PILLAR BIOSCIENCES

Physician Insert:

ONCO/Reveal™ Dx Lung and Colon Cancer Assay

FOR IN VITRO DIAGNOSTIC USE

Caution: Federal law restricts this device to sale by or on the order of a physician

EPIDEMIOLOGY OF NON-SMALL CELL LUNG CANCER AND COLORECTAL CANCER

COLORECTAL CANCER (CRC)

Colorectal cancer (CRC) is the third leading cause of cancer death after lung and prostate (men) or breast (women) cancers. In 2020, an estimated 104,610 new cases (52,340 in men and 52,270 in women) of colon cancer and 43,340 new cases (25,960 in men and 17,380 in women) of rectal cancer will be diagnosed in the United States, and a total of 53,200 people will die from these cancers (1). Studies by the American Cancer Society suggest that 55% of CRC in the United States can be attributed to modifiable risk factors such as smoking, body weight, exercise, consumption of red or processed meat, low calcium intake, heavy alcohol consumption, and very low intake of fruits, vegetables, and fiber. The prognosis for patients recently diagnosed with CRC is relatively good with 64% of patients (across all stages) surviving for 5 years past diagnosis. The mortality rate has decreased by 54% between 1970 and 2017 largely because of changing risk factors, increased screening, and improvements in treatment regimens (1).

NON-SMALL CELL LUNG CANCER (NSCLC)

Lung cancer is the leading cause of cancer death in the United States (1) and is the 7th leading cause of death in non-smokers (2) with such cases accounting for 10-15% of all cases (3). 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 (1). About 60% of patients present with late stage disease (4). The outlook for lung cancer patients is poor— during the period 2009-2015, only 19% of all patients diagnosed with lung cancer live 5 years or more after diagnosis. The number is slightly higher for NSCLC (24%) and shows great gender bias. However, after decades of gradual increase in age-adjusted lung cancer death rates, a plateau occurred during the years 1990-2000, and the rates of lung cancer have fallen steadily to roughly 32 (females) and 44 (males) deaths per 100,000. Much of the decline is attributed to a decrease in the use of cigarettes (5).

THE ROLE OF TESTING IN PATIENT TREATMENT

The development of HER2 testing as a companion to trastuzumab treatment is considered to be the first example of a co-developed companion diagnostic (CDx) test. Since the approval of that test, there are

nearly 20 FDA-approved CDx devices and dozens of lab-developed tests for oncology, speaking to the success of this approach for cancer treatment (6). For the most current list of cleared or approved CDx devices, go to: https://www.fda.gov/medicaldevices/productsandmedicalprocedures/invitrodiagnostics/ucm301431.htm.

GENETIC COMPANION DIAGNOSTIC TESTS FOR TARGETED CRC THERAPY SELECTION

Monoclonal antibodies to epidermal growth factor receptor (EGFR) are targeted therapies for CRC that require knowledge of the mutational status of genes in the EGFR signaling pathway as predictive biomarkers of response to these therapies (7,8,9). Clinical trial data demonstrated that CRC patients with activating mutations of *KRAS* affecting codons 12 and 13 did not benefit from anti-EGFR monoclonal antibody therapy (7,8,9). Subsequent studies expanded the biomarker list to include *BRAF*, *NRAS*, *PIK3CA*, and *PTEN*, but no guidance currently exist regarding interpretation for the additional biomarkers (10). For the most current information on the association between biomarkers and therapeutic outcomes, refer to the drug labelling information available at Drugs@FDA on the FDA website.

The Kirsten ras (*KRAS*) oncogene homolog gene is altered in approximately 44% of CRC patients with activating mutations to codons 12 and 13 representing roughly half of the alterations (11).

The most current list of CDx tests approved by FDA for detection of *KRAS* wild-type to select CRC patients who may benefit from treatment with targeted therapies is given at https://www.fda.gov/medicaldevices/productsandmedicalprocedures/invitrodiagnostics/ucm301431.htm.

GENETIC COMPANION DIAGNOSTIC TESTS FOR TARGETED NSLC THERAPY SELECTION

Lung cancers are divided into two main histologic subtypes: non-small cell lung cancer (NSCLC, 84% of cases) and small cell lung cancer (SCLC, 13% of cases) (1). Recent research has identified a number of genetic alterations associated with the effectiveness of various therapeutics (12). A multiplexed technology, such as next-generation sequencing, is recommended by IASLC/AMP NSCLC testing guidelines when specimen tissue is limited. For the most current information on the association between biomarkers and therapeutic outcomes, refer to the drug labelling information available at Drugs@FDA on the FDA website.

Epidermal growth factor receptor (*EGFR*) gene is altered in 23% of NSCLC patients. Specific mutations in the *EGFR* gene result in the activation of the tyrosine kinase domain and are associated with sensitivity to a class of small molecule compounds collectively known as tyrosine kinase inhibitors (TKIs). These include erlotinib, osimertinib, gefitinib, dacomitinib, and afatinib.

EGFR exon 19 deletions are found in approximately 9% and L858R mutations in approximately 6% of US patients (11). Multiple trails have shown that erlotinib, osimertinib, gefitinib, dacomitinib, or afatinib TKIs should be used in patients with *EGFR* exon 19 deletions and L858R mutation instead of standard first-line chemotherapy.

The most current list of CDx tests approved by FDA for detection of *EGFR* alterations to identify NSCLC patients who may benefit from treatment with targeted therapies is given at

https://www.fda.gov/medicaldevices/productsandmedicalprocedures/invitrodiagnostics/ucm301431.htm.

OVERVIEW OF THIS TEST

INDICATIONS FOR USE

The ONCO/Reveal™ Dx Lung and Colon Cancer Assay (O/RDx-LCCA) is a qualitative next generation sequencing based in vitro diagnostic test that uses amplicon-based target enrichment technology for detection of single nucleotide variants (SNVs) and deletions in 2 genes from DNA isolated from formalin-fixed paraffin-embedded (FFPE) non-small cell lung cancer (NSCLC) and colorectal cancer (CRC) tumor tissue specimens. The test is intended as a companion diagnostic to identify patients with NSCLC or CRC who may benefit from treatment with the targeted therapies listed in Table 1 in accordance with the approved therapeutic product labeling. The O/RDx-LCCA is intended to be used on the Illumina MiSeqDx® instrument.

Table 1 List of Somatic Variants for Therapeutic Use

Indication	Gene	Variant	Targeted therapy
Colorectal Cancer (CRC)	*KRAS*	*KRAS* wild-type (absence of mutations in codons 12 and 13)	ERBITUX® (cetuximab), or VECTIBIX® (panitumumab)
Non-Small Cell Lung Cancer (NSCLC)	*EGFR*	Exon 19 In Frame Deletions and Exon 21 L858R Substitution Mutations	EGFR Tyrosine Kinase Inhibitors approved by FDA*

*For the most current information about the therapeutic products in this group, go to: https://www.fda.gov/medicaldevices/productsandmedicalprocedures/invitrodiagnostics/ucm301431.htm

CONTRAINDICATIONS

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

TEST PERFORMANCE CHARACTERISTICS

Clinical performance of the O/RDx-LCCA was established by demonstrating non-inferiority to FDA-approved companion diagnostic tests that established clinical validity directly via clinical trial. Two clinical concordance studies were conducted to support the CDx claims indicated in Table 1 of the Indications for Use statement for *EGFR* Exon 19del/L858R in NSCLC and *KRAS* wild-type (absence of mutation in codons 12 and 13) in CRC. The performance of this test was shown to be non-inferior with a non-inferiority margin of 4% for the detection of claimed *EGFR* mutations and 5% for the detection of claimed *KRAS* mutations.

The analytical performance characteristics of the O/RDx-LCCA were established using DNA derived from FFPE NSCLC and CRC tumor tissue specimens. Studies included reportable CDx SNV and deletion variant representatives of *EGFR* and *KRAS* genes indicated in Table 1 of the intended use statement. The

contrived samples (cell line, reference cell line standards in genomic DNA, FFPE and formalin-compromised DNA formats) were used only in cross-contamination study and reagent kit stability studies.

Analytical performance for accuracy was established using an externally validated NGS reference method. The observed positive and negative percent agreements were both 100% for CDx mutations when results were separated by gene at the variant level. The positive percent agreement was 100% for both *EGFR* and *KRAS* CDx variants, and the negative percent agreement was 96.8% and 98% for *EGFR* and *KRAS* CDx variants, respectively, when results were binned by gene at the specimen level.

A Limit of Blank (LoB) of zero was determined across 70 independent sample libraries prepared from four FFPE specimens each of normal (non-tumor) colon and normal (non-tumor) lung tissue. No false positives were reported for the CDx variants.

The limit of detection (LoD) for each positive variant detected by the O/RDx-LCCA was estimated using the hit rate approach where LoD is defined as the lowest VAF with 100% hit rate. A total of 4 clinical NSCLC and CRC specimens were evaluated, which included CDx SNVs and a deletion/insertion (DelIns) variant which is a complex mutation with a deletion followed by an insertion. The originally estimated LoD for each variant was based on the conservative hit rate approach where the assay produced 100% positive calls. Since adequate dilutions were not tested in the original LoD study, additional dilutions with one dilution level below 100% hit rate for all samples were tested in a second study to determine the lowest VAF with 100% hit rate.

The minimum tumor fraction required to support the robustness of the O/RDx-LCCA was evaluated. The resulting data show robustness of O/RDx-LCCA in samples with tumor content above 10% at 30 ng DNA input. The data supports O/RDx-LCCA requirement of 30% tumor content.

The recommended DNA input range of the O/RDx-LCCA is between 30 ng to 80 ng. The DNA input range was evaluated at 5, 10, 20, 40, 80, and 160 ng in duplicate using DNA extracted from 10 FFPE samples containing reportable CDx SNV and deletion variant representatives of *EGFR* and *KRAS* genes indicated in Table 1 of the intended use statement. The data showed that 10-80 ng of DNA input for the O/RDx-LCCA produced accurate results (at the variant level: PPA=100.0% [95% CI: 95.4%, 100%] (80/80), NPA=100.0% [95% CI: 99.9%, 100%] (9999/10000)).

To evaluate the potential impact of interfering substances on the performance of the O/RDx-LCCA, four CRC and four NSCLC FFPE specimens including reportable CDx SNV and deletion variant representatives of *EGFR* and *KRAS* genes indicated in Table 1 of the intended use statement were evaluated in the presence of exogenous and endogenous substances. No impact on the performance of the O/RDx-LCCA was observed for each substance and at each level tested.

Retrospective analyses of impact of necrotic tissue content in FFPE samples from clinical validation and analytical accuracy studies demonstrated that the performance of the O/RDx-LCCA is robust within the recommended range of necrotic content less than 50%.

To assess intra-run cross-contamination, 24 replicates of a positive cell line sample containing *EGFR* L858R at ~50% VAF and 24 replicates of NTC were processed on the same plate in a checkerboard format. No false positive calls (0/24, 0%) were detected in all NTC samples. Therefore, no cross-contamination was observed.

An *in-silico* cross-reactivity analysis was performed to evaluate the specificity of the primers used in the OR/Dx-LCCA. The results demonstrated that the primers are specific for the intended targeted sequences.

Test reproducibility was demonstrated across three test sites using a sample panel that included analytical targets near their originally established LoD. Testing included multiple reagent lots, operators, equipment, and was conducted over multiple test dates. The observed positive and negative percent agreements were 100% for all tests performed and no significant differences were detected between sites, reagent lots, or equipment used. An additional single-site precision study that was conducted using clinical samples with target CDx variants near their LoD levels based on the second LoD study (see above for LoD) demonstrated a positive percent agreement of 99.2% by site and 98.6% and 100% for *EGFR* and for *KRAS* on a gene-level, respectively, and a negative percent agreement of 100% on a site-level and on a gene-level for both genes. An additional 3-site reproducibility study is planned with samples carrying CDx variants and covering different *EGFR* Exon 19 deletions, *EGFR* Exon 21 L858R mutations, and *KRAS* codon 12/13 variants at the newly defined LoD levels to supplement the existing studies such that the assay precision is demonstrated to be robust near the true LoD levels of variants that are detected by O/RDx-LCCA.

A study evaluating performance of three commercially available FFPE tissue extraction kits was conducted because extraction kits are not included in the O/RDx-LCCA kit. The results demonstrate that the 3 commercially available FFPE extraction kits yield DNA with comparable quality and quantity to generate reliable results when used with O/RDx-LCCA.

The tolerances encompassing the library preparation and sequencing workflow steps were assessed, which correspond to the test's most critical steps that could lead to assay failure. The testing of different conditions for the assay's most critical steps resulted in zero failures and 100% agreement across conditions.

Three separately manufactured kit lots including all components of the O/RDx-LCCA were stored according to the storage conditions specified in product labeling. The data currently available support at least 13 months of stability for O/RDx-LCCA kit components for all 3 lots evaluated. The shelf-life stability will continue to be evaluated to extend the shelf-life stability claim. The stability of the reagents that was further evaluated in an additional study by testing FFPE clinical specimens with target CDx variants near the LoD levels based on second LoD study (see above for LoD) and using three representative reagent kit lots aged a minimum of 17 months demonstrated 100% detection rate across all three lots.

The reagent kit stability studies were performed as one large study that included data points for in-use freeze-thaw stability and transport stability testing under recognized summer and winter profiles for international shipments. The data demonstrate that all kit components show acceptable transport stability at the simulated time points. The in-use stability study evaluated both open vial stability and freeze-thaw stability. The data demonstrate in-use stability for at least 5 freeze-thaw cycles.

The stability of FFPE clinical samples (section and block) was assessed using both retrospective and a real-time analysis. No significant trend in poorer overall performance with increasing FFPE block age was observed, and robust assay performance was observed for samples over 10 years old.

Stability of FFPE curls was assessed at baseline, 30 days and 60 days to support stability at 30 days. FFPE curls were stable, as measured by PPA/NPA analysis, at both the 30-day and 60-day time points, supporting a claim of a 30-day stability.

Stability of DNA extracted from FFPE clinical samples using QIAGEN QIAamp FFPE extraction kit was assessed after storage at 4°C or -20°C, and after 5 cycles of freeze-thaw. Stability at 4°C was assessed after 60 days, 8 months, and 8.25 months and stability at -20°C was assessed after 6 months and 6.5 months. Data supports a claim of DNA storage stability at 8 months at 4°C and 6 months at -20°C. The DNA stability at -20°C will continue to be evaluated to extend the stability claim. The data supports a DNA freeze-thaw stability claim of 5 cycles.

The workflow for the O/RDx-LCCA incorporates several optional stopping points to hold assay intermediates. The stability of the intermediate products was evaluated by incorporating two optional stopping points specified in the assay instructions for use. The study results support the conclusion that the 60-day hold of Gene-Specific PCR (GS-PCR) products and 90-day hold on indexed libraries at recommended storage condition did not result in a decrease in O/RDx-LCCA performance.

GUIDE TO THE INTERPRETATION OF TEST RESULST

Test results should be interpreted in the context of tumor histopathology, clinical findings, patient and treatment history, and available laboratory data. Interpretations of any mutations detected by the test and treatment recommendations should be made by a board-certified molecular pathologist.

Additional information may be obtained from NCCN Guidelines and CAP/IASLC/AMP NSCLC Testing Guidelines or the ASCP/CAP/AMP/ASCO Testing Guidelines.

TEST LIMITATIONS

1. The ONCO/Reveal™ Dx Lung and Colon Cancer Assay has only been validated for use with CRC and NSCLC tumor tissues. Test only the indicated tissue types.
2. The ONCO/Reveal™ Dx Lung and Colon Cancer Assay has been validated with DNA extracted from NSCLC and CRC FFPE tissues.
3. Use of this product should be limited to personnel trained in the techniques of Next-Generation Sequencing library preparation and the use of the Illumina MiSeqDx® instrument.
4. Only the Illumina MiSeqDx® instrument installed with Pillar LC-HS module has been validated for use with this assay.
5. Only the PiVAT® software has been validated for use with this assay.
6. Quantification of FFPE extracted DNA and prepared libraries in this assay has been validated with Qubit™ dsDNA HS Assay Kit.
7. The ONCO/Reveal™ Dx Lung and Colon Cancer Assay only determines the presence or absence of the *KRAS* and *EGFR* mutations listed in Table 1 of the Intended Use.
8. Targeted molecular testing can only provide information for the targeted regions. A negative test result cannot rule out the possibility of other mutations with clinical utility outside of the target

region. For example, samples with results reported as "No mutation detected" may harbor *KRAS* and *EGFR* variants not reported by the assay.

9. A negative "No mutation detected" result does not rule out the presence of a mutation that may be present but below the limits of detection of this test (3.0-3.7%) (see Analytical Sensitivity: Limit of Detection section in Assay Instructions for Use).
10. This assay does not interrogate all variants or genes (*NRAS*) that confer resistance to cetuximab and panitumumab.
11. The ONCO/Reveal™ Dx Lung and Colon Cancer Assay is not to be used for the diagnosis of any disease.

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PATENTS AND TRADEMARKS

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