *IDH1* R132 variant locations. (Table 10).

Clinical Study

To evaluate the ability of the Oncomine Dx Target Test to identify five *IDH1* biomarkers in FFPE cholangiocarcinoma tumor specimens, 168 specimens from patients tested positive and 181 specimens tested negative using Sanger assay were tested using Oncomine Dx Target Test to demonstrate positive percent agreement (PPA) and negative percent agreement (NPA) concordance with Sanger assay as validated reference detection method.

The study demonstrated PPA of 99.4%, NPA of 96.5%, and OPA of 97.9%, excluding invalids and no calls; and PPA level of 97.0%, NPA of 90.6% and OPA of 93.7% including no calls. A summary of the data is included in Table 11. For details, see the User Manual.

Table 10. Reproducibility results

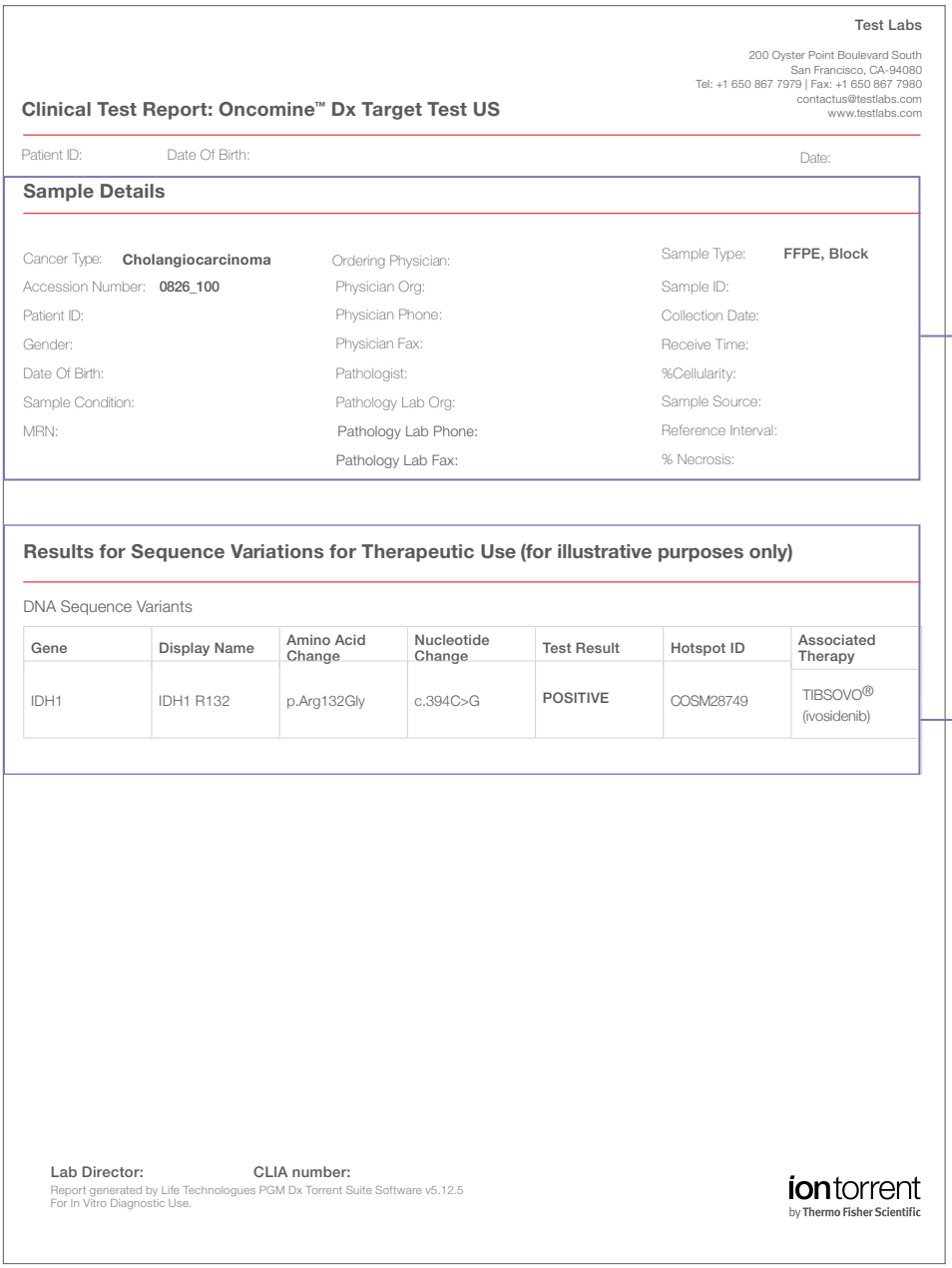
Sample COSMIC ID, Variant	No. of valid sample results	Call rate (95% CI)	
		Including no calls	Excluding no calls
D1 COSM28747, R132C	36	100% (90.3%, 100%)	100% (90.3%, 100%)
D2 COSM28747, R132C	36	97.2% (85.5%, 99.9%)	100% (90.0%, 100%)
D3 COSM28749, R132G	36	100% (90.3%, 100%)	100% (90.3%, 100%)
D4 COSM28749, R132G	36	100% (90.3%, 100%)	100% (90.3%, 100%)
D5 COSM28750, R132L	36	100% (90.3%, 100%)	100% (90.3%, 100%)
D6 COSM28750, R132L	35	57.1% (39.4%, 73.7%)	76.9% (56.4%, 91.0%)
D1-D6 All Variants, R132	215	92.6% (88.2%, 95.7%)	97.1% (93.7%, 98.9%)
D7 Wild-Type	36	100% (90.3%, 100%)	100% (90.3%, 100%)

Table 11. Concordance between the Oncomine Dx Target Test and reference method for *IDH1* R132 mutations

Variants for therapy selection	Validated comparator methods	Excluding invalid results and no-calls			Including invalid results and no-calls		
		Positive percent agreement	Negative percent agreement	Overall percent agreement	Positive percent agreement	Negative percent agreement	Overall percent agreement
***IDH1* R132**	Validated Sanger assay	99.4% (163/164)	96.5% (164/170)	97.9% (327/334)	97.0% (163/168)	90.6% (164/181)	93.7% (327/349)

Oncomine Dx Target Test Report for Cholangiocarcinoma (CC)

The Clinical Test Report for the Oncomine Dx Target Test is automatically generated as a PDF and incorporates relevant patient, sample, and test information required to help ensure high performance standards, and to assist with regulatory compliance and quality control. The test results are presented in two parts: companion diagnostic biomarker results with associated therapy indications, and analytically detected biomarker results in a separate section. The report is laboratory information management system (LIMS) system-compatible and customizable for sample details.



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Clinical Test Report: Oncomine™ Dx Target Test US

Patient ID: Date Of Birth: Date:

Sample Details

Cancer Type: **Cholangiocarcinoma**	Ordering Physician:	Sample Type: **FFPE, Block**
Accession Number: **0826_100**	Physician Org:	Sample ID:
Patient ID:	Physician Phone:	Collection Date:
Gender:	Physician Fax:	Receive Time:
Date Of Birth:	Pathologist:	%Cellularity:
Sample Condition:	Pathology Lab Org:	Sample Source:
MRN:	Pathology Lab Phone:	Reference Interval:
	Pathology Lab Fax:	% Necrosis:

Results for Sequence Variations for Therapeutic Use (for illustrative purposes only)

DNA Sequence Variants

Gene	Display Name	Amino Acid Change	Nucleotide Change	Test Result	Hotspot ID	Associated Therapy
IDH1	IDH1 R132	p.Arg132Gly	c.394C>G	POSITIVE	COSM28749	TIBSOVO® (ivosidenib)

Lab Director: **CLIA number:**

Report generated by Life Technologues PGM Dx Torrent Suite Software v5.12.5
For In Vitro Diagnostic Use.

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1

Section 1. Includes the patient ID, date of birth, date of the report, and specifics such as the cancer type, sample type and quality, source, and pathologic characteristics customizable by the lab.

2

Section 2. Includes results of the companion diagnostic markers, with associated therapy indications.

Figure 5. Example of Oncomine Dx Target Test report format.

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Ordering information

Product	Cat. No.
Oncomine Dx Target Test includes:	A51695
Ion Torrent Dx FFPE Sample Preparation Kit	A32445
Oncomine Dx Target Test, Controls, and Diluent Kit	A49756
Ion PGM Dx Library Kit	A49758
Ion PGM Dx OneTouch Template Kit	A49759
Ion PGM Dx Sequencing Kit	A49760
Ion PGM Dx 318 Chip Kit	A18937
Oncomine Dx Target Test User Guides and Assay Definition File	A51694
Ion PGM Dx Instrument System includes:	**A25511**
• Ion PGM Dx Sequencer	
• Ion OneTouch Dx Instrument	
• Ion PGM Dx System Installation and Training Kit	
• Ion PGM Dx Chip Minifuge	
• Ion PGM Wireless Scanner	
• Ion Torrent Server with Ion PGM Dx Software Pack v5.12.5 (Torrent Suite Dx Software v5.12.5 and Torrent Suite Assay Development Software v5.12.5)	

Find out more at **thermofisher.com/oncomine-dxtarget**

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Abbreviated Intended 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), insertions, and deletions in 23 genes from DNA and fusions in *ROS1* and *RET* from RNA isolated from formalin-fixed, paraffin-embedded (FFPE) tumor tissue samples from patients with non–small cell lung cancer (NSCLC) and *IDH1* R132 mutations from FFPE tumor tissue samples from patients with cholangiocarcinoma (CC) using the Ion PGM Dx System (MAN0018810).

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Oncomine Dx Target Test

Epidemiology of lung cancer

Lung cancer is the leading cause of cancer deaths in the United States [1]. In 2020, an estimated 228,820 new cases (116,300 in men and 112,520 in women) of lung and bronchial cancer will be diagnosed, and 135,720 deaths (72,500 in men and 63,220 in women) are estimated to occur because of the disease [2]. Only 19.4% of all patients with lung cancer live 5 years or more after diagnosis [3].

Genetic companion diagnostic testing for targeted therapy selection in NSCLC

Lung cancer comprises two main histologic subtypes: non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC). Over the past decade, several biomarkers associated with therapeutic benefit have emerged for NSCLC. To obtain comprehensive molecular biomarker profiling, multiplexing technology such as next-generation sequencing (NGS) is recommended by IASLC/AMP* NSCLC testing guidelines, given the limited tissue.

For the most current information concerning the essential biomarkers for lung cancer and their association with therapeutic outcomes, refer to the therapeutic labels available at “Drugs@FDA” on the FDA website.

EGFR: *EGFR* exon 19 deletions and the L858R mutation are found in approximately 10% of Caucasian patients with NSCLC and up to 50% of Asian NSCLC patients [4]. These mutations result in activation of the tyrosine kinase domain, and are associated with sensitivity to small molecule tyrosine kinase inhibitors (TKIs), such as erlotinib, gefitinib, and afatinib [5]. Data show that erlotinib, gefitinib, or afatinib (instead of standard first-line chemotherapy) should be used in patients with *EGFR* exon 19 deletions and the L858R mutation [6-11]. *EGFR* companion diagnostic tests have been approved by the FDA for specific drug indications, including the *therascreen*™ *EGFR* RGQ PCR Kit by Qiagen for gefitinib and afatinib, the cobas™ *EGFR* Mutation Test v2 by Roche for erlotinib, and the Ion Torrent™ Oncomine™ Dx Target Test by Thermo Fisher Scientific for gefitinib.

EGFR*: EGFR* exon 20 insertions are much less common and seen in approximately 2% of non-squamous NSCLC (in the Caucasian population), or 12% of all *EGFR* mutations [32]. *EGFR* exon 20 insertions are typically represented by in-frame insertion of 3 to 21 base pairs, or 1 to 7 amino acids, involving codons 761 to 775 [33]. NSCLC with *EGFR* exon 20 insertions, with the exception of the A763_Y764insFQEA variant, do not typically respond to first- and second-generation tyrosine kinase inhibitors (TKIs) or anti–PD-L1 treatments [32]. Over 60 unique variants of *EGFR* exon 20 insertions have been identified through comprehensive genomic profiling, the majority of which are rare variants [32]. Both amivantamab-vmjw and mobocertinib are approved by the FDA to treat metastatic NSCLC patients with *EGFR* exon 20 insertions who have received prior platinum-based chemotherapy. The Oncomine Dx Target Test is approved by the FDA as a companion diagnostic test for detection of *EGFR* exon 20 insertions.

BRAF: It is estimated that *BRAF* mutations occur in about 3–5% of patients with NSCLC [17]. Dabrafenib in combination with trametinib is approved by the FDA to treat NSCLC patients with a *BRAF* V600E mutation. The Oncomine Dx Target Test is approved by the FDA as a companion diagnostic test for detection of *BRAF* V600E mutation.

ERBB2/HER2: *ERBB2/HER2* mutations, largely exon 20 in-frame insertions, have been described as an oncogenic driver alterations in 2%-4% of NSCLC [34-37], associated with the initiation and progression of adenocarcinoma [38]. Fam-trastuzumab deruxtecan-nxki has been approved by the FDA to treat NSCLC patients with *ERBB2/HER2* activating mutations. The Oncomine Dx Target Test is approved by the FDA as a companion diagnostic test for detection of *ERBB2/HER2* activating mutations.

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* International Association for the Study of Lung Cancer/Association for Molecular Pathology

For *In Vitro* Diagnostic Use.

ALK: It is estimated that 2–7% of patients with NSCLC have an *ALK* gene rearrangement [12]. Crizotinib is approved by the FDA to treat people with NSCLC that has spread to other parts of the body and is caused by either an *ALK* fusion or a *ROS1* fusion. Molecular diagnostic testing using FISH and immunohistochemistry (IHC), which are the standard methods for *ALK* in NSCLC, have been approved by the FDA for detecting *ALK* fusions and *ALK* expression, respectively [13,14]. While NGS can also be used to assess the presence of an *ALK* fusion, the Oncomine Dx Target Test does not detect *ALK* fusions. Two *ALK* companion diagnostic tests have been approved by the FDA for use with crizotinib, including the Vysis™ *ALK* Break Apart FISH Probe Kit by Abbott Molecular, and the VENTANA™ *ALK* (D5F3) CDx Assay by Roche.

ROS1: It is estimated that *ROS1* fusions occur in about 1–2% of patients with NSCLC [15]. *ROS1* is very similar to *ALK* (77% amino acid sequnece homology in the ATP binding sites of the tyrosine kinase domain) and both are members of the insulin receptor family. Crizotinib is very effective for NSCLC patients with *ROS1* rearrangements [16]. The Oncomine Dx Target Test is approved by the FDA as a companion diagnostic test for detection of *ROS1* fusions.

RET: *RET* fusions occur in ~1–2% of lung carcinomas [18]. Pralsetinib is approved by the FDA to treat NSCLC patients with *RET* fusions. The Oncomine Dx Target Test is approved by FDA as a companion diagnostic test for detection of *RET* fusions.

Epidemiology of Cholangiocarcinoma

Cholangiocarcinoma, i.e., bile duct cancer, is classified according to its anatomical location in relation to the liver, being either introhepatic or extrahepatic. Extrahepatic cholangiocarcinomas, aka perihilar (or referred to as Klatskin tumor) or distal bile duct cancers, are more common than intrahepatic cholangiocarcinomas.[19] Each year, about 8,000 people in the United States are diagnosed with cholangiocarcinoma.[20] Average 5-year survival rate (2008-2014) is estimated at about 8% for intrahepatic bile duct cancers, with 24% for localized and as low as 1% for metastatic, and 10% for extrahepatic bile duct cancers, at 13% for localized and only 1% for metastatic tumors.[21]

IDH1/2: *IDH1/2* mutations are the most commonly observed genomic alteration found in intrahepatic cholangiocarcinomas (10-23% of cases). [22-28] Mutations in *IDH1* have been found in approximately 70% of Grade 2 to 3 gliomas,[29] 50% of chondrosarcomas,[30] and up to 20% of cholangiocarcinomas.[31] Ivosidenib has been approved by FDA for the treatment of adult patients with locally advanced or metastatic cholangiocarcinoma who have been previously treated with gemcitabine- or fluorouracil (5-FU)-based regimens with an *IDH1* mutation as detected by an FDA-approved test. For more information of the biomarker and therapeutic outcomes, refer to the therapeutic labels available at Drugs@FDA on the FDA website.

Test intended use/indications for use

The Oncomine Dx Target Test is a qualitative *in vitro* diagnostic (IVD) test that uses high-throughput targeted, parallel sequencing technology to detect single-nucleotide variants (SNVs), insertions, and deletions in 23 genes from DNA and fusions in *ROS1* and *RET* from RNA isolated from formalin-fixed, paraffin-embedded (FFPE) tumor samples from patients with NSCLC, and *IDH1* R132 mutations from FFPE tumor tissue samples from patients with CC using the Ion PGM™ Dx System.

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

.**Table 1. Variants for therapeutic use.**

Non-small Cell Lung Cancer (NSCLC)

Gene	Variant status	Targeted therapy
BRAF	*BRAF* V600E	TAFINLAR® (dabrafenib) in combination with MEKINIST® (trametinib)
EGFR	*EGFR* L858R, exon 19 deletions	IRESSA® (gefitinib)
EGFR	*EGFR* exon 20 insertions	EXKIVITY™ (mobocertinib)) RYBREVANT™ (amivantamab-vmjw)
ERBB2/ HER2	*ERBB2/HER2* activating mutations (SNVs and exon 20 insertions)	ENHERTU® (fam-trastuzumab deruxtecan-nxki)
RET	*RET* fusions	GAVRETO™ (pralsetinib)
ROS1	*ROS1* fusion	XALKORI® (crizotinib)

Cholangiocarcinoma (CC)

Gene	Variant status	Targeted therapy
IDH1	*IDH1* R132C *IDH1* R132G *IDH1* R132H *IDH1* R132L *IDH1* R132S	TIBSOVO® (ivosidenib)

Safe and effective use has not been established for selecting therapies using this device for the variants other than those listed in Table 1. 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. Safety and effectiveness of these three genes and four variants have not been established and they are not intended to be used to direct therapy.

Table 2. Variants with established analytical performance only.

Gene	Variant ID	Amino acid change	Nucleotide change
KRAS	COSM512	p.Gly12Phe	c.34_35delGGinsTT
KRAS	COSM516	p.Gly12Cys	c.34G>T
MET	COSM707	p.Thr1010Ile	c.3029C>T
PIK3CA	COSM754	p.Asn345Lys	c.1035T>A

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

Test performance and characteristics - NSCLC

Oncomine Dx Target Test detects over 300 variants in 23 genes, with active clinical trials and/or having demonstrated association with NSCLC in the literature. Summary of reported variants in NSCLC:

DNA: *AKT1*, *ALK*, *BRAF*, *CDK4*, *DDR2*, *EGFR*, *ERBB2*, *ERBB3*, *FGFR2*, *FGFR3*, *HRAS*, *KIT*, *KRAS*, *MAP2K1*, *MAP2K2*, *MET*, *MTOR*, *NRAS*, *PDGFRA*, *PIK3CA*, *RAF1*, *RET,* and *ROS1*

RNA: *RET* and *ROS1*

Analytical validation of the Oncomine Dx Target Test was established through a series of studies to assess the accuracy, sensitivity, specificity, and reproducibility of the assay for the detection of SNVs, deletions, insertions, and fusions [39].

Based on the data observed, the test demonstrated a limit of detection (LoD) of between 6–8% allele frequencies for DNA variants, 4.8–5.2% allele frequencies for *EGFR* insertions, 4.8-5.0% allele frequencies for *ERBB2/HER2* exon 20 insertions, 4.5-5.8% allele frequencies for *ERBB2/ HER2* SNVs, 516 fusion reads for *ROS1* fusions, and 405 fusion reads for *RET* fusions, with 95% confidence.

Additionally, based on the representative variants that were tested in the accuracy study, the test detected variants with 98.5% positive percent agreement (PPA) and 100% negative percent agreement (NPA) against validated reference methods (excluding no-calls).

Six studies were conducted to evaluate the repeatability and reproducibility of the test for DNA variants, *EGFR* exon 20 insertions, *ERBB2/HER2* SNVs and exon 20 insertions, *ROS1* fusions, and *RET* fusions. Repeatability is >94% for DNA variants, 100% for *EGFR* exon 20 insertions, 100% for *ERBB2/HER2* SNVs and exon 20 insertions, 100% for *ROS1* fusions, and >98% for *RET* fusions. Reproducibility is >99% for DNA variants, *EGFR* exon 20 insertions, *ERBB2/ HER2* SNVs and exon 20 insertions, *ROS1* fusions, and *RET* fusions (excluding no calls and unknowns).

Method comparison studies evaluated the concordance of the test for the detection of *BRAF* V600E, *EGFR* exon 19 deletions, L858R, and exon 20 insertions, *ERBB2/HER2* activating mutations (SNVs and exon 20 insertions), *ROS1* fusions, and *RET* fusions, using a *BRAF* V600E PCR assay, *therascreen*™ *EGFR* PCR kit, , *ROS1* FISH assay, and validated NGS assays, respectively. The studies show:

- 100% overall percent agreement (OPA), positive percent agreement (PPA) and negative percent agreement (NPA) for *BRAF*, *EGFR* exon 20 insertions, and *ROS1* fusions
- 99% OPA, PPA, and NPA for *EGFR* exon 19 deletions and L858R
- 99% OPA, 100% PPA, and 99% NPA for *ERBB2/HER2* activating mutations (SNVs and exon 20 insertions)
- 92% OPA, 91% PPA, and 92% NPA for *RET* fusions

Test performance and characteristics - CC

Oncomine Dx Target Test only reports *IDH1* R132 in CC. Analytical validation of the Oncomine Dx Target Test in CC was established through LoD and precision studies [39].

Based on the data observed, the test confirmed a limit of detection (LoD) of 4.5-5.7% allele frequencies for 5 *IDH1* R132 mutations, including 4.5% for R132C, 5.7% for R132G, 4.9% for R132H, 5.1% for R132L, and 5.3% for R132S. Repeatability and reproducibility study demonstrated overall positive call rate of 92.6% for the *IDH1* R132 variants when including no calls and 97.1% when excluding no calls. The negative call rates for the *IDH1* WT sample were 100%.

A clinical concordance study was conducted to evaluate the ability of the Oncomine Dx Target Test to identify five *IDH1* biomarkers in FFPE cholangiocarcinoma tumor specimen compared to a validated Sanger assay. The study shows 99.4% positive percent agreement (PPA), 96.5% negative percent agreement (NPA), and 97.9% overall percent agreement (OPA) excluding invalid results and no-calls.

Guide to interpreting results

Test results should be interpreted in the context of pathological evaluation of tumors, treatment history, clinical findings, and other laboratory data.

All clinical interpretations of the variants detected should be made by a board-certified pathologist or equivalent. It is recommended that the physician ordering the test consult with a board-certified pathologist. Patients are advised to seek information from their oncologist or certified health care provider.

Additional information may be obtained from NCCN Guidelines™ and IASLC/AMP NSCLC testing guidelines.

The molecular profile of a tumor can vary between primary and metastatic sites, as well as change over time in response to treatment, leading to the development of mutations that could confer resistance to therapeutic agents.

Test limitations and warnings

The test is designed to interrogate over 300 variants in 23 genes associated with NSCLC and one gene in CC. However, when certain quality metrics and controls established for the specimen testing are not met, accuracy of the test cannot be assured and therefore mutation status in the exons is reported. Variants detected by the panel that are not clinically or analytically validated should not be used for selecting treatment.

- This test does not detect genomic copy number variants.
- The Oncomine Dx Target Test does not detect *ALK* fusions.
- This test does not detect structural variants in genes other than *ROS1 and RET*.
- Rare polymorphisms exist that could lead to false-negative or false-positive results.
- A negative (wild-type) result does not rule out the presence of a mutation that is below the limits of detection of this assay (6–8%).
- The product is designed to detect a targeted set of known variants in the genes. New variants that are not included in the test may be discovered in the future.
- For NSCLC, the Oncomine Dx Target Test assay definition file includes prevalent but not all rare or newly identified *RET* isoforms, *ROS1* isoforms, *EGFR* exon 20 insertions, and *ERBB2/HER2* activating mutations (SNVs and exon 20 insertions). The Oncomine Dx Target Test may miss rare or newly identified:
    - *RET* isoforms carried by a subset of patients who may derive benefit from GAVRETO™ (pralsetinib)
    - *ROS1* isoforms carried by a subset of patients who may derive benefit from XALKORI® (crizotinib)
    - *EGFR* exon 20 insertions carried by a subset of patients who may derive benefit from EXKIVITY™ (mobocertinib) or RYBREVANT™ (amivantamab-vmjw)
    - *ERBB2/HER2* activating mutations (SNVs and exon 20 insertions) carried by a subset of patients who may derive benefit from ENHERTU® (fam-trastuzumab deruxtecan-nxki)

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39. Oncomine Dx Target Test User Guide

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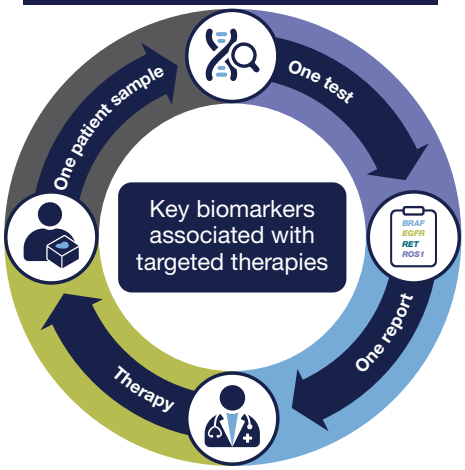
Tell your oncologist about Oncomine Dx Target Test

A new paradigm in testing for targeted therapies in NSCLC and CC

This test is now reimbursed by Medicare and the top 40 commercial payers, covering over 200 million US lives.

The Ion Torrent™ Oncomine™ Dx Target Test is the first targeted next-generation sequencing (NGS) *in vitro* diagnostic test for non–small cell lung cancer (NSCLC) and cholangiocarcinoma (CC), simultaneously delivering multiple biomarker results for multiple targeted therapies from one sample within 4 days.

- **Identify patients for multiple therapies**—one test indicated as a companion diagnostic (CDx) device to aid in selecting NSCLC and CC patients for treatment with targeted therapies.
- **Multiple biomarkers from one limited sample**—one test for detection of 23 genes, minimizing the risk of depleting tissues and requiring additional biopsies. Based on Ion AmpliSeq™ technology, the required input is as low as 10 ng of DNA and RNA.
- **One workflow, helps save time**—laboratory results can be generated within four days.
- **Established performance**—concordance with FDA approved or validated reference methods based on FISH, PCR, Sanger, or NGS was established for all CDx biomarkers (excluding no-calls or unknowns):
  - 100% overall percent agreement (OPA), positive percent agreement (PPA) and negative percent agreement (NPA) for *BRAF*, *EGFR* exon 20 insertions, and *ROS1* fusions
  - 99% OPA, PPA, and NPA for *EGFR* exon 19 deletions and L858R 99%
  - OPA, 100% PPA, and 99% NPA for *ERBB2/HER2* activating mutations (SNVs and exon 20 insertions)
  - 92% OPA, 91% PPA, and 92% NPA for *RET* fusions
  - 98% OPA, 99% PPA, and 97% NPA for *IDH1*



Cancer type	Gene	Targeted therapies
NSCLC	*BRAF*	TAFINLAR® (dabrafenib) in combination with MEKINIST® (trametinib)
	EGFR L858R, exon 19 deletions	IRESSA® (gefitinib)
	EGFR exon 20 insertions	EXKIVITY™ (mobocertinib) RYBREVANT™ (amivantamab-vmjw)
	ERBB2/HER2 activating mutations (SNVs and exon 20 insertions)	ENHERTU® (fam-trastuzumab deruxtecan-nxki)
	RET	GAVRETO™ (pralsetinib)
	ROS1	XALKORI® (crizotinib)
CC	*IDH1*	TIBSOVO® (ivosidenib)

Figure 1. List of genes for therapeutic use.

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A complete and flexible system

The Oncomine Dx Target Test is used in conjunction with the Ion PGM™ Dx System, which includes a complete NGS system of instruments, reagents, and software, now validated with the Oncomine Dx Target Test for somatic mutation reporting for FFPE samples (see Figure 2 for workflow). The Ion PGM Dx sequencing system is a Class II Medical Device and incorporates combined functionality, with both "IVD Mode" for molecular diagnostic tests and "Assay Development Mode" for clinical research. The system also facilitates 21 CFR Part 11 compliance, with role-based workflows, sample and reagent tracking, QC metrics, and audit trails.

Oncomine Dx Target Test—gene content

The Oncomine Dx Target Test includes targets for cancer-associated genes. Seven biomarkers are companion diagnostics to aid in selecting patients for approved targeted therapies, including six in NSCLC and one in CC, while remaining genes are currently being investigated in clinical trials and may be potentially actionable in the future as referenced in Figure 3.

Oncomine Dx Target Test—report

The Oncomine Dx Target Test report is automatically generated as a PDF and incorporates relevant patient, sample, and test information required to help ensure high performance standards, regulatory compliance, and quality control. The test results are presented in two parts: companion diagnostic biomarker results with associated therapy indication (Figure 4), and other analytically detected biomarker results in a separate section (not shown). The report is laboratory information management system (LIMS) compatible.

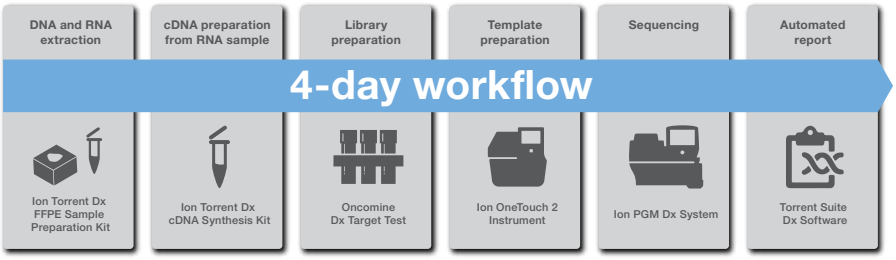

Figure 2. The Oncomine Dx Target Test utilizes a single streamlined NGS workflow for detecting cancer-associated biomarkers, incorporating reagents, instrument systems, and bioinformatics. The turnaround time from FFPE sample to report is 4 days.

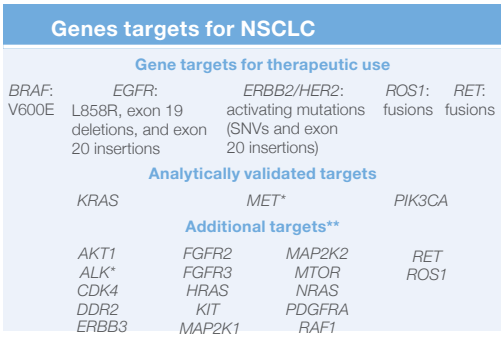

Figure 3. Complete gene list. * The test reports fusion/translocation variants for *ROS1* and *RET* only. The test only reports *ALK* and *MET* mutations. ** Performance for the additional gene target variants has been validated based on a representative method. Only *IDH1* is reported for CC.

NSCLC results for sequence variations for therapeutic use (for illustrative purposes only; *EGFR*, *BRAF*, *ERBB2/HER2*, *ROS1*, and *RET* are mutually exclusive)

DNA sequence variants

Gene	Display name	Amino acid change	Nucleotide change	Test result	Hotspot ID	Associated therapy
EGFR	*EGFR* L858R	p.Leu858Arg	c.2573T>G	POSITIVE	COSM6224	IRESSA® (gefitinib)
EGFR	*EGFR* exon 20 insertions	p.Ala767_Ser768 insSerValAsp	c.2311_2312ins GCGTGGACA	POSITIVE	COSM13428	EXKIVITY™(mobocertinib) RYBREVANT™(amivantamab-vmjw)
BRAF	*BRAF* V600E	p.Val600Glu	c.1799T>A	POSITIVE	COSM476	TAFINLAR® + MEKINIST® (dabrafenib in combination with trametinib)
ERBB2	*ERBB2* exon 20 insertions	p.Gly776delins LeuCys	c.2326_2326del GinsCTTT	POSITIVE	COSM12554	ENHERTU® (fam-trastuzumab deruxtecan-nxki)

Gene fusions (RNA)

Gene	Display name	Test result	Associated therapy
ROS1	*ROS1* fusions	POSITIVE	XALKORI® (crizotinib)
RET	*RET* fusions	POSITIVE	GAVRETO™ (pralsetinib)

CC results for sequence variations for therapeutic use (for illustrative purposes only)

DNA sequence variants

Gene	Display name	Amino acid change	Nucleotide change	Test result	Hotspot ID	Associated therapy
IDH1	*IDH1* R132	p.Arg132Gly	c.394C>G	POSITIVE	COSM28749	TIBSOVO® (ivosidenib)

Figure 4. Example of Oncomine Dx Target Test report format. The report includes a section with results of the validated biomarkers and information about relevant treatment indication, as well as a separate section with the other biomarkers not validated for treatment selection (not shown).

Find out more at **thermofisher.com/oncomine-dxtarget**

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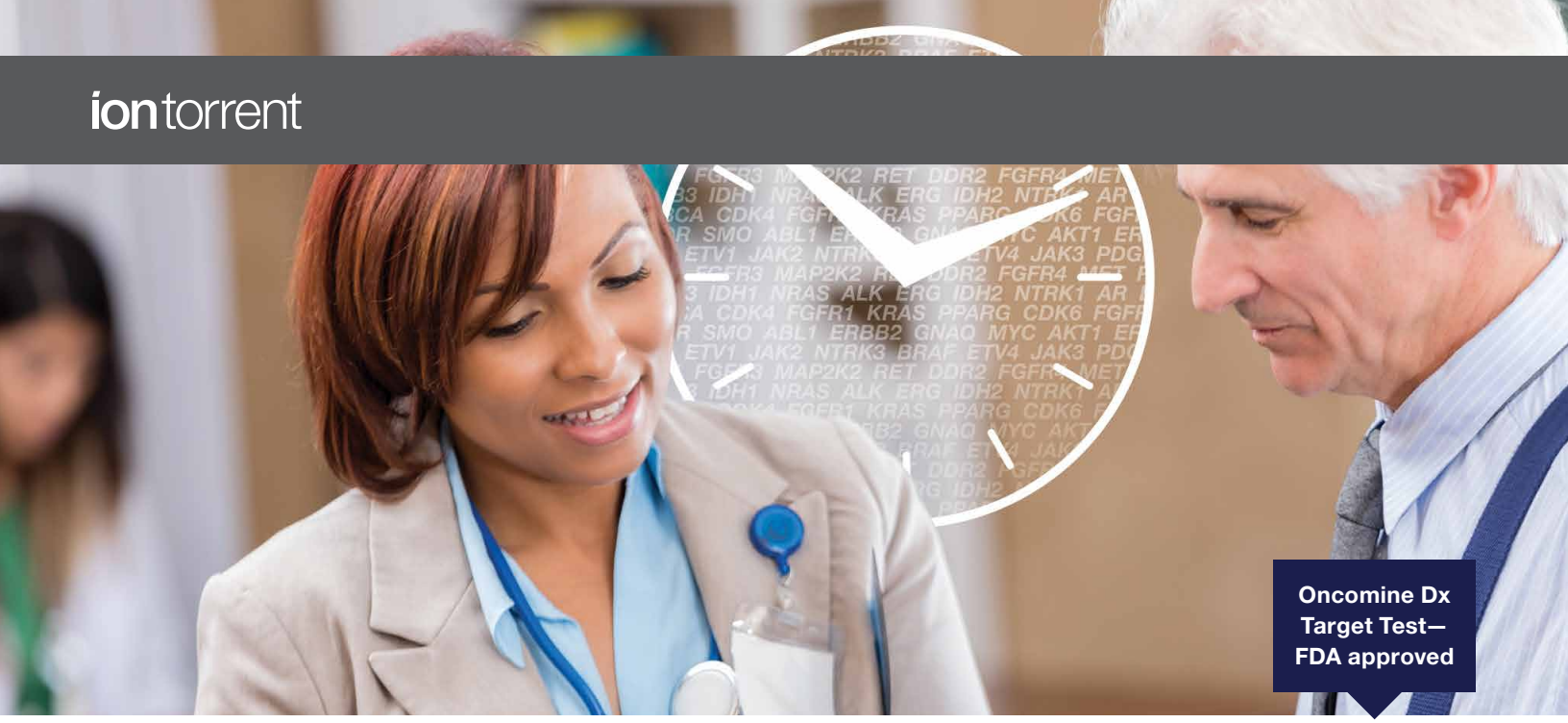


Ask your pathologist about Oncomine Dx Target Test

A new paradigm in testing for targeted therapies in NSCLC and CC

The Ion Torrent™ Oncomine™ Dx Target Test is the first targeted next-generation sequencing (NGS) *in vitro* diagnostic test for non–small cell lung cancer (NSCLC) and cholangiocarcinoma (CC), simultaneously delivering multiple biomarker results for multiple targeted therapies from one sample within 4 days.

Did you know:

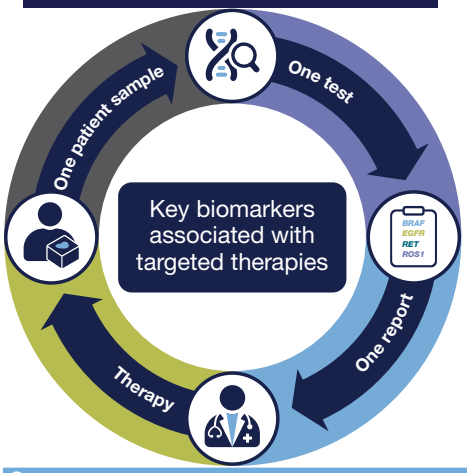
- Many biopsy samples are so small that they cannot be analyzed by some NGS tests, especially panels containing hundreds of genes, leading to tissue exhaustion
- It can take several weeks to get results with alternative NGS tests, potentially delaying treatment decision

Choosing the right NGS test can make a difference for your patient

The Oncomine Dx Target Test is an FDA-approved NGS CDx test that can:

- Identify patients for multiple therapies—one test indicated as a companion diagnostic (CDx) device to aid in selecting NSCLC and CC patients for treatment with targeted therapies
- Accept small samples (10 ng DNA and RNA), for more patients to potentially access targeted therapies
- Generate results in a laboratory within four days, enabling faster treatment decisions

This test is now reimbursed by Medicare and the top 40 commercial payers, covering over 200 million US lives.



Cancer type	Gene	Targeted therapies
NSCLC	*BRAF*	TAFINLAR® (dabrafenib) in combination with MEKINIST® (trametinib)
	EGFR L858R, exon 19 deletions	IRESSA® (gefitinib)
	EGFR exon 20 insertions	EXKIVITY™ (mobocertinib) RYBREVANT™ (amivantamab-vmjw)
	ERBB2/HER2 activating mutations (SNVs and exon 20 insertions)	ENHERTU® (fam-trastuzumab deruxtecan-nxki)
	RET	GAVRETO™ (pralsetinib)
	ROS1	XALKORI® (crizotinib)
CC	*IDH1*	TIBSOVO® (ivosidenib)

Figure 1. List of genes for therapeutic use.

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For *In Vitro* Diagnostic Use.

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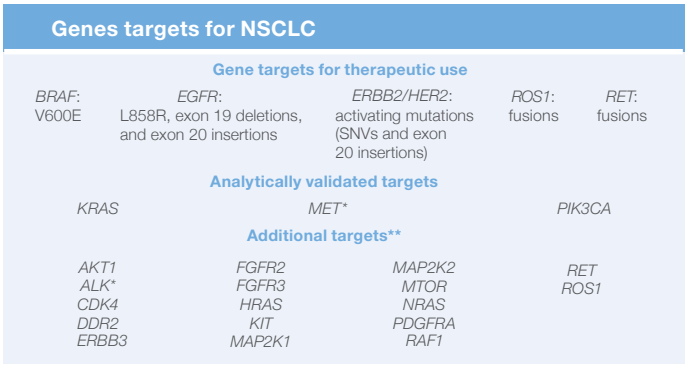

Figure 2. Complete gene list. * The test reports fusion/translocation variants for *ROS1* and *RET* only. The test only reports *ALK* and *MET* mutations. ** Performance for the additional gene target variants has been validated based on a representative method. Only *IDH1* is reported for CC.

Oncomine Dx Target Test—performance

Concordance with FDA approved or validated reference methods based on FISH, PCR, Sanger, or NGS was established for all CDx biomarkers (excluding no-calls or unknowns):

- 100% overall percent agreement (OPA), positive percent agreement (PPA) and negative percent agreement (NPA) for *BRAF*, *EGFR* exon 20 insertions, and *ROS1* fusions
- 99% OPA, PPA, and NPA for *EGFR* exon 19 deletions and L858R
- 99% OPA, 100% PPA, and 99% NPA for *ERBB2/HER2* activating mutations (SNVs and exon 20 insertions)
- 92% OPA, 91% PPA, and 92% NPA for *RET* fusions
- 98% OPA, 99% PPA, and 97% NPA for *IDH1*

Oncomine Dx Target Test—report

NSCLC results for sequence variations for therapeutic use (for illustrative purposes only; *EGFR*, *BRAF*, *ERBB2/HER2*, *ROS1*, and *RET* are mutually exclusive)

DNA sequence variants

Gene	Display name	Amino acid change	Nucleotide change	Test result	Hotspot ID	Associated therapy
EGFR	*EGFR* L858R	p.Leu858Arg	c.2573T>G	POSITIVE	COSM6224	IRESSA® (gefitinib)
EGFR	*EGFR* exon 20 insertions	p.Ala767_Ser768 insSerValAsp	c.2311_2312ins GCGTGGACA	POSITIVE	COSM13428	EXKIVITY™(mobocertinib) RYBREVANT™(amivantamab-vmjw)
BRAF	*BRAF* V600E	p.Val600Glu	c.1799T>A	POSITIVE	COSM476	TAFINLAR® + MEKINIST® (dabrafenib in combination with trametinib)
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Gene fusions (RNA)

Gene	Display name	Test result	Associated therapy
ROS1	*ROS1* fusions	POSITIVE	XALKORI® (crizotinib)
RET	*RET* fusions	POSITIVE	GAVRETO™ (pralsetinib)

CC results for sequence variations for therapeutic use (for illustrative purposes only)

DNA sequence variants

Gene	Display name	Amino acid change	Nucleotide change	Test result	Hotspot ID	Associated therapy
IDH1	*IDH1* R132	p.Arg132Gly	c.394C>G	POSITIVE	COSM28749	TIBSOVO® (ivosidenib)

Figure 3. Example of Oncomine Dx Target Test report format. The report includes a section with results of the validated biomarkers and information about relevant treatment indication, as well as a separate section with the other biomarkers not validated for treatment selection (not shown).

If your pathology laboratory does not perform the Oncomine Dx Target Test, you can send samples to one of these reference laboratories.

Reference lab	Telephone number	Website
NeoGenomics Laboratories, Inc.	866-776-5907	**neogenomics.com**
OncoCyte	615-639-0710	**oncocyte.com**

Find out more at **thermofisher.com/oncomine-dxtarget**

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Oncomine IVD solutions—it's about time

Get faster answers for more NSCLC and CC patients

When your patients' samples are analyzed with the NGS-based Ion Torrent™ Oncomine™ Dx Target Test in your own hospital laboratory, you can get answers within aweek, even from small biopsies, allowing you to make faster therapeutic decisions for more patients. No need to rely on external laboratories. Now you can bring precision oncology closer to your patients and fully own their care journey.

Find out more at **thermofisher.com/oncomine-dxtarget**

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Oncomine IVD solutions—it's about time

Test faster to enable faster treatment

When your patients' samples are analyzed with the NGS-based Ion Torrent™ Oncomine™ Dx Target Test in your own hospital laboratory, you can get answers within a week, even from small biopsies, allowing you to make faster therapeutic decisions for more patients. Ask your pathologist about Ion Torrent™ Oncomine™ IVD solutions.

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Oncomine IVD solutions— faster answers for more patients

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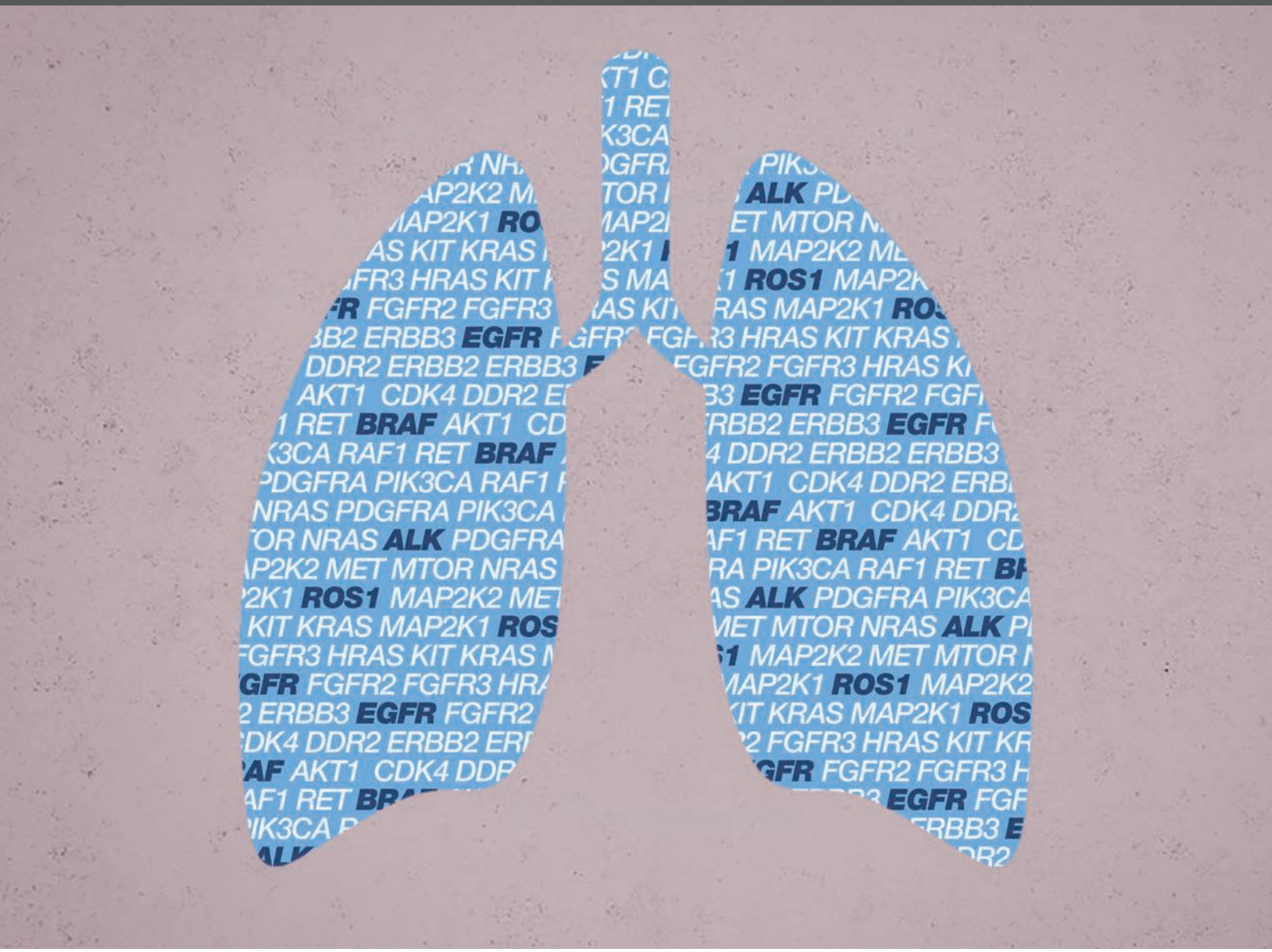

Ask for Oncomine IVD solutions

Get faster answers for more NSCLC patients

When your patients' samples are analyzed with the NGS-based Ion Torrent™ Oncomine™ Dx Target Test in your own hospital laboratory, you can get answers within a week, even from small biopsies, allowing you to make faster therapeutic decisions for more patients. No need to rely on external laboratories. Now you can bring precision oncology closer to your patients and fully own their care journey.

Find out more at **thermofisher.com/oncomine-dxtarget**

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Oncomine Dx Target Test—US

A new paradigm in testing for targeted therapies in NSCLC and CC

In the era of personalized medicine, molecular profiling has become essential for the treatment of cancer patients. With an increasing number of genomic alterations becoming clinically relevant, sequential testing of individual mutations becomes a significant challenge for clinical laboratories. Next-generation sequencing (NGS), which can detect multiple alterations at once from a small amount of tissue, offers a solution.

Request more info

Download flyer for clinicians ›
Download flyer for laboratory professionals ›
Download brochure for laboratory professionals ›

The Oncomine Dx Target Test is currently available in the following commercial labs:

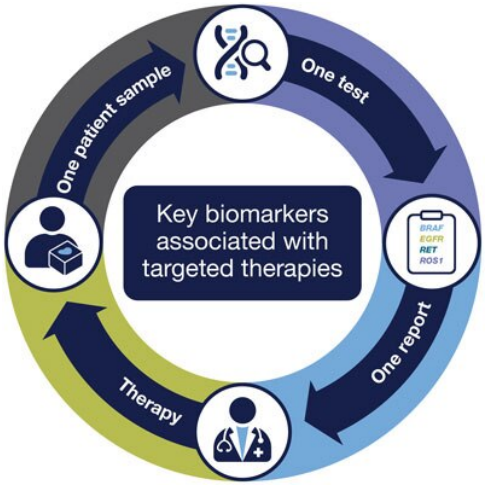
Reference lab	Telephone number	Website
NeoGenomics Laboratories, Inc.	866-776-5907	**neogenomics.com**
OncoCyte	615-639-0710	**oncocyte.com**

One test that can expedite treatment selection decisions

The Ion Torrent Oncomine Dx Target Test is the first targeted NGS-based *in vitro* diagnostic test for non-small cell lung cancer (NSCLC) and cholangiocarcinoma (CC) , simultaneously delivering multiple biomarker results for multiple targeted therapies from one sample within four days.

Cancer type	Gene	Targeted therapies
NSCLC	*BRAF*	TAFINLAR® (dabrafenib) in combination with MEKINIST® (trametinib)
	EGFR L858R and exon 19 deletions	IRESSA® (gefitinib)
	EGFR exon 20 insertions	EXKIVITY™ (mobocertinib) RYBREVANT™ (amivantamab-vmjw)
	ERBB2/HER2 activating mutations (SNVs and exon 20 insertions)	ENHERTU® (fam-trastuzumab deruxtecan-nxki)
	RET	GAVRETO™ (pralsetinib)
	ROS1	XALKORI® (crizotinib)
CC	*IDH1*	TIBSOVO® (ivosidenib)

Figure 1. List of genes for therapeutic use.



- **Identify patients for multiple therapies**—one test indicated as a companion diagnostic (CDx) device to aid in selecting NSCLC and CC patients for treatment with targeted therapies
- **Multiple biomarkers from one limited sample**—one test for detection of 23 genes, minimizing the risk of depleting tissues and requiring additional biopsies. Based on Ion AmpliSeq™ technology, the required input is as low as 10 ng DNA and RNA.
- **One workflow, helps save time**—laboratory results can be generated within 4 days
- **Established performance**—Concordance with FDA approved or validated reference methods based on FISH, PCR, Sanger, or NGS was established for all CDx biomarkers (excluding no-calls or unknowns):
  - 100% overall percent agreement (OPA), positive percent agreement (PPA) and negative percent agreement (NPA) for *BRAF*, *EGFR* exon 20 insertions, and *ROS1* fusions
  - 99% OPA, PPA, and NPA for *EGFR* exon 19 deletions and L858R
  - 99% OPA, 100% PPA, and 99% NPA for *ERBB2/HER2* activating mutations (SNVs and exon 20 insertions)
  - 92% OPA, 91% PPA, and 92% NPA for *RET* fusions
  - 98% OPA, 99% PPA, and 97% NPA for *IDH1*

Technical and validation data for the Oncomine Dx Target Test ›

Oncomine Dx Target Test content

Gene targets included for NSCLC				
Gene targets for therapeutic use				
BRAF: V600E	*EGFR:* L858R, exon 19 deletions, and exon 20 insertions	*ERBB2/HER2:* activating mutations (SNVs and exon 20 insertions)	*ROS1:* fusions	*RET:* fusions
Analytically validated targets				
KRAS	*MET**	*PK3CA*		
Additional targets**				
AK1	*ERBB3*	*KIT*	*NRAS*	*ROS1*
*ALK**	*FGFR2*	*MAP2K1*	*PDGFRA*	
CDK4	*FGFR3*	*MAP2K2*	*RAF1*	
DDR2	*HRAS*	*MTOR*	*RET*	

Figure 2. Complete gene list. *The test reports fusion/translocation variants for *ROS1* and *RET* only. The test only reports *ALK* and *MET* mutations. **Performance for the additional gene target variants has been validated based on a representative method. Only *IDH1* is reported for CC.

The power of next generation sequencing

Next-generation sequencing (NGS) can sequence hundreds to thousands of genes and detect multiple biomarkers at the same time. The sequencing takes place in a chip that contains millions of wells (flow cells) with separate sequencing reactions taking place in each well, allowing many genes to be sequenced at once and multiple variations to be detected simultaneously, unlike traditional companion diagnostic technologies such as FISH, IHC, or PCR, which only analyze one target gene at the time.

Learn how Ion Torrent NGS technology works in short video

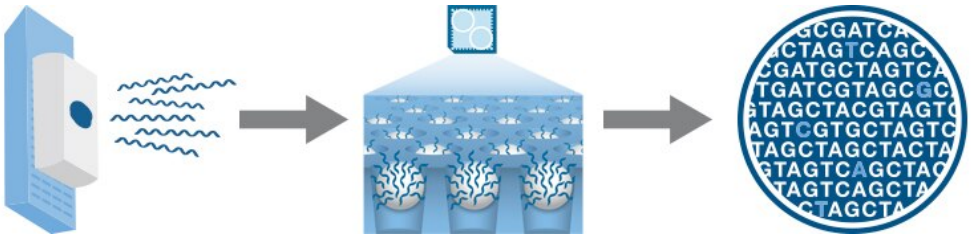


Figure 3. The NGS process starts with extraction of the DNA and/or RNA, which is processed in the chip in the Ion PGM Dx instrument, and results are analyzed and reported by a dedicated bioinformatics solution.

Oncomine Dx Target Test report

The Oncomine Dx Target Test Clinical Test Report is automatically generated as a PDF and incorporates relevant patient, sample, and test information required to help ensure high-performance standards, regulatory compliance, and quality control. The test results are presented in two-parts: companion diagnostic marker results with associated therapy indications and cancer driver analytical-only biomarker results in a separate section. The report is customizable and LIMS system-compatible.

Download an example of an Oncomine Dx Target Test report ›

NSCLC results for sequence variations for therapeutic use (for illustrative purposes only: *EGFR*, *BRAF*, *ERBB2*/*HER2*, *ROS1*, and *RET* are mutually exclusive)

DNA Sequence Variants

Gene	Display name	Amino acid change	Nucleotide change	Test result	Hotspot ID	Associated therapy
EGFR	*EGFR* L858R	p.Leu858Arg	c.2573T>G	POSITIVE	COSM6224	IRESSA® (gefitinib)
EGFR	*EGFR* exon 20 insertions	p.Ala767_Ser768insSerValAsp	c.2311_2312ins GCGTGGACA	POSITIVE	COSM13428	EXKIVITY™ (mobocertinib) RYBREVANT™ (amivantamab-vmjw)
BRAF	*BRAF* V600E	p.Val600Glu	c.1799T>A	POSITIVE	COSM476	TAFINLAR® + MEKINIST® (dabrafenib in combination with trametinib)
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Gene Fusions (RNA)

Gene	Display name	Test result	Associated therapy
ROS1	*ROS1* Fusion	POSITIVE	XALKORI® (crizotinib)
RET	*RET* Fusions	POSITIVE	GAVRETO™ (pralsetinib)

CC results for sequence variations for therapeutic use (for illustrative purposes only)

DNA Sequence Variants

Gene	Display name	Amino acid change	Nucleotide change	Test result	Hotspot ID	Associated therapy
IDH1	*IDH1* R132	p.Arg132Gly	c.394C>G	POSITIVE	COSM28749	TIBSOVO® (ivosidenib)

Figure 4. An example of the Oncomine Dx Target Test report format. The report includes a section with results of the validated biomarkers and information about relevant treatment indication, as well as a section with the other biomarkers not validated for treatment selection (not shown).

Request more information

Request a quote

Abbreviated Intended 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), insertions, and deletions in 23 genes from DNA and fusions in *ROS1* and *RET* from RNA isolated from formalin-fixed, paraffin-embedded (FFPE) tumor samples from patients with non-small cell lung cancer (NSCLC) and *IDH1* R132 mutations from FFPE tumor tissue samples from patients with cholangiocarcinoma (CC) using the Ion PGM Dx System.

Test limitations and warnings

- Use of this product must be limited to personnel trained in the techniques of PCR, NGS, and the use of the Oncomine Dx Target Test and the Ion PGM Dx System.
- The Oncomine Dx Target Test has only been validated for use with NSCLC and CC FFPE tumor slide specimens.
- The Oncomine Dx Target Test has been validated to detect the following somatic mutations: RNA fusions, single-nucleotide variations (SNVs), multi-nucleotide variations (MNVs), and deletions of 3, 6, 9, 12, 15, and 18 base pairs (bps), and insertions of 3, 6, 9, and 12 base pairs (bps) from DNA..
- The Oncomine Dx Target Test is only validated for use with the Ion PGM Dx System and the Veriti Dx 96-Well Thermal Cycler.
- The Oncomine Dx Target Test is only validated for use with 10 ng each of DNA and RNA per sample. Input amounts lower or higher than 10 ng are not recommended.
- Both the DNA and RNA from a single sample extraction must meet the concentration requirements specified in the procedure. Do not use DNA from one extraction with RNA from a different extraction.
- The effects of potential variations in FFPE specimen fixation have not been evaluated.
- Extraction from FFPE sample curls has not been evaluated.
- A potential source of contamination in the procedure is nucleic acid from previous sample processing steps. Follow good laboratory practices and all precautions and guidelines in these user guides to avoid cross-contamination between samples.
- The Oncomine Dx Target Test is a qualitative test. The test is not for quantitative measurements of percent mutation.
- The Ion OneTouch Rack Kit has only been designed to work with GeneMate SnapStrip 8-Strip 0.2 mL PCR Tubes. Tubes from other manufacturers may not fit properly in the rack, resulting in a higher risk of user error.
- For NSCLC, the Oncomine Dx Target Test assay definition file includes prevalent but not all rare or newly identified *RET* isoforms, *ROS1* isoforms, *EGFR* exon 20 insertions and *ERBB2*/*HER2* activating mutations (SNVs and exon 20 inseertions). The Oncomine Dx Target Test may miss rare or newly identified:
    - *RET* isoforms carried by a subset of patients who may derive benefit from GAVRETO™ (pralsetinib)
    - *ROS1* isoforms carried by a subset of patients who may derive benefit from XALKORI® (crizotinib)
    - *EGFR* exon 20 insertions carried by a subset of patients who may derive benefit from EXKIVITY™ (mobocertinib) or RYBREVANT™ (amivantamab-vmjw)
    - *ERBB2*/*HER2* activating mutations (SNVs and exon 20 insertions) carried by a subset of patients who may derive benefit from ENHERTU® (fam-trastuzumab deruxtecan-nxki)

Oncomine Dx Target Test Technical and Validation Information

Established performance

The Ion Torrent Oncomine Dx Target Test is the first targeted next-generation sequencing (NGS) *in vitro* diagnostic test simultaneously delivering multiple biomarker results to aid selection of targeted therapies for NSCLC and CC patients. Concordance with FDA approved or validated reference methods based on FISH, PCR, Sanger, or NGS was established for *EGFR*, *BRAF*, *ERBB2*/*HER2*, *ROS1,* and *RET* in NSCLC, and for *IDH1* in CC. The variants for *KRAS*, *MET*, and *PIK3CA* were analytically validated in NSCLC. The safety and effectiveness of these three genes have not been established and they are not intended to be used to direct therapy. The performance of all other variants identified by the test, other than clinically validated therapeutic variants and analytically validated variants, was validated based on a representative method.

Request more info

Oncomine Dx Target Test content

Gene targets included for NSCLC

Gene targets for therapeutic use				
BRAF: V600E	*EGFR:* L858R, exon 19 deletions, and exon 20 insertions	*ERBB2*/*HER2:* activating mutations (SNVs and exon 20 insertions)	*ROS1:* fusions	*RET:* fusions
Analytically validated targets				
KRAS	*MET**	*PK3CA*		
Additional targets**				
AK1	*ERBB3*	*KIT*	*NRAS*	*ROS1*
*ALK**	*FGFR2*	*MAP2K1*	*PDGFRA*	
CDK4	*FGFR3*	*MAP2K2*	*RAF1*	
DDR2	*HRAS*	*MTOR*	*RET*	

Figure 1. Complete gene list. *The test reports fusion/translocation variants for *ROS1* and *RET* only. The test only reports *ALK* and *MET* mutations. ** Performance for the additional gene target variants has been validated based on a representative method. Only *IDH1* is reported for CC

Clinical concordance for companion diagnostics markers for targeted therapies selection - NSCLC

Method comparison studies evaluated the accuracy of the Oncomine Dx Target Test for the detection of *BRAF* V600E, *EGFR* exon 19 deletions, L858R, and exon 20 insertions, *ERBB2*/*HER2* activating mutations (SNVs and exon 20 insertions), *ROS1* fusions, and *RET* fusions, using a *BRAF* V600E PCR assay, therascreen™ *EGFR* PCR Kit, *ROS1* FISH assay, and NGS assays respectively. A summary of the concordance studies results are included in Table 1. For details see the User Manual.

Variants for therapy selection	Validated comparator methods	Excluding no calls or unknowns*			Including no calls or unknowns*		
		Positive percent agreement	Negative percent agreement	Overall percent agreement	Positive percent agreement	Negative percent agreement	Overall percent agreement
BRAF V600E	Validated *BRAF* V600E qPCR test	100% (67/67)	100% (114/114)	100% (181/181)	91.8% (67/73)	97.4% (114/117)	95.3% (181/190)
EGFR	Therascreen™ *EGFR* PCR Kit	98.6% (71/72)	99.2% (120/121)	99.0% (191/193)	81.6% (71/87)	96.8% (120/124)	90.5% (191/211)
EGFR exon 19 deletions		97.6% (41/42)	99.3% (147/148)	99.0% (188/190)	74.6% (41/55)	94.2% (147/156)	89.1% (188/211)
EGFR exon 21 L858R		100% (30/30)	100% (167/167)	100% (197/197)	93.8% (30/32)	93.3% (167/179)	93.4% (197/211)
EGFR exon 20 insertions	Validated NGS Assay	100% (54/54)	100% (95/95)	100% (149/149)	98.2% (54/55)	90.5% (95/105)	93.1% (149/160)
	Validated NGS Assay 2	100% (46/46)	100% (63/63)	100% (109/109)	97.9% (46/47)	91.3% (63/69)	94.0% (109/116)
ERBB2/HER2 activating mutations (SNVs and exon 20 insertions)	Validated NGS Assay	100% (38/38)	99.1% (108/109)	99.3% (146/147)	97.4% (38/39)	92.3% (108/117)	93.6% (146/156)
ROS1 fusions	Validated *ROS1* FISH test	100% (9/9)	100% (62/62)	100% (71/71)	90.0% (9/10)	88.6% (62/70)	88.8% (71/80)
RET fusions	Validated NGS Assay	90.9% (40/44)	91.8% (101/110)	91.6% (141/154)	90.9% (40/44)	91.8% (101/110)	91.6% (141/154)

* No-calls are for DNA variants and unknowns are for RNA fusions

Table 1. Method comparison between Oncomine Dx Target Test and reference methods for five companion diagnostic biomarkers in NSCLC.

Analytical validation performance - NSCLC

The Oncomine Dx Target Test also detects DNA sequence variations in an additional 19 genes in NSCLC. The variants for *KRAS*, *MET*, and *PIK3CA* have been analytically validated. Safety and effectiveness of these three genes have not been established and they are not intended to be used to direct therapy. The performance of all other variants identified by the test, other than clinically validated therapeutic variants and analytically validated variants, has not been directly demonstrated and was validated based on a representative method.

Limit of detection

Six LoD studies were performed to evaluate DNA variants, *ROS1* fusions, *RET* fusions, *EGFR* exon 20 insertions, and *ERBB2*/*HER2* SNVs and exon 20 insertions.

Study I: The limit of detection (LoD) was evaluated for 14 representative DNA variants representing 3 variant categories detected by the Oncomine Dx Target Test. The LoD is the lowest allele frequency of SNV, multi-nucleotide polymorphism (MNP), or deletion variants, that can be detected at least 95% of the time. The study demonstrated that the Oncomine Dx Target Test can detect DNA variants with allele frequencies between 6 and 8%.

Study II: The LoD was calculated for 2 clinical *ROS1* RNA fusion variants using the updated RNA library preparation workflow, and determined at 516 fusion reads.

Study III: The LoD was calculated for 2 clinical *RET* fusion variants using the updated RNA library preparation workflow, and determined at 405 fusion reads.

Study IV: The LoD was calculated for 2 clinical *EGFR* exon 20 insertion positive samples, and determined to be 4.8–5.2% allele frequencies.

Study V: The LoD was calculated for 2 clinical *ERBB2/HER2* exon 20 insertion positive samples, and determined to be 4.8-5.0% allele frequencies.

Study VI: The LoD was calculated for 2 clinical *ERBB2/HER2* SNV positive samples, and determined to be 4.5-5.8% allele frequencies.

Accuracy

To evaluate the ability of the Oncomine Dx Target Test DNA and RNA panels to identify somatic variants in human specimens, 290 FFPE tumor samples were analyzed using the Oncomine Dx Target Test to demonstrate positive percent agreement (PPA) and negative percent agreement (NPA) concordance with validated reference detection methods.

The following reference detection methods were used:

- Validated NGS method, to detect SNV and deletion hotspot variants
- Validated *ROS1* FISH test, to detect *ROS1* fusions

The study demonstrated variant level PPA of 98.5%, NPA of 100%, and OPA of 100%, excluding invalids no-calls; and PPA level of 98.5%, NPA of 96.8%, and OPA of 96.8% including no-calls. A summary of the data are included in Table 2. For details see the User Manual.

Variant level measure of agreement	**Percent agreement (N) excluding no-calls**	**Percent agreement (N) including no-calls**
Positive percent agreement	98.5% (195/198)	98.5% (195/198)
Negative percent agreement	100.0% (118,155/118,159)	96.8% (118,155/122,012)
Overall percent agreement	100.0% (118,350/118,357)	96.8% (118,350/122,210)

Table 2. Variant level accuracy study results

Reproducibility

Six reproducibility studies were performed to evaluate DNA variants, *ROS1* fusions, *RET* fusions, *EGFR* exon 20 insertions, and *ERBB2*/*HER2* SNVs and exon 20 insertions.

Study I:

The reproducibility and repeatability of the Oncomine Dx Target Test was evaluated for 30 representative variants from 18 DNA samples. The study was designed to evaluate within-run precision performance (repeatability) and variability across sites, operators, and instruments (reproducibility). Due to the large number of variants detected by the test and the rarity of some of the variants, a representative variant approach was used. Variants were selected in the following categories:

- Simple SNVs
- Complex SNVs and MNPs, including SNVs in di- or tri-nucleotide repeat regions and SNVs in high-GC (>60%) or low-GC (<40%) content regions
- Deletions (including deletions of 6, 9, 15, and 18 bp)

Excluding no calls, the percent of correct calls is >96%. The estimate of repeatability at each DNA variant location across all the samples was ≥98.8% (95% CI lower limit of ≥97.5%). A summary of results of the assay reproducibility study are included in Table 3. For details see the User Manual.

Description	**No. of variants**	**Call rate excluding no-calls**		**Call rate including no-calls**	
		Mean	**Median**	**Mean**	**Median**
DNA positive variants (positive calls)	46	96.60%	97.10%	94.50%	95.80%
WT DNA variant locations (negative calls)	872	96.10%	95.00%	96.10%	95.00%

Table 3. Assay reproducibility study I.

Study II:

An additional study was performed to evaluate the reproducibility and repeatability of the Oncomine Dx Target Test for 6 representative variants from 11 DNA samples and 4 RNA samples. 1 WT DNA sample and 4 WT RNA samples were included in the study.

The study was designed to evaluate within-run precision performance (repeatability) and variability across sites, operators, and instrument platforms (reproducibility). The updated RNA library preparation workflow was used. Due to the large number of variants detected by the test and the rarity of some variants, a representative variant approach was used.

Variants were selected in the following categories:

- 15 bp deletion
- Simple SNVs
- Complex SNVs and MNPs
- Fusions

Excluding no calls, the estimate of repeatability at each DNA variant location across all the samples was ≥94.4% (95% CI lower limit of ≥72.7%). The estimate of repeatability at each RNA clinical variant location was 100%. A summary of the results of Study II is included in Table 4 and 5.

Description	No. of variants	Call rate excluding no-calls		Call rate including no-calls	
		Mean	Median	Mean	Median
DNA positive variants (positive calls)	11	99%	100%	98%	99%
WT DNA variant locations (negative calls)	367	100%	100%	99%	100%

Table 4. Study II—assay reproducibility study results (DNA variants)

Description	No. of variants	Call rate including or excluding unknowns	
		Mean	Median
ROS1 positive variants (positive calls)	4	100%	100%
WT RNA variant locations (negative calls)	4	99%	100%

Table 5. Study II—assay reproducibility study results (*ROS1* fusions)

Study III:

An additional study was performed to evaluate the reproducibility and repeatability of the Oncomine Dx Target Test for 4 *RET* fusion positive samples and 2 *RET* fusion-negative samples. The study was designed to evaluate within-run precision performance (repeatability) and variability across sites, operators, and instrument platforms (reproducibility). The updated RNA library preparation workflow was used. Excluding unknowns, estimates of the repeatability ranged from 98.1% to 100% for two *RET* variants. A summary of the results of Study III is included in Table 6.

Description	No. of variants	Call rate including or excluding unknowns	
		Mean	Median
RET positive variants (positive calls)	4	99%	100%
WT RNA variant locations (negative calls)	2	100%	100%

Table 6. Study III—assay reproducibility study results (*RET* fusions)

Study IV:

A study was performed to evaluate the reproducibility and repeatability of the Oncomine™ Dx Target Test for detection of *EGFR* exon 20 insertion variants using FFPE DNA from 2 *EGFR* variant-positive samples (blended with WT clinical samples) and 2 *EGFR* variant-negative (WT) samples. The study was designed to evaluate within-run precision performance (repeatability) and variability across sites, operators, and instrument platforms (reproducibility). Excluding no-calls, estimates of the repeatability is 100% for both *EGFR* exon 20 insertion variants. A summary of the results of Study IV is included in Table 7.

Description	No. of variants	Call rate excluding no-calls		Call rate including no-calls	
		Mean	Median	Mean	Median
EGFR insertion positive variants (positive calls)	2	100%	100%	100%	100%
WT DNA variants (negative calls)	2	100%	100%	100%	100%

Table 7. Study IV—assay reproducibility study results (*EGFR* exon 20 insertions)

Study V:

A study was performed to evaluate the reproducibility and repeatability of the Oncomine™ Dx Target Test for detection of *ERBB2/HER2* exon 20 insertion variants using FFPE DNA from 2 *ERBB2/HER2* variant-positive samples. The study was designed to evaluate within-run precision performance (repeatability) and variability across sites, operators, and instrument platforms (reproducibility). Excluding no-calls, estimates of the repeatability is 100% for both *ERBB2/HER2* exon 20 insertion variants. A summary of the results of Study V is included in Table 8.

Description	No. of variants	Call rate excluding no-calls		Call rate including no-calls	
		Mean	Median	Mean	Median
ERBB2/HER2 insertion positive variants (positive calls)	2	100%	100%	98.6%	100%
WT DNA variants (negative calls)	2	100%	100%	100%	100%

Table 8. Study V—assay reproducibility study results (*ERBB2/HER2* exon 20 insertions)

Study VI:

A study was performed to evaluate the reproducibility and repeatability of the Oncomine™ Dx Target Test for detection of *ERBB2/HER2* SNV variants using FFPE DNA from 3 *ERBB2/HER2* variant-positive samples and 4 negative samples. The study was designed to evaluate within-run precision performance (repeatability) and variability across sites, operators, and instrument platforms (reproducibility). Excluding no-calls, estimates of the repeatability is 100% for four *ERBB2/HER2* SNV variants. A summary of the results of Study VI is included in Table 9

Description	No. of variants	Call rate excluding no-calls		Call rate including no-calls	
		Mean	Median	Mean	Median
ERBB2/HER2 SNV positive variants (positive calls)	4	100%	100%	100%	100%
WT DNA variants (negative calls)	4	98.3%	100%	98.3%	98.4%

Table 9 . Study VI—assay reproducibility study results (*ERBB2/HER2* SNVs)

Clinical concordance for companion diagnostics marker for targeted therapies selection - CC

A clinical concordance study was conducted to evaluate the ability of the Oncomine Dx Target Test to identify five *IDH1* biomarkers in FFPE cholangiocarcinoma tumor specimen compared to a validated Sanger assay The study demonstrated OPA of 97.9%, excluding invalids and no calls. A summary of the data is included in Table 8.

Table 8. Concordance between the Oncomine Dx Target Test and reference method for *IDH1* R132

Variants for therapy selection	Validated comparator methods	Excluding invalid results and no-calls			Including invalid results and no-calls		
		Positive percent agreement	Negative percent agreement	Overall percent agreement	Positive percent agreement	Negative percent agreement	Overall percent agreement
***IDH1* R132**	Validated Sanger assay	99.4% (163/164)	96.5% (164/170)	97.9% (327/334)	97.0% (163/168)	90.6% (164/181)	93.7% (327/349)

Analytical validation performance - CC

Limit of detection

The limit of detection (LoD) was evaluated for 5 *IDH1* R132 variants detected by the Oncomine Dx Target Test. The LoD is the lowest allele frequency of SNV that can be detected at least 95% of the time. The study demonstrated LoD of the 5 *IDH1* R132 variants ranged from 4.5-5.7% allele frequencies, including 4.5% for R132C, 5.7% for R132G, 4.9% for R132H, 5.1% for R132L, and 5.3% for R132S.

Assay reproducibility

The reproducibility and repeatability of *IDH1* R132 variant detection using Oncomine Dx Target Test were assessed with 1 *IDH1* WT sample and 3 *IDH1* R132 variant positive samples at two allele frequency (AF) levels. Testing was performed at 4 testing sites, each site had 2 PGM Dx instrument systems, 2 operators, and using 4 lots of reagents. The overall positive call rate for *IDH1* R132 variants was 92.6% when including no calls and 97.1% when excluding no calls. The negative call rate for IDH1 WT sample were 100% at all *IDH1* R132 variant locations. (Table 9).

Table 9. Reproducibility results

Sample COSMIC ID, Variant	No. of valid sample results	Call rate (95% CI)	
		Including no calls	Excluding no calls
D1 COSM28747, R132C	36	100% (90.3%, 100%)	100% (90.3%, 100%)
D2 COSM28747, R132C	36	97.2% (85.5%, 99.9%)	100% (90.0%, 100%)
D3 COSM28749, R132G	36	100% (90.3%, 100%)	100% (90.3%, 100%)
D4 COSM28749, R132G	36	100% (90.3%, 100%)	100% (90.3%, 100%)
D5 COSM28750, R132L	36	100% (90.3%, 100%)	100% (90.3%, 100%)
D6 COSM28750, R132L	35	57.1% (39.4%, 73.7%)	76.9% (56.4%, 91.0%)
D1-D6 All Variants, R132	215	92.6% (88.2%, 95.7%)	97.1% (93.7%, 98.9%)
D7 Wild-Type	36	100% (90.3%, 100%)	100% (90.3%, 100%)

A complete and flexible system

The Oncomine Dx Target Test is used in conjunction with the Ion PGM Dx System, which includes a complete NGS system of instruments, reagents, and software. The Ion PGM Dx System was initially validated using challenging germline variants and is now additionally validated with the Oncomine Dx Target Test for somatic mutation reporting for FFPE tissue samples. The Ion PGM Dx sequencing system is a Class II 510 K Medical Device and incorporates combined functionality, with both "IVD Mode" for molecular diagnostic tests and "Assay Development Mode" for clinical research. The system also facilitates 21CFR Part 11 compliance, role-based workflows, sample and reagent tracking, QC metrics, and audit trails.

The Oncomine Dx Target Test workflow—all results in 4 days

The Oncomine Dx Target Test workflow is a fully validated IVD workflow from beginning to end and includes all the reagents, consumables, instruments, and software to perform the test. It is possible to run 1–6 samples per run, plus 2 controls within 4 days (Figure 2).

Figure 2. The Oncomine Dx Target Test utilizes a single streamlined NGS workflow for detecting cancer-associated biomarkers, incorporating reagents, instrument systems, and bioinformatics. The turnaround time, from FFPE sample to report, is 4 days.

Request more information

Abbreviated Intended 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), insertions, and deletions in 23 genes from DNA and fusions in *ROS1* and *RET* from RNA isolated from formalin-fixed, paraffin-embedded (FFPE) tumor samples from patients with non-small cell lung cancer (NSCLC) and *IDH1* R132 mutations from FFPE tumor tissue samples from patients with cholangiocarcinoma (CC) using the Ion PGM Dx System.

Test limitations and warnings

- Use of this product must be limited to personnel trained in the techniques of PCR, NGS, and the use of the Oncomine Dx Target Test and the Ion PGM Dx System. The Oncomine Dx Target Test has only been validated for use with NSCLC and CC FFPE tumor slide specimens.
- The Oncomine Dx Target Test has been validated to detect the following somatic mutations: RNA fusions, single-nucleotide variations (SNVs), multi-nucleotide variations (MNVs), deletions of 3, 6, 9, 12, 15, and 18 base pairs (bps), and insertions of 3, 6, 9, and 12 base pairs (bps) from DNA.
- The Oncomine Dx Target Test is only validated for use with the Ion PGM Dx System and the Veriti Dx 96-Well Thermal Cycler.
- The Oncomine Dx Target Test is only validated for use with 10 ng each of DNA and RNA per sample. Input amounts lower or higher than 10 ng are not recommended.
- Both the DNA and RNA from a single sample extraction must meet the concentration requirements specified in the procedure. Do not use DNA from one extraction with RNA from a different extraction.
- The effects of potential variations in FFPE specimen fixation have not been evaluated.
- Extraction from FFPE sample curls has not been evaluated.
- A potential source of contamination in the procedure is nucleic acid from previous sample processing steps. Follow good laboratory practices and all precautions and guidelines in these user guides to avoid cross-contamination between samples.
- The Oncomine Dx Target Test is a qualitative test. The test is not for quantitative measurements of percent mutation.
- The Ion OneTouch Rack Kit has only been designed to work with GeneMate SnapStrip 8-Strip 0.2 mL PCR Tubes. Tubes from other manufacturers may not fit properly in the rack, resulting in a higher risk of user error.
- For NSCLC, the Oncomine Dx Target Test assay definition file includes prevalent but not all rare or newly identified *RET* isoforms, *ROS1* isoforms, *EGFR* exon 20 insertions, and *ERBB2*/*HER2* activating mutations (SNVs and exon 20 insertions). The Oncomine Dx Target Test may miss rare or newly identified:
  - *RET* isoforms carried by a subset of patients who may derive benefit from GAVRETO™ (pralsetinib)
  - *ROS1* isoforms carried by a subset of patients who may derive benefit from XALKORI® (crizotinib)
  - *EGFR* exon 20 insertions carried by a subset of patients who may derive benefit from EXKIVITY™ (mobocertinib) or RYBREVANT™ (amivantamab-vmjw)
  - *ERBB2*/*HER2* activating mutations (SNVs and exon 20 insertions) carried by a subset of patients who may derive benefit from ENHERTU® (fam-trastuzumab deruxtecan-nxki)

For In Vitro Diagnostic Use.