PILLAR BIOSCIENCES

CE IVD

Pillar® ONCO/Reveal™ Dx Lung and Colon Cancer Assay

One assay for six lung and colon cancer therapies

The first next-generation sequencing (NGS)-based companion diagnostic (CDx) kit to guide prescription of both colorectal cancer (CRC)-targeted therapies Erbitux® and Vectibix®, and non-small cell lung cancer (NSCLC)-targeted therapies Tarceva®, Gilotrif®, Iressa®, and Vizimpro®.

Assay Highlights

One Test, Multiple Therapies

- Establishes eligibility for two CRC therapies and four NSCLC therapies
- Detects clinically relevant mutations in KRAS for CRC and EGFR for NSCLC

Robust Chemistry

- Requires just 10ng DNA
- Sensitive down to just 9.8% tumor content
- Robust to up to 50% contaminating necrotic tissue

Rapid Workflow

- Single-tube workflow with only 2 purification steps
- From sample to sequencer in <8 hours
- Sample to actionable report in <4 days

Powerful bioinformatics

- Detects clinically relevant variants down to 1.6% VAF
- Run time <8 hours
- Calls clinically actionable SNVs and indels

Introduction

Colorectal cancer (CRC) represents a significant health issue as it is the most common gastrointestinal (GI) tract cancer worldwide with over 1.2 million new diagnoses each year. It is the third most common cancer diagnosis in both men and women. Increased understanding of the genetic and genomic changes in CRC has helped direct therapies and predict response, as evident in patients with KRAS mutations. Mutations in codons 12 and 13 of the KRAS gene are associated with resistance to monoclonal antibodies cetuximab and panitumumab that target the EGF receptor (EGFR).

Lung cancer is the second most common cancer in both men and women. Approximately 85% of lung cancer is non-small cell lung cancer (NSCLC). Tyrosine kinase inhibitors (TKIs) that inhibit EGFR are now standard of care for first-line treatment in patients with metastatic NSCLC whose tumors harbor an EGFR mutation. TKIs in clinical practice include erlotinib, gefitinib, afatinib, and dacomitinib.

Assay Details

Table 1. Intended uses

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 Deletions Exon 21 L858R	Tarceva (erlotinib), Gilotrif (afatinib), Iressa (gefitinib), or Vizimpro (dacomitinib)

Table 2. List of Variants with Established Analytical Performance Only

Gene	Variant ID	Cancer	Nucleotide Change
EGFR	T790M	NSCLC	c.2369C>T
EGFR	G719A	NSCLC	c.2156G>C
EGFR	G719C	NSCLC	c.2154_2155delinsTT; c.2155G>T
EGFR	G719D	NSCLC	c.2156G>A
EGFR	G719S	NSCLC	c.2155G>A
EGFR	Exon 20 In-frame Insertions	NSCLC	Multiple
BRAF	V600E	NSCLC	c.1799T>A; c.1799_1800delinsAA
KRAS	Exon 2 Mutation	NSCLC	Multiple
KRAS	A59E	CRC	c.176C>A
KRAS	A59G	CRC	c.176C>G
KRAS	A59T	CRC	c.175G>A
KRAS	A59S	CRC	c.175G>T
KRAS	Q61E	CRC	c.181C>G
KRAS	Q61H	CRC	c.183A>C ; c.183A>T
KRAS	Q61K	CRC	c.180_181delinsAA ; c.180_181inv ; c.181C>A
KRAS	Q61L	CRC	c.182A>T ; c.182_183delinsTC ; c.182_183delinsTG ; c.182_183inv
KRAS	Q61R	CRC	c.182A>G ; c.182_183delinsGC ; c.182_183delinsGT
KRAS	K117N	CRC	c.351A>C ; c.351A>T
KRAS	A146T	CRC	c.436G>A
KRAS	A146P	CRC	c.436G>C
KRAS	A146V	CRC	c.437C>T
BRAF	V600E	CRC	c.1799T>A ; c.1799_1800delinsAA

Make treatment decisions faster with multiplex testing

The Pillar ONCO/Reveal Dx Lung and Colon Cancer Assay is the first companion diagnostic assay kit that detects somatic mutations in DNA derived from formalin-fixed paraffin-embedded (FFPE) non-small cell lung cancer (NSCLC) and colorectal cancer (CRC) tumor tissue. The test simultaneously detects clinically relevant mutations in KRAS (absence of mutations in codons 12 and 13) for CRC and EGFR (exon 19 deletions and Exon 21 L858R) for NSCLC in a single assay. The assay is intended to be used to select patients with NSCLC or CRC that may benefit from treatment with the targeted therapies listed in *Table 1* in accordance with the approved therapeutic product labeling.

Multiple treatment decisions from one workflow
Testing multiple sample types at the same time allows for treatment decisions in four days for both NSCLC and CRC patients simultaneously.

In-house testing
Up to 48 libraries (2 controls and 46 patient samples) can be sequenced on a single MiSeq™Dx cartridge (Illumina Cat. No. 20037124), enabling clinical labs to bring testing in-house instead of sending to third-party labs.

Reduce repeat testing
With low sample-input requirements and extremely robust and sensitive variant detection, the ONCO/Reveal Dx Lung and Colon Cancer Assay reduces the need for repeat testing.

Single-day library preparation

The ONCO/Reveal Dx Lung and Colon Cancer Assay uses Pillar Bioscience's proprietary SLIMamp® chemistry to enable amplification of overlapping amplicons in a single tube. SLIMamp chemistry uses primers that prohibit the formation of undesirable amplicons by creating inhibitory stem loop structures.

Unlike traditional amplicon-based library preparation, SLIMamp chemistry prevents nonspecific amplification, even in highly multiplexed PCR reactions. This approach decreases contamination risks and minimizes the amount of required input DNA to just 10ng (*see Figure 1*).

The SLIMamp workflow is also designed for fast library prep. The single-tube workflow consists of just two PCR steps and two cleanup steps, so you can go from sample to sequencer in under 8 hours (*see Figure 2*).

Figure 1. SLIMamp chemistry overview

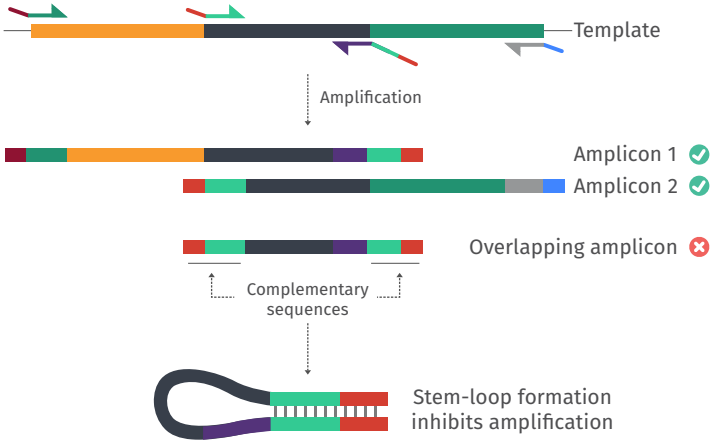

Figure 2. SLIMamp workflow overview

Single day data analysis

The Pillar PiVAT® bioinformatics platform that accompanies the ONCO/Reveal Dx Lung and Colon Cancer Assay is maximized for efficiency and sensitivity, delivering answers in less than 8 hours for variant allele frequencies down to 1.6% with minimal computing resources. The test is accompanied by a workstation (additional purchase required) for intuitive, cyber-secure reporting. The platform's built-in sample sheet tool also makes batching easy.

Concordant with previously approved assays

Clinical verification and validation studies were conducted with the ONCO/Reveal Dx Lung and Colon Cancer Assay using FFPE tissues from NSCLC and CRC tumors. The assay demonstrated concordance with the FDA-approved cobas® EGFR Mutation Test and the FDA-approved therascreen® KRAS test (*see Table 3*).

Table 3. Concordance with relevant IVD-cleared assays

Gene	PPA[a]	NPA[b]
EGFR	(91/91) 100%	(163/166) 98.2%
KRAS	(79/81) 97.5%	(103/104) 99.0%

[a]Number of samples with mutation according to Pillar ONCO/Reveal Dx Lung and Colon Cancer Assay divided by the number of samples with mutation according to comparator
[b]Number of samples identified as wild-type by Pillar ONCO/Reveal Dx Lung and Colon Cancer Assay divided by the number of samples identified as wild-type by comparator

Reproducible across a variety of testing conditions

ONCO/Reveal Dx Lung and Colon Cancer Assay reproducibility was evaluated at 3 sites with 2 operators at each site performing 3 runs on non-consecutive days (*see Table 4*). One sequencing instrument and 2 reagent lots were used at each site. Each panel member was tested in 4 replicates in each run for total of 360 possible results.

Table 4. Reproducibility data

Gene	True Positives
KRAS variant	(144/144) 100%
EGFR variant	(144/144) 100%
BRAF variant	(72/72) 100%

Straightforward reporting

Table 5. Example of report data
Sample Library ID: *[sample_library_ID] [sample_library_ID_barcode]*

Variants Detected for Therapeutic Use
Companion Diagnostic (CDx) Associated Findings

Indication	Gene	Exon	Nucleotide Change	Amino Acid Change	Variant Frequency (%)	FDA-Approved Therapeutic Options
[CRC/NSCLC]	[Gene]	[Exon]	[NC]	[AC]	[VAF]	[Therapy]

Other Alterations and Biomakers Identified
Results reported in this section are not part of the CDx claim of the ONCO/Reveal Dx Lung and Colon Cancer Assay and may not be perscriptive or conclusive for labeled use of any specific therapeutic product

Gene	Exon	Nucleotide Change	Amino Acid Change	Variant Frequency (%)
[Gene]	[Exon]	[NC]	[AC]	[VAF]

MiSeqDx Instrument

This assay is intended to be sequenced on a MiSeqDx instrument (Illumina Cat. No. DX-410-1001) loaded with a special Pillar LRM module.

Ordering information

Product	Part Number
ONCO/Reveal Dx Lung and Colon Cancer Assay, 48 reaction kit	HDA-LC-2002-48
Cyber secure ONCO/Reveal Dx Lung and Colon Cancer Assay PiVAT workstation	SFW-2005
Pillar MiSeqDx LRM Module	SW-0001

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