Trublood® the no risk biopsy

Lung Comprehensive

## Patient Details

| | | |
|---|---|---|
| Name | : | Mr. ABC |
| Patient ID | : | --- |
| Age | : | 52 Years |
| Gender | : | Male |
| Address | : | --- |
| Referring Doctor | : | Dr. XYZ |

## Specimen Details

| | | |
|---|---|---|
| Specimen Type | : | Blood |
| Draw Date | : | 10-Aug-2022 / 06.20 AM |
| Accession Date | : | 12-Aug-2022 / 09.15 AM |
| Report Date | : | 22-Aug-2022 / 03.10 PM |

### Specimen Analysis Summary:

**Blood**

| | | |
|---|---|---|
| cf Total Nucleic acids | : | 52 Genes (SNAs \| Indels \| CNAs \| Fusion Transcripts) |
| CTC | : | EpCAM \| PanCK \| Napsin A \| TTF1 \| CD45 \| CK7 \| P40 P63 \| Chromogranin A |

### Clinical Details:

Mr. ABC, a 75-year old male patient, presented with right mediastinal mass and supraclavicular lymph nodes.

### Principal reason for ordering the test:

To evaluate a case suspected of lung carcinoma.

Summary

## Summary and Interpretation

### Test result for Circulating Tumor Cells (CTCs)

| | | |
|---|---|---|
| ☐ Negative | ☑ Positive | - - - |

### Type of Malignancy

| | | |
|---|---|---|
| ☑ Adenocarcinoma | ☐ Squamous Cell Carcinoma | ☐ Adenosquamous Carcinoma |
| ☐ Neuroendocrine Carcinoma | ☐ Indeterminate | |

### Summary of Immunocytochemistry Analysis

| | | |
|---|---|---|
| ☑ EpCAM | ☑ PanCK | ☑ TTF1 |
| ☑ Napsin A | ☐ CD45 | ☑ CK7 |
| ☐ p40 | ☐ p63 | ☐ Chromogranin A |

### Test result for Circulating Tumor DNA (ctDNA)

| Biomarker with Results | Therapies with Potential Benefit | Therapies with Potential Lack of Benefit |
|---|---|---|
| **EGFR**<br>Exon 19 deletion<br>p.L747_P753delinsS<br>(MAF 0.58% at 26840X) | ☑ Osimertinib ☑ Gefitinib<br>☑ Erlotinib ☑ Dacomitinib<br>☑ Afatinib | - - - |

## Test Interpretation and Advice

Positive expression of EpCAM, PanCK and negative expression of CD45 indicates presence of CTCs (Circulating tumor cells) in given blood sample. Positive expression of TTF1, Napsin A, CK7 indicates adenocarcinoma of lung.

EGFR Exon 19 deletion, p.L747_P753delinsS, was detected in the cell free DNA (cfDNA) analysis as performed for targeted genes by Next Generation Sequencing (NGS) assay.

Consultation with treating physician is advised.

## Guide to Interpretation of (CTC) Test Results

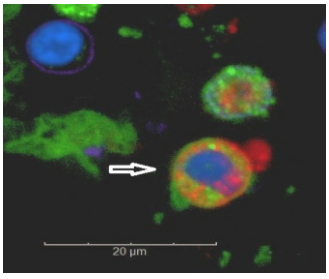
The Trublood comprehensive test analyzes Circulating Tumor Cells (CTCs) in peripheral blood and is intended to aid in the diagnosis of cancer.

- "Negative"
  No CTCs associated with suspected malignancy are detected in the given sample.
- "Positive"
  CTCs associated with suspected malignancy are detected in the given sample. Individuals with positive test result are advised to consult their physician / clinician for appropriate guidance / workup.
- "Indeterminate"
  Type of malignancy could not be determined. This may be due to any other malignancy in which markers are inadequately expressed.

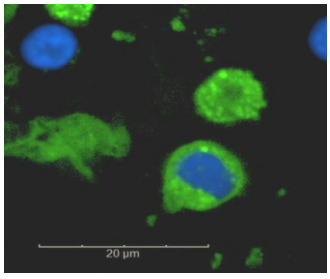
☑ Sample text here ☐ Sample text here

## Immunocytochemistry (ICC) Analysis

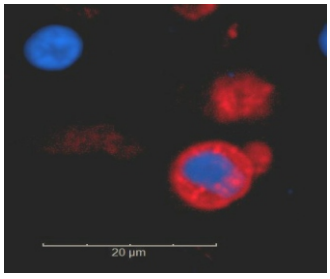
Immunocytochemistry performed on the isolated Circulating Tumor Cells (CTCs) showed the following profile:



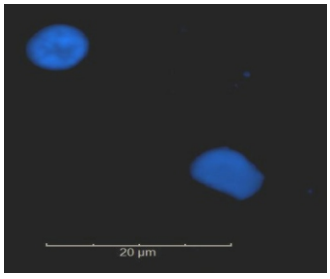

CTC(EpCAM+PanCK+CD45-): **Positive**



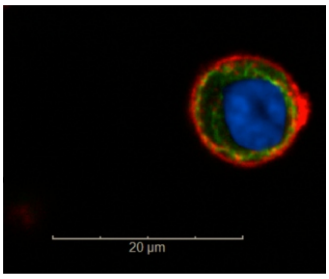

PanCK: **Positive**



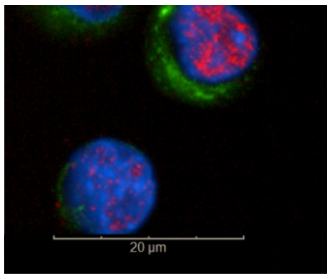

EpCAM: **Positive**



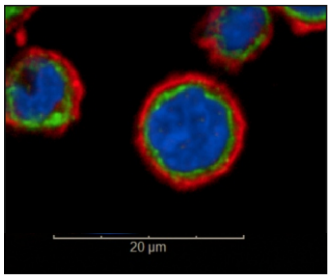

CD45: **Negative**



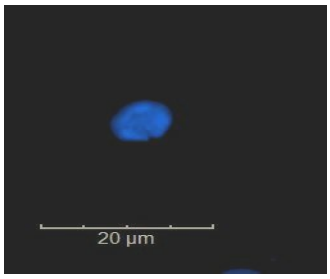

Napsin A: **Positive**



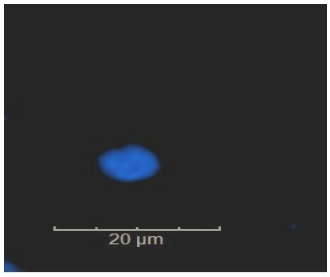

TTF-1: **Positive**



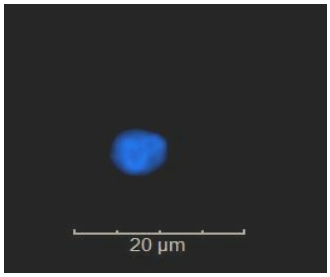

CK7: **Positive**




Chromogranin A: **Negative**




p63: **Negative**




p40: **Negative**

## Methods

Peripheral blood collected from the patient is treated with 'CellWizard™' Tumor Cell Isolation medium for detection and characterization of captured CTCs, if any. Analysis is performed as follows.

| Immunocytochemistry Markers (Internally Validated) : Marker (Clone) | | |
|---|---|---|
| **PanCK** (REA831) | **CK7** (OV-TL 12/30) | **Chromogranin A** (LK2H10+PHE5) |
| **EpCAM** (REA764) | **Napsin A** (Tmu-ad02) | **p63** (4A4) |
| **CD45** (REA747) | **TTF-1** (8G7G3/1) | **p40** (BC28) |

Circulating Tumor Cells (CTCs) which are defined as cells in the peripheral blood that are EpCAM and PanCK positive and CD45 negative are harvested using a proprietary medium and are characterized thereafter with enlisted antibodies. If CTCs are found in the sample submitted for the test, the sample is assessed to be positive and if no CTCs are found, the sample is assessed to be negative. For our publications pertaining to Trublood, please visit http://datarpgx.com/publications/.

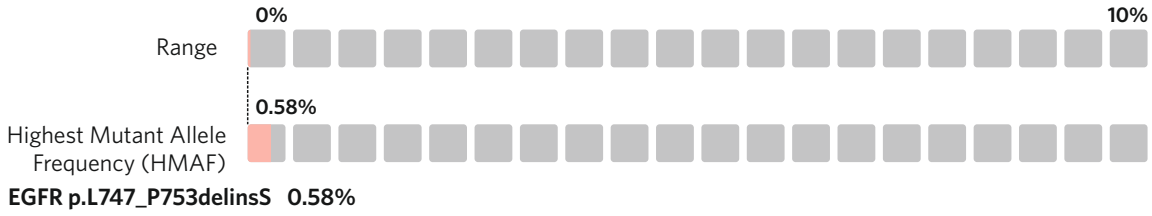
The Trublood comprehensive test is a Laboratory Developed Test and has not been submitted for approval by the US FDA. The test is however carried out in a laboratory which is certified to be compliant with ISO 15189:2012, ISO 27001:2013 and ISO 9001:2015 and is also accredited by the College of American Pathologists (CAP) and Clinical Laboratory Improvement Amendments (CLIA).

**Note:** Please refer to Interpretation Summary.

## Cell Free DNA (cfDNA) Analysis

Mutation load of 0.58% was detected in the cell free nucleic acids isolated from patient's plasma.

**Highest Mutant Allele Frequency** Mutant Fraction Wildtype Fraction




**EGFR p.L747_P753delinsS 0.58%**

## cfDNA Variant Details

| Marker Gene/s (Transcript ID) | Variant | |
|---|---|---|
| **EGFR** (NM_005228.5) | **Exon 19 deletion c.2240_2257del TAAGAGAAGCAACATCTC, p.L747_P753delinsS; [p.(Leu747_Pro753delinsSer)]** | **Category :** Tier I (Level A) **Result :** Positive |

EGFR mutations are found in 30% to 50% of lung adenocarcinomas, with the most common mutation being deletion in exon 19 (Ex19 in 45% patients) including p.L747_P753delinsS (6%) (Furuyama et al, 2013; Karachaliou et al., 2015; Su et al., 2017; Zheng et al., 2017; Grosse et al., 2019).

EGFR exon 19 deletion mutation is suggestive of response to anti-EGFR-tyrosine kinase inhibitors (TKIs), Osimertinib, Erlotinib, Afatinib, Gefitinib and Dacomitinib. These drugs are USFDA approved for the treatment of patients with metastatic non-small cell lung cancer (NSCLC) whose tumors have epidermal growth factor receptor (EGFR) exon 19 deletions or exon 21 (L858R) substitution mutations (Popat, 2018; Ramalingam et al., 2018; NCCN guidelines, 2022). As per NCCN guidelines, Erlotinib plus Ramucirumab, Erlotinib plus Bevacizumab are also recommended as a standard of care therapy for EGFR exon 19 deletion mutation positive non-small cell lung cancer (NCCN guidelines, 2022).

EGFR p.L747_P753delinsS is also designated as delL747-P753insS. This alteration leads to deletion of seven amino acids in the protein kinase domain of the EGFR protein from amino acids 747 to 753, combined with the insertion of a serine (S) at the same location and results in an increased EGFR kinase activity (Mulloy et al., 2007; Kancha et al., 2009; Karachaliou et al., 2015).

## Method

Cell free nucleic acids are analyzed for mutations and fusions detection using semiconductor based Next Generation Sequencing technology. Cell free nucleic acids extracted from the plasma of submitted specimen are subjected to target enrichment by multiplex PCR amplification using Oncomine lung panel.

Analytical validation of this test has shown sensitivity of 94.72% and specificity of 97.88%. Lower limit of detection of the mutations targeted is 0.1% and variants present below 0.1% may not be detectable with this assay, whereas analytical sensitivity is 97.14% and specificity is 93.75% for SNV, CNV and Fusion.

The Trublood comprehensive test is a Laboratory Developed Test and has not been submitted for approval by the US FDA. The test is however carried out in a laboratory which is certified to be compliant with ISO 15189:2012, ISO 27001:2013 and ISO 9001:2015 and is also accredited by the College of American Pathologists (CAP) and Clinical Laboratory Improvement Amendments (CLIA).

## Clinical Performance

The Trublood comprehensive test for Lung Cancer is a 'Single Laboratory Developed Test' for diagnosis and management of Lung Cancer. The test has been validated by Datar Cancer Genetics through the 'RESOLUTE' and 'TRUEBLOOD' clinical trial (Registration No. CTRI/2019/01/017219 and CTRI/2019/03/017918 respectively).

The test has a detection rate of 89.8% as validated on samples from 1378 Lung Cancer patients [adenocarcinoma, squamous cell carcinoma and neuroendocrine tumors]. The test has a specificity of 97.0% as determined by the screening of 22030 asymptomatic individuals.

## Classification of Somatic Variants (cfDNA)

**Criteria**

The criteria/guidance used in this report is in accordance with the guidelines provided by the American College of Medical Genetics and Genomics (ACMG) for the interpretation and reporting of sequence variants in cancer. Somatic sequence variations are categorized into four tiers based on their clinical significance (Li et al., 2017).

**Tier I:** Variants/biomarkers with strong clinical significance (therapeutic, prognostic and/or diagnostic)

**Level A evidence:** FDA approved therapies or standard guidelines for a specific tumor type.

**Level B evidence:** Statistically significant studies with consensus for specific tumor type.

**Tier II:** Biomarkers with potential clinical significance (therapeutic, prognostic and/or diagnostic)

**Level C evidence:** FDA approved therapies or standard guidelines for a different tumor type (off-label use of the drug). An inclusion criteria for clinical trials.

**Level D evidence:** No consensus among different studies.

**Tier III:** Biomarker whose association with cancer is not evident from available literature and is not frequently present in general population.

**Tier IV:** Biomarker whose association with cancer has not been reported till date and is frequently present in general population. This category of variants is not included in this report as per guidelines.

**Disclaimer**

Results of ICC (antigen expression on CTCs) may vary from that of primary tumor tissue and over time due to tumor heterogeneity and other biological processes. Further, certain conditions such as active inflammatory diseases, medications, exposure to radiation, UV induced sunburn etc. may interfere with accuracy of assay results. Other potential sources of error include, but are not limited to, sample contamination / degradation or pre-analytical deviations.

The Trublood comprehensive test is performed pursuant to symptoms leading to suspicion of malignancy, such as clinical features, imaging etc. Decisions on patient care and treatment must be based on the independent medical judgement of the treating physicians, taking into consideration all available and relevant information concerning the patient's condition, such as personal and family history, physician's examination as well as information from other pertinent diagnostic tests. A treating physician's decisions should not be based on a single test or solely on the information contained in this report.

This report should be read as a whole and used and acted upon only by a registered / licensed medical practitioner under the relevant law who is duly qualified to practice medicine.

This is not a prescription.

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****End of Report****

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## Notes

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2. Trublood is trademark owned by Datar Cancer Genetics and are the subject matter of intellectual property rights applications worldwide.

## Important Note

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- Please read the Disclaimer section carefully.

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