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PATIENT'S DETAILS AND CLINICAL HISTORY

Name	:	Mr. A. B. C.	Collection On	:	10/04/2019
Sex	:	Male	Received On	:	10/04/2019
Age	:	54 Years	Report On	:	19/04/2019 03:50 PM
Address	:	---	Specimen Type	:	18 mL EDTA Blood, **8 mL Blood in DCGLTubes**
			Referred By	:	**Dr. X. Y. Z.**

Mr. A. B. C. , a 54-year-old male patient is a case of lung adenocarcinoma. Positive for CK7 and negative for TTF-1, Napsin A on IHC. Other details as per records.

SAMPLE PROCESSING SUMMARY

Peripheral blood in special collection tubes was used for comprehensive exosomal gene expression study, cell free nucleic acids analysis, Copy Number Variations (CNVs), microRNA expression profile, Circulating Tumor Cell (CTC) detection, chemosensitivity and pharmacogenetics analysis.

REPORT HIGHLIGHTS

THERAPIES WITH POTENTIAL BENEFIT - FDA APPROVED THERAPIES IN CANCERS

THERAPY	BIOMARKER
Osimertinib+	EGFR activating mutation
Dacomitinib+	EGFR activating mutation
Erlotinib+	EGFR activating mutation
Gefitinib+	EGFR activating mutation
Afatinib+	EGFR activating mutation
Paclitaxel+	TUBB2A overexpression; Chemosensitivity
Vinorelbine+	TUBB2A overexpression; Chemosensitivity
Vinblastine*	TUBB2A overexpression; Chemosensitivity
Eribulin*	TUBB2A overexpression
Cabazitaxel*	TUBB2A overexpression
Ixabepilone*	TUBB2A overexpression
Vincristine*	TUBB2A overexpression
Irinotecan*	TOP1 overexpression; Chemosensitivity
Ruxolitinib*	JAK2 overexpression
5-Fluorouracil*	Chemosensitivity
Cisplatin*	Chemosensitivity

THERAPIES WITH POTENTIAL LACK OF BENEFIT

THERAPY	BIOMARKER
Docetaxel+	Chemosensitivity#
Topotecan*	Chemosensitivity#

POTENTIAL LACK OF BENEFIT FROM IMMUNE CHECKPOINT INHIBITORS

2.96 mutations/Mb	Cell free tumor mutation burden (CMB)
Negative	MLH1, MSH2, MSH6, PMS1, PMS2 gene mutations

THERAPIES WITH INCREASED RISK OF TOXICITY

THERAPY	BIOMARKER
Carboplatin	ERCC1, MTHFR
Cisplatin	XPC, ERCC1
Oxaliplatin	ERCC1
Methotrexate	ABCB1, MTHFR

INDICATIONS FOR NONCONVENTIONAL DRUGS

THERAPY	BIOMARKER
Quercetin	WNT pathway activation- APC mutation
Celecoxib	WNT pathway activation- APC mutation

Carboplatin*	Chemosensitivity
Mitomycin*	Chemosensitivity
Dacarbazine*	Chemosensitivity
Etoposide*	Chemosensitivity
Gemcitabine+	Chemosensitivity
Pemetrexed+	Chemosensitivity
Doxorubicin*	Chemosensitivity

MUTATION LOAD AND CTC

10.2%	Mutation load
02 CTCs/ ml	Number of CTCs detected

PROGNOSTIC IMPLICATIONS

PROGNOSIS	BIOMARKER
Adverse	TP53, APC mutations; miR-134-5p, let-7c-5p, miR-146a-5p, miR-140-3p, miR-195-5p, miR-30a-3p, miR-126-5p, miR-142-5p
Favorable	miR-145-5p, miR-29c-5p

THERAPIES WITH LABELED RISK OF TOXICITY

THERAPY	BIOMARKER
Belinostat	UGT1A1
Dabrafenib	G6PD
Erlotinib	UGT1A1
Fluoropyrimidines	DPYD
Gefitinib	CYP2D6
Gemcitabine	NT5C2
Irinotecan	UGT1A1
Mercaptopurine	TPMT, NUDT15
Nilotinib	UGT1A1
Pazopanib	UGT1A1
Rasburicase	G6PD
Regorafenib	UGT1A1
Thioguanine	TPMT, NUDT15
Trametinib	G6PD
Vincristine	CEP72

+ US FDA approved for treatment of lung cancer.
* as may be found appropriate by treating physician as off-label therapy.
$ evidence based on pre-clinical studies.
# Refer to more therapies with potential lack of benefit based on Correkt-Chemo analysis on pg. 30

COMPREHENSIVE PATHWAY PERTURBATION IN PRIMARY TUMOR

PATHWAYS IN CANCER

Data on KEGG graph
Rendered by Pathview

THERAPEUTIC IMPLICATIONS (Exosomal RNA)

Therapeutic implications section of the report briefly describes the genetic alterations with potential implications to the patient's treatment and cancer management. The findings were taken from each of the analytes for clinical interpretation.

Table 1: List of drugs approved for cancers with potential benefit to the patient based on exosomal gene expression analysis

Genetic alterations	Result	Reference / FDA
TUBB2A	▲ +2.47	Upregulation of TUBB2A is suggestive of potential benefit from Docetaxel, Paclitaxel, Vinorelbine, Eribulin, Cabazitaxel, Ixabepilone, Vinblastine and Vincristine (Stanton et al., 2011; Mukhtar et al., 2014). Docetaxel, Paclitaxel and Vinorelbine are USFDA approved for the treatment of multiple cancers including non-small cell lung cancer. Vinblastine is USFDA approved for the treatment of breast cancer, testicular cancer, choriocarcinoma, Hodgkin lymphoma, Kaposi sarcoma and Non- Hodgkin lymphoma (NHL). Vinblastine is recommended as standard of care therapy for NSCLC as per NCCN guidelines (NCCN Clinical practice guidelines in oncology version 3, 2019). Eribulin is USFDA approved for treatment of metastatic breast cancer. A randomized, open-label, multicenter, phase III study of Eribulin showed activity in the third-line setting and had a manageable safety profile in patients with advanced non-small cell lung cancer. The objective response rate was 12% for Eribulin and 15% for treatment of physician's choice (TPC). Clinical benefit rate and disease control rate were Eribulin, 57%; TPC, 55% and Eribulin, 63%; TPC, 58% (Katakami et al, 2017). Cabazitaxel is USFDA approved for treatment of patients with hormone-refractory metastatic prostate cancer. Results of a multicentre phase II trial of Cabazitaxel in patients with advanced non-small cell lung cancer progressing after Docetaxel based chemotherapy suggest that Cabazitaxel exhibits activity in these patients with a substantial but manageable toxicity profile (Kotsakis et al., 2016).

Ixabepilone is approved to be used alone or with Capecitabine in treatment of breast cancer.
A phase II trial of Ixabepilone and Carboplatin with or without Bevacizumab in patients with previously untreated advanced non-small-cell lung cancer demonstrated the treatment benefits were consistent with those achieved with other modern platinum-doublet regimens. The objective response rates were 29% and 50% for Ixabepilone/Carboplatin (cohort A) and Ixabepilone/Carboplatin/Bevacizumab (cohort B), respectively. After median follow up of 17.5 months (A) and 15.7 months (B), median progression free survivals were A-5.3 months and B-6.7 months, with median overall survivals of 9.3 months 13.2 months, respectively (Spigel et al., 2012).
A phase II clinical trial of the Epothilone B analog, Ixabepilone, in patients with non small-cell lung cancer whose tumors have failed first-line platinum based chemotherapy demonstrated that single-agent ixabepilone had clinically relevant activity and an acceptable safety profile. Ixabepilone administered as a single 32 mg/m(2) 3-hour infusion (77 patients; arm A) or a 6 mg/m(2) 1-hour infusion daily for 5 consecutive days (69 patients; arm B) in a 3-week cycle showed objective response rate was 14.3% in arm A and 11.6% in arm B. Median duration of response was 8.7 months in arm A and 9.6 months in arm B. Median time to progression was 2.1 months for arm A and 1.5 months for arm B. Median survival was 8.3 months for arm A, and 7.3 months for arm B; the 1-year survival rate (both cohorts) was 38% (Vansteenkiste et al., 2007).

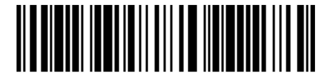
Vincristine is USFDA approved for acute leukemia, Hodgkin lymphoma, neuroblastoma, non-Hodgkin lymphoma, rhabdomyosarcoma and Wilms tumor.
A study on Gemcitabine and Vincristine in NSCLC demonstrated that this combination was an effective outpatient regimen with low myelotoxicity for stage IV non-small cell lung cancer. The overall response rate was 16/40 (40%) (N = 40); with 2 complete and 14 partial responses; additional 14 patients had minor responses or stable disease. Median duration of remission was 4.5 months, and the median survival was 9 months (Zwitter et al., 2001).

TOP1	▲	+2.29	Upregulation of TOP1 is suggestive of potential benefit from Irinotecan and Topotecan (Holcombe et al, 2004; Meisenberg et

			al, 2014; Meisenberg et al, 2015). Topotecan is USFDA approved for treatment of patients with multiple cancers including small cell lung cancer. A phase III study comparing oral Topotecan to intravenous Docetaxel in patients with pretreated advanced non-small-cell lung cancer demonstrated activity in these patients. Median survival was 27.9 weeks with Topotecan and 30.7 weeks with Docetaxel. The median time to progression was 11.3 weeks with Topotecan versus 13.1 weeks with Docetaxel. The overall response rate was 5% in each group (Ramlau et al., 2006). A phase I dose escalation study of Vinorelbine and Topotecan combination chemotherapy in patients with recurrent lung cancer demonstrated that potential activity for the treatment of patients with advanced NSCLC and SCLC (Beldner et al., 2007). Irinotecan is USFDA approved for treatment of patients with colorectal cancer. A phase II study of Irinotecan as a third- or fourth-line treatment for advanced non-small cell lung cancer demonstrated that Irinotecan monotherapy is effective for advanced NSCLC patients (Matsubara et al., 2013). Irinotecan combined with Bevacizumab showed favorable antitumor activity in heavily pretreated patients with NSCLC (Wills et al., 2017).
JAK2		+2.60	Upregulation of JAK2 is suggestive of potential benefit from Ruxolitinib (Harrison et al., 2012). Ruxolitinib is USFDA approved for polycythemia vera and intermediate and high risk myelofibrosis. A placebo-controlled phase II study for first-line treatment of patients with advanced nonsquamous non-small cell lung cancer and systemic inflammation demonstrated that Ruxolitinib at 15 mg b.i.d. had an acceptable safety profile in combination with Pemetrexed and Cisplatin (Giaccone et al., 2018). A pre-clinical study reported that Ruxolitinib treatment overcomes Cisplatin resistance in non-small-cell lung cancer (Hu et al., 2014).

Table 2: List of non-conventional drugs that may provide therapeutic benefit to this patient based on exosomal gene expression analysis

Genetic alterations	Result	Drugs	Interpretation / References
None Detected			



POTENTIAL LACK OF BENEFIT FROM OTHER DRUGS

Table 3: List of FDA approved drugs that may not provide therapeutic benefit to this patient based on exosomal gene expression analysis.

Markers	Result	Drugs Without Benefit	Interpretation / References
TYMS	▲ +2.34	Pemetrexed, Capecitabine, 5-Fluorouracil	Upregulation of TYMS is suggestive of lack of resistance from Capecitabine, 5-fluorouracil (5-FU) and Pemetrexed. Pemetrexed and its polyglutamated derivatives inhibit thymidylate synthase (TYMS), dihydro-folate reductase (DHFR), and glycinamide ribonucleotide transformylase (GART), all of which are involved in the denovo biosynthesis of thymidine and purine nucleotides. Antimetabolite agents, including Pemetrexed, induce an imbalance in the cellular nucleotide pool and inhibit nucleic acid biosynthesis that results in arresting the proliferation of tumor cells and inducing cell death (Hazarika et al., 2005; Chattopadhyay et al., 2007; Obata et al., 2013; Hamal et al, 2018). Pemetrexed is USFDA approved for lung carcinoma and mesothelioma. Capecitabine is USFDA approved for treatment of metastatic breast and colorectal cancer. 5-Fluorouracil (5-FU) is USFDA approved for breast, colorectal, gastric (stomach) and pancreatic cancer.

GLOBAL GENE EXPRESSION HIGHLIGHTS

- Out of 20805 number of protein coding genes analyzed in the blood sample, **8489** genes were expressed in the analyzed blood sample.
- **2957** genes were found to be differentially regulated in the blood sample.

Table 4: Top Cancer Pathways Detected in Blood

Top Cancer Pathways Detected in Blood	
Pathway Name	Differetially Expressed Genes (DE) (%)
Cytosolic DNA-sensing pathway	26.56
B cell receptor signaling pathway	25.35
TNF signaling pathway	24.07
NF-kappa B signaling pathway	23.16
Toll-like receptor signaling pathway	23.08
Non-small cell lung cancer	22.73
RIG-I-like receptor signaling pathway	21.43
NOD-like receptor signaling pathway	20.83
FoxO signaling pathway	20.45
Jak-STAT signaling pathway	20.37
HIF-1 signaling pathway	20
Natural killer cell mediated cytotoxicity	19.55

MISMATCH REPAIR (MMR) GENE MUTATIONS

Analysis of the mismatch repair (MMR) genes, MLH1, MSH2, MSH6, PMS1 and PMS2, did not detect any mutations.

Literature-based evidence suggests that loss of mismatch repair function via germline or somatic mutation confers the microsatellite instability (MSI) phenotype that is associated with high TMB and response to immune-checkpoint inhibitors (Richman et al, 2015; Lee et al, 2016; Viale et al, 2017; Mouw et al, 2017).

A study on sporadic primary non-small-cell lung cancers (NSCLC) for MSI by Lawes and his colleagues demonstrated no correlation between MSI and pathological features such as size, histotype, stage, grade and lymph-node metastasis (Lawes et al., 2003). However, a review of microsatellite alterations in primary lung cancer suggested that MSI may only be causatively associated with the initiation of molecular changes, which may later lead to neoplasia (Shen et al., 2014).

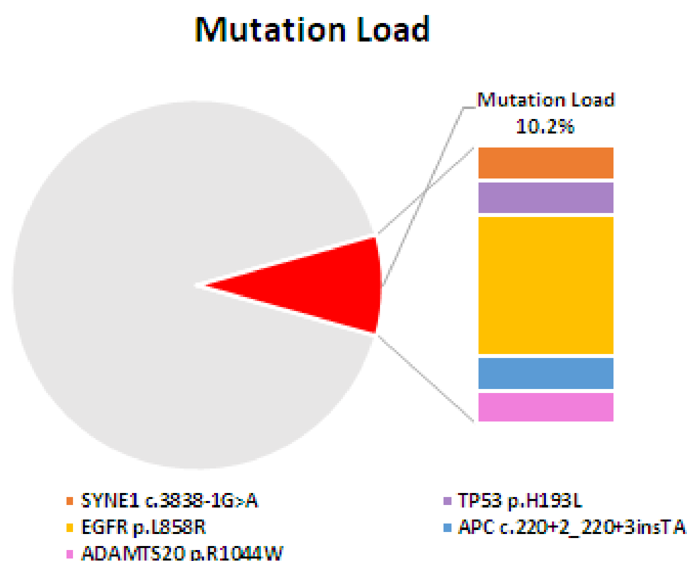
SOMATIC GENOME ALTERATIONS (CELL FREE NUCLEIC ACIDS)

Cell free Tumor Mutation Burden (CMB) of the blood is: 2.96 mutations/Mb. CMB was calculated based on the allelic fraction of the somatic mutations detected by Next Generation Sequencing analysis of 411 genes.

Tumor mutation burden (TMB) is an informative biomarker for predicting response to immunotherapy in a number of malignancies. The median tumor mutation burden (TMB) (n=11855) for lung adenocarcinoma is reported to be 6.3 mutations/Mb, while the maximum TMB is 755.0 mutations/Mb (95% Confidence Interval, 11.8 - 12.9) (Chalmers et al., 2017). Analysis of tumor mutational burden (TMB) across more than 100,000 clinical cancer specimens, including lung adenocarcinoma, suggests that patients with TMB <20 mutations/Mb may not derive benefit from immunotherapy drugs (Chalmers et al., 2017; Samstein et al., 2019). Recently the combination of nivolumab (Opdivo) plus Ipilimumab (Yervoy) has been USFDA approved for the frontline treatment of patients with advanced non–small cell lung cancer (NSCLC) with tumor mutational burden (TMB) ≥10 mutations per megabase (mut/Mb) (Hellman et al., 2018; NCCN guidelines, 2019).

Therefore, in this case, the patient may not derive potential benefit from immunotherapy drugs based on low cell free tumor mutation burden (2.96 mutations/Mb) and no pathogenic/ likely pathogenic mutations detected in the analysed MMR genes in the submitted sample.

Next-generation sequencing analysis for mutations and amplifications of 411 oncogenes and tumor suppressor genes was carried out on submitted blood sample.



1. Mutation Load of 10.2% was detected in the cell free nucleic acids isolated from patient's plasma.

2. The presence of activating mutation in EGFR gene, p.L858R [c.2573T>G, p.(Leu858Arg)] detected in cell free nucleic acids analysis, is suggestive of potential benefit from anti-EGFR tyrosine kinase inhibitors (TKIs) Osimertinib, Dacomitinib, Erlotinib, Gefitinib and Afatinib.

3. Loss-of-function mutation in the APC gene is suggestive of potential benefit from non-conventional drugs, Quercetin and Celecoxib.

4. Mutations detected in TP53 and APC genes are associated with an adverse prognosis in lung adenocarcinoma.

5. The clinical significance of the mutations detected in ADAMTS20 and SYNE1 genes in lung adenocarcinoma is currently not known, as per available literature.

Table 5: List of mutations found in cell-free nucleic acids analysis in lung cancer.

Markers (Transcript ID)	Result	Significance	Category
EGFR (NM_005228.3) c.2573T>G, p.L858R; [p.(Leu858Arg)]	Positive	EGFR mutations are found in 30% to 50% of lung adenocarcinomas, with the most common mutations being deletion in exon 19 (Ex19 in 45% patients) and a mutation in exon 21 p.L858R (Ex21 in 40% patients) (Yang et al, 2011; Jin et al, 2016; Imianitov et al, 2017, Zheng et al, 2017). EGFR p.L858R mutation is suggestive of response to anti-EGFR tyrosine kinase inhibitors (TKIs) Osimertinib, Dacomitinib, Erlotinib, Gefitinib and Afatinib. 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 21 (L858R) substitution mutation (Popat, 2018; Ramalingam et al., 2018; NCCN guidelines, version 3, 2019). EGFR p.L858R mutation results in an amino acid substitution at position 858 in EGFR, from a leucine (L) to an arginine (R). It is the most common sensitizing mutation to EGFR-TKI therapies. This mutation occurs within exon 21, which encodes part of the kinase domain. The p.L858R mutation increases the kinase activity of EGFR, leading to hyperactivation of downstream pro-survival signaling pathways (Sordella et al., 2004; Mitsudomi et al., 2010; Kobayashi et al., 2013). It is reported in tumors of lung, esophagus, breast and upper aerodigestive tract. The EGFR gene encodes a transmembrane glycoprotein that is a member of the protein kinase superfamily. This protein is a receptor for members of the epidermal growth factor family. It is a cell surface protein that binds to epidermal growth factor. Binding of the protein to a ligand induces receptor dimerization and tyrosine auto-phosphorylation and leads to cell proliferation. Mutations in this gene are associated with lung cancer.	Tier I (Level A)
TP53 (NM_000546.5) c.578A>T, p.H193L; [p.(His193Leu)]	Positive	Mutations in TP53 are frequently found in lung adenocarcinoma and are associated with an adverse prognosis as well as resistance to chemotherapy (Mogi et al, 2011; Jincui et al, 2016). TP53 p.H193L mutation is reported in patients with non-small	Tier I (Level B)

		cell lung cancer (Lee et al., 2010). This mutation lies within the DNA-binding domain of the TP53 protein (Bode and Dong, 2004). TP53 p.H193L has been identified in the scientific literature (Levy et al., 2011), but has not been biochemically characterized and therefore, its effect on TP53 protein function is unknown. It is reported in tumors of oesophagus, upper aerodigestive tract, breast, ovary, haematopoietic and lymphoid system. The TP53 gene provides instructions for making a protein called tumor protein p53 (or p53). This protein acts as a tumor suppressor, which means that it regulates cell division by keeping cells from growing and dividing too fast or in an uncontrolled way. Because p53 is essential for regulating cell division and preventing tumor formation, it has been nicknamed the "guardian of the genome".	
APC (NM_000038.5) c.220+2_220+3insTA	Positive	Loss-of-function mutations in the APC gene are not common in lung cancers but reported to be involved in pathogenesis of lung cancer (Ohgaki et al., 2004). APC loss stabilizes beta-catenin and constitutively activates the pathway even in the absence of a WNT signal and therefore suggestive of potential benefit from non-conventional drugs such as Quercetin and Celecoxib (Schneikert et al, 2007; Hankey et al, 2018). Preclinical studies reported that Quercetin inhibits cancer growth through inhibition of Wnt/β-catenin signalling pathway (Shan et al., 2009; Amado et al., 2011). Celecoxib, a COX-2 inhibitior, results in decreased cell proliferation and potentiation of chemotherapy This gene encodes a tumor suppressor protein that acts as an antagonist of the Wnt signaling pathway. It is also involved in other processes including cell migration and adhesion, transcriptional activation, and apoptosis. This protein also helps ensure that the number of chromosomes in a cell is correct following cell division. The APC protein accomplishes these tasks mainly through association with other proteins, especially those that are involved in cell attachment and signaling. Disease-associated mutations tend to be clustered in a small region designated the mutation cluster region (MCR) and result in a truncated protein product.	Tier I (Level B)

ADAMTS20 (NM_025003.3) c.3130C>T, p.R1044W; [p.(Arg1044Trp)]	Positive	ADAMTS20 is reported to be a pro-survival molecule act as an oncogene in certain cancers (Llamazares et al., 2003). ADAMTS20 p.R1044W mutation is reported in tumors of skin and prostate. However, its clinical significance in lung adenocarcinoma is not yet known. The protein encoded by this gene is a member of the ADAMTS family of zinc-dependent proteases. The encoded protein has a signal peptide that is cleaved to release the mature peptide, which is secreted and found in the extracellular matrix. This protein may be involved in tissue remodeling.	Tier III
SYNE1 (NM_182961.3) c.3838-1G>A	Positive	Mutations in SYNE1 gene are reported in certain cancer (Cheng et al., 2015). However, the clinical significance of SYNE1 c.3838-1G>A mutation in lung adenocarcinoma is not yet known. This gene encodes a spectrin repeat containing protein expressed in skeletal and smooth muscle, and peripheral blood lymphocytes, that localizes to the nuclear membrane.	Tier III

*Please refer to criteria for classification of somatic variants on pg. 35

COPY NUMBER VARIATIONS

Figure 1: Ideogram of selected chromosomes showing copy number alterations detected in the submitted sample

Markers (Cytoband)	Result	Interpretation	Category
(11q13.4 - 11q13.5)	Loss	Loss of chromosomal region at 11q is reported in patients with lung adenocarcinoma (Cheng et al., 1990; Rasio et al., 1995). However, its clinical significance of this alteration in lung adenocarcinoma is not yet known.	Tier III

*Please refer to criteria for classification of somatic variants on pg. 35

NEGATIVE FINDINGS

Markers	Alteration type	Result	Interpretation
EGFR (NM_005228)	p.T790M; [p.(Thr790Met)]	Negative	EGFR p.T790M [p.(Thr790Met)] mutation was not detected on cell free nucleic acids analysis in the submitted sample. It is the most common secondary EGFR mutation that causes resistance to EGFR TKIs. The EGFR p.(Thr790Met) mutation has been reported in about 60% of patients with disease progression after initial response to Erlotinib, Gefitinib, or Afatinib (Fujita et al, 2012; Stewart et al, 2015; NCCN Guidelines, Non-small cell lung cancer version 3, 2019).
KRAS (NM_004985)	Single nucleotide alterations and indels in codons 12, 13, 61 and others in the tested exons	Negative	KRAS mutations were not detected on cell free nucleic acids analysis in the submitted sample in the exons tested. Approximately 15-25% of patients with lung adenocarcinoma have tumor associated KRAS mutations. They have also been associated with a poorer prognosis as well as resistance to chemotherapy and EGFR TKIs (Stewart et al, 2015; Lovly et al, 2015; NCCN Guidelines, Non-small cell lung cancer version 3, 2019). The KRAS gene provides instructions for making a protein called K-Ras that is involved primarily in regulating cell division. As part of a signaling pathway known as the RAS/MAPK pathway, the protein relays signals from outside the cell to the cell's nucleus. These signals instruct the cell to grow and divide or to mature and take on specialized functions, like differentiation. It belongs to a class of genes known as oncogenes.
BRAF (NM_004333)	Single nucleotide alterations and indels in codons 469, 594, 596, 600 and others in the tested	Negative	BRAF mutations were not detected on cell free nucleic acids analysis in the submitted sample in the exons tested. BRAF mutations in NSCLC are uncommon and seen in

	exons		less than 5% of cases. Mechanisms of resistance to TKI therapy in EGFR mutated NSCLC includes mutations in BRAF (Stewart et al, 2015; Lovly et al, 2015; NCCN Guidelines, Non-small cell lung cancer version 3, 2019). The BRAF gene provides instructions for making a protein that helps transmit chemical signals from outside the cell to the cell's nucleus. It encodes a protein which is part of a signaling pathway known as the RAS/MAPK pathway, which controls several important cell functions like cell division, differentiation, and secretion.
MET (NM_000245)	Single nucleotide alterations and indels in the tested exons, including those resulting in exon 14 skipping	Negative	MET mutations were not detected on cell free nucleic acids analysis in the submitted sample in the exons tested. MET alterations that result in exon 14 skipping are found in lung cancer in both the presence and absence of MET amplification. Exon 14 skipping results in the deletion of the juxtamembrane domain of MET, which leads to enhanced signaling through the MET receptor pathway. Both preclinical and case report evidence suggest that tumors harboring MET with exon 14 alterations and/or MET amplifications have increased sensitivity to MET inhibitors (Lovly et al, 2017; NCCN Guidelines, Non-small cell lung cancer version 3, 2019). This gene encodes a member of the receptor tyrosine kinase family of proteins and the product of the proto-oncogene MET. The encoded preproprotein is proteolytically processed to generate alpha and beta subunits that are linked via disulfide bonds to form the mature receptor. Binding of its ligand, hepatocyte growth factor, induces dimerization and activation of the receptor, which plays a role in cellular survival, embryogenesis, and cellular migration and invasion. Mutations, amplification and overexpression of this gene are associated with multiple human cancers.
ERBB2/Her2 (NM_004448)	Single nucleotide alterations and indels in exon 20 and	Negative	ERBB2/Her2 mutations were not detected on cell free nucleic acids analysis in the submitted sample in the exons tested.

other tested exons	
	ERBB2/Her2 mutations in NSCLC are uncommon and seen in 2-4% of cases. In cohorts of EGFR/KRAS/ALK-negative NSCLC specimens, the frequency of HER2 mutations is 6%. Preclinical data suggest that the presence of mutation is associated with primary resistance to the first-generation EGFR TKIs, Erlotinib and Gefitinib. However, cells expressing the HER2 exon 20 mutations are sensitive to the irreversible dual EGFR and HER2 TKIs Neratinib and Afatinib (Lovly et al, 2015). The ERBB2 gene encodes a member of the epidermal growth factor (EGF) receptor family of receptor tyrosine kinases. This protein has no ligand binding domain of its own and therefore cannot bind growth factors. However, it does bind tightly to other ligand-bound EGF receptor family members to form a heterodimer, stabilizing ligand binding and enhancing kinase-mediated activation of downstream signaling pathways, such as those involving mitogen-activated protein kinase and phosphatidylinositol-3 kinase.

FUSION ANALYSIS

Table 6: Fusion analysis in cell free nucleic acids

Marker (Transcript ID)	Alteration	Result	Interpretation
ALK (NM_004304)	EML4-ALK KIF5B-ALK KLC1-ALK HIP1-ALK TPR-ALK	Negative	ALK fusions were not detected in the cell free nucleic acids analysis in the submitted sample. Approximately 3-7% of lung tumors harbor ALK fusions. Multiple different ALK rearrangements have been described in NSCLC. The majority of these ALK fusion variants are comprised of portions of the echinoderm microtubule-associated protein-like4 (EML4) gene with the ALK gene. In the vast majority of cases, ALK rearrangements are non-overlapping with other oncogenic mutations found in NSCLC (NCCN Guidelines, Non-small cell lung cancer version 3, 2019). Crizotinib, Ceritinib, Lorlatinib, Alectinib and Brigatinib are approved by the USFDA for non-small cell lung cancer with ALK rearrangements.
ROS1 (NM_002944)	CD74-ROS1 TPM3-ROS1 LRIG3-ROS1 GOPC-ROS1 SDC4-ROS1 SLC34A2-ROS1 EZR-ROS1	Negative	ROS1 fusions were not detected in the cell free nucleic acids analysis in the submitted sample. Approximately 2% of lung tumors harbor ROS1 fusions. Several different ROS1 rearrangements have been described in NSCLC. These include SLC34A2-ROS1, CD74-ROS1, EZR-ROS1, TPM3-ROS1, and SDC4-ROS1 (NCCN Guidelines, Non-small cell lung cancer version 3, 2019). Crizotinib, Lorlatinib and Ceritinib are approved by the USFDA for non-small cell lung cancer with ROS1 rearrangements.
RET (NM_020975)	KIF5B-RET CUX1-RET CCDC6-RET	Negative	RET fusions were not detected in the cell free nucleic acids analysis in the submitted sample. Approximately 1% of lung cancers harbour RET rearrangements. Cabozantinib and Vandetanib are emerging targets for tumors with RET rearrangements

	(NCCN Guidelines, Non-small cell lung cancer version 3, 2019).

PHARMACOGENETIC REPORT FOR ONCOLOGY DRUGS - SNAPSHOT

Drug with Contraindication

None

Drug with Increased Risk of Toxicity

Cisplatin

Carboplatin

Oxaliplatin

Methotrexate

Drug with Labelled Toxicity

Belinostat

Dabrafenib

Erlotinib

Fluoropyrimidines

Gefitinib

Gemcitabine

Irinotecan

Mercaptopurine

Nilotinib

Pazopanib

Rasburicase

Regorafenib

Thioguanine

Trametinib

Vincristine

PHARMACOGENETIC REPORT FOR ONCOLOGY DRUGS

Table 7: Analysis of pharmacogenetics markers for oncology drugs.

Gene Analysis Results	Interpretation
Cisplatin ERCC1; rs11615 AG XPC; rs2228001 GG	Evidence level : Level 1B,2B The patient has unfavorable genotypes in the analysed XPC and ERCC1 gene variants. Patients with such genotype may have an increased risk of toxicity including hearing loss, neutropenia and nephrotoxicity when treated with Cisplatin (Sakano et al., 2010; Khrunin et al., 2010; Tzvetkov et al., 2011).
Carboplatin ERCC1; rs11615 AG MTHFR; rs1801133 GG	Evidence level : Level 2A,2B The patient has an unfavorable genotype in the analysed ERCC1 gene variant. Patients with this genotype may have an increased risk of nephrotoxicity, when treated with Carboplatin (Patiño-García et al., 2009; Khrunin et al., 2010; Tzvetkov et al., 2011).
Oxaliplatin ERCC1; rs11615 AG	Evidence level : Level 2B The patient has an unfavorable genotype in ERCC1 gene. Patients with such genotype when treated with Oxaliplatin may have an increased risk for nephrotoxicity (Khrunin et al., 2010; Tzvetkov et al., 2011).
Methotrexate ABCB1; rs1045642 AA MTHFR; rs1801133 GG	Evidence level : Level 2A The patient has an unfavorable genotype in the analysed ABCB1 gene variant. Patients with such genotype when treated with Methotrexate, may have an increased concentrations of the drug and an increased risk of toxicity (Suthandiram et al., 2014).
5-Fluorouracil DPYD; *1/*1	Evidence level : Level 1A The patient has a normal metabolizer status for DPYD gene leading to normal DPYD activity. Labelled risk for 5-Fluorouracil toxicity. Use as directed (Fluorouracil FDA Label).
Belinostat UGT1A1; *1/*1	Evidence level : Level 1A The patient has a normal metabolizer status for UGT1A1 gene leading to reference UGT1A1 activity. Such genotype does not affect the clearance of Belinostat. Use as directed (Belinostat FDA Label).

Capecitabine
DPYD; *1/*1

Evidence level : Level 1A

The patient has a normal metabolizer status for DPYD gene leading to normal DPYD activity.
Labelled risk for Capecitabine toxicity. Use as directed (Capecitabine FDA Label).

Dabrafenib
G6PD; wildtype/wildtype

Evidence level : Level 1A

The patient is not a carrier of G6PD deficient genotype.
Patients with such genotype who are treated with Dabrafenib may have a reduced risk of hemolysis (Dabrafenib FDA Label).

Erlotinib
UGT1A1; *1/*1

Evidence level : Level 1A

The patient has a normal metabolizer status for UGT1A1.
Patients with such genotype who are treated with Erlotinib may have an average risk of hyperbilirubinemia. Use as directed (Erlotinib EMA Label).

Gefitinib
CYP2D6; *2/*2

Evidence level : Level 1A

The patient has a normal metabolizer status for CYP2D6.
Patients with such genotype who are treated with Gefitinib may have normal metabolism of Gefitinib. Use as directed (Gefitinib FDA Label).

Gemcitabine
NT5C2; rs11598702 CT

Evidence level : Level 2B

The patient has a favorable genotype in the analysed variant of NT5C2 gene.
Patients with the such genotype may have an increased clearance of Gemcitabine and a decreased risk of toxicity (Mitra et al., 2012).

Irinotecan
UGT1A1; *1/*1

Evidence level : Level 1A

The patient has a normal metabolizer status for UGT1A1.
Patients with such genotype who are treated with Irinotecan -based regimens may have an average risk of neutropenia, diarrhea, or asthenia (Irinotecan FDA Label).

Mercaptopurine
NUDT15; *1/*1
TPMT; *1/*1

Evidence level : Level 1A

The patient is a normal metabolizer for TPMT and NUDT 15 genes.
Patients with such metabolizer status who are treated with Mercaptopurine may have an increased inactivation of Mercaptopurine and a decreased risk of developing severe, life-threatening myelotoxicity. Use as directed. Start with normal starting dose and adjust doses of Mercaptopurine based on disease-specific guidelines (Mercaptopurine FDA Label).

Nilotinib
UGT1A1; *1/*1

Evidence level : Level 1A

The patient has a normal metabolizer status for UGT1A1.
Patients with such genotype who are treated with Nilotinib may have an average risk of hyperbilirubinemia. Use as directed (Nilotinib FDA Label).

Drug / Genotype	Interpretation
Pazopanib UGT1A1; *1/*1	Evidence level : Level 1A The patient has a normal metabolizer status for UGT1A1. Patients with such genotype who are treated with Pazopanib may have an average risk of hyperbilirubinemia. Use as directed (Pazopanib FDA Label).
Rasburicase G6PD; wildtype/wildtype	Evidence level : Level 1A The patient is not a carrier of G6PD deficient genotype. Patients with such genotype who are treated with Rasburicase may have a reduced risk of hemolysis (Rasburicase FDA Label).
Regorafenib UGT1A1; *1/*1	Evidence level : Level 1A The patient has a normal metabolizer status for UGT1A1. Patients with such genotype who are treated with Regorafenib may have an average risk of hyperbilirubinemia. Use as directed (Regorafenib EMA Label).
Tegafur DPYD; *1/*1	Evidence level : Level 1A The patient has a normal metabolizer status for DPYD gene leading to normal DPYD activity. Labelled risk for Tegafur toxicity. Use as directed (Fluorouracil FDA Label).
Thioguanine NUDT15; *1/*1 TPMT; *1/*1	Evidence level : Level 1A The patient is a normal metabolizer for TPMT and NUDT 15 genes. Patients with such metabolizer status who are treated with Thioguanine may have an increased inactivation of Thioguanine and a decreased risk of developing severe, life-threatening myelotoxicity. Use as directed. Start with normal starting dose and adjust doses of Thioguanine based on disease-specific guidelines. Allow 2 weeks to reach steady state after each dose adjustment (Thioguanine FDA Label).
Trametinib G6PD; wildtype/wildtype	Evidence level : Level 1A The patient is not a carrier of G6PD deficient genotype. Patients with such genotype who are treated with Trametinib may have a reduced risk of hemolysis (Trametinib FDA Label).
Vincristine CEP72; rs924607 CC	Evidence level : Level 2B The patient has a favorable genotype in the analysed variant of CEP72 gene. Patients with such genotypes who are treated with Vincristine may have a decreased, but not absent, risk of peripheral nervous system diseases (Diouf et al., 2015).

*Please refer to criteria of classification for pharmacogenetic analysis on pg. 35

CIRCULATING TUMOR CELL DETECTION

Circulating Tumor Cells (CTCs): **DETECTED**

No of CTCs: **02 CTCs/** ml peripheral blood

CTCs are defined as CK+ve, EPCAM+ve, CD45-ve cells

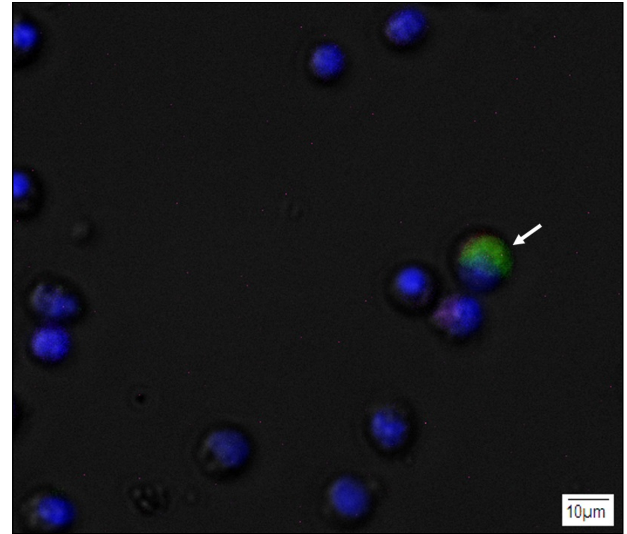


Figure 2: Fluorescent microscopic image of CTC (arrow indicating 01 of 02 CTCs)

INTERPRETATION

02 CTCs/ ml peripheral blood detected in the submitted sample.

RECOMMENDATION

Cell Alert™ may be done every three months to monitor disease status.

CORREKT-CHEMO ANALYSIS

Chemosensitivity assay performed on circulating tumor cells (CTCs) indicates the effectiveness of chemotherapeutic drugs in descending order of efficacy for the patient's cancer cells. Tumor marker staining for Napsin A showed more than 40% positivity on cultured CTCs. Depending on viable tumor cell availability, single/combinations of drugs mentioned below were tested.

% Cell Death	>= 25%	< 25%
Response Level$	Response	No response

RESPONSE TO CYTOTOXIC DRUG/DRUG COMBINATIONS

Sr.No.	Drug/Drugs Combination	% Cell Death	Recommendation$
1	5-Fluorouracil	90	Response
2	Vinblastine	82	Response
3	Cisplatin	80	Response
4	Carboplatin	79	Response
5	Mitomycin	74	Response
6	Dacarbazine	63	Response
7	Vinorelbine	57	Response
8	Etoposide	57	Response
9	Paclitaxel	55	Response
10	Irinotecan	52	Response
11	Gemcitabine	50	Response
12	Pemetrexed	48	Response
13	Doxorubicin	37	Response

Drugs showing no response (<25% cell death)

Drug	% Cell Death	Drug	% Cell Death
Cyclophosphamide	No response	Decitabine	No response
Docetaxel	No response	Epirubicin	No response
Methotrexate	No response	Temozolomide	No response
Topotecan	No response		

Drugs Already Administered to Patient

Sr.No.	Drug/Drugs Combination	% Cell Death
1	Pemetrexed + Carboplatin	Not tested
2	Carboplatin	79
3	Pemetrexed	48

*Please refer to the list of drugs tested for correkt chemo on pg. 41

EXOSOMAL micro-RNAs

Table 8: Analysis of exosomal microRNAs detected in the submitted sample.

Markers	Result	Interpretation
miR-134-5p	▼ -7.16	miR-134 functions as a potent tumor suppressor in non-small cell lung cancer (NSCLC) and plays a role in epithelial to mesenchymal transition. Therefore, its downregulation is suggestive of an adverse prognosis (Li et al, 2012).
let-7c-5p	▼ -7.16	Reduced expression of let-7 correlates with an adverse outcome in lung cancers (Takamizawa et al, 2004).
miR-146a-5p	▼ -4.64	miR-146a is downregulated in non-small cell lung cancer tissues and low expression is correlated with advanced clinical TNM stages and distant metastasis in lung cancer (Chen et al., 2013).
miR-140-3p	▼ -4.32	Downregulation of miR-140-3p is significantly associated with lymphatic invasion, distant metastasis, TNM stage, tumor grade and is potential prognostic biomarker of NSCLC (Qu et al., 2016). Therefore it is an indicative of adverse prognosis.
miR-195-5p	▼ -3.88	miR-195 has been reported to function as a tumor suppressor in various cancers, including non-small cell lung cancer (NSCLC). The expression of miR-195 is lower in tumors than in adjacent normal tissues, which is associated with poor survival in both lung adenocarcinoma and squamous cell carcinoma patients (Yu et al, 2018).
miR-126-5p	▼ -2.55	Downregulation of miR-126-5p is associated with angiogenesis, cell proliferation, invasion and metastasis in lung cancer, thus indicating an adverse prognosis (Li et al., 2017).
miR-30a-3p	▼ -2.79	Low level of miR-30a is negatively correlated to tumor size, lymphatic metastasis, clinical TNM stage, pathological grading, histological classification and shorter survival time (Tang et al., 2015; Luan et al., 2018). Therefore, its downregulation is suggestive of an adverse prognosis.
miR-142-5p	▲ +3.07	miR-142-5p is reported to induce cancer stem cell-like properties through PD-L1 expression via the PTEN pathway (Wan et al., 2018). Therefore its upregulation is indicative of an adverse prognosis.
miR-145-5p	▲ +2.98	Overexpression of miR-145 is reported to inhibit human non-small cell lung cancer growth by dual-targeting RIOK2 and NOB1 (Liu et al., 2018).

miR-29c-5p	▲ +2.99	MiR-29c is a tumor suppressor gene found to suppress tumors in NSCLC patients and its low levels correlated with shorter relapse-free survival of patients treated with radiotherapy (Arechaga- Ocampo et al., 2017). Therefore, its upregulation is suggestive of favorable prognosis.

VARIANT ALLELE FRACTION AND COVERAGE (CELL FREE NUCLEIC ACIDS)

Variant (Transcript ID)	Genomic co-ordinates	Allele fraction	Coverage (X)
SYNE1 (NM_182961.3) c.3838-1G>A,	chr6:152763381C>T	1.3	3036
TP53 (NM_000546.5) c.578A>T, p.H193L	chr17:7578271T>A	2.2	5963
EGFR (NM_005228.3) c.2573T>G, p.L858R	chr7:55259515T>G	10.2	4741
APC (NM_000038.5) c.220+2_220+3insTA, p.L858R	chr5:112102110InsTA	1.2	2239
ADAMTS20 (NM_025003.3) c.3130C>T, p.R1044W	chr12:43825266G>A	1.0	9795

Due to minimum coverage or no sequence, the presence or absence of variants contained within the target regions listed below could not be meaningfully assessed.

MAP2K2 [NM_030662], Exon 6, Codons 194-221; CTNNB1 [NM_001904], Exon 3, Codons 5-46; PIK3CA [NM_006218], Exon 8, Codons 418-422; KIT [NM_000222], Exon 14, Codons 670-697; NRAS [NM_002524], Exon 3, Codons 38-63

CRITERIA FOR CLASSIFICATION OF SOMATIC VARIANTS

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.

CRITERIA OF CLASSIFICATION FOR PHARMACOGENETIC ANALYSIS

Each variant-drug combination can be graded based on the measure of confidence in the association and the strength of prescribing recommendation.

- **Level 1:** Evidence based on pharmacogenetics guidelines or well-established association studies
- **Level 2:** Evidence of moderate variant-drug association from studies.
- **Level 3:** Evidence suggests no consensus among different studies.

DRUG METABOLIZER STATUS CATEGORIES

Based on the different combination of haplotypes an individual inherits in each drug metabolizing gene, a drug metabolizer status can be predicted. There are 4 different drug metabolizer status types:

- **Poor Metabolizers (also called "PM"),** Poor metabolizers have two non-functional alleles and therefore have little to no enzyme activity.
- **Intermediate Metabolizers (also called "IM"),** Intermediate metabolizers have one non-functional allele and one normally functioning allele, and therefore have decreased enzyme activity.
- **Normal Metabolizers (also called "NM")** Normal metabolizers have 2 normally functioning alleles and therefore have normal enzyme activity.
- **Ultra-Rapid Metabolizers (also called "UM").** Ultra-rapid metabolizers have one or more alleles which result in increased enzyme activity compared to extensive metabolizers.

The impact of each metabolizer type on medication response depends on the role of the enzyme in the metabolism of the specific drug in question. For example, for a drug that is inactivated by the enzyme, an ultra-rapid metabolizer may need a higher dose of the drug to reach a therapeutic range while for another drug, that is activated by the enzyme; ultra-rapid metabolizer status may be associated with increased exposure to the drug and therefore an increased risk of adverse drug reactions.

METHODS AND LIMITATIONS

Cell free nucleic acid (cfDNA) was analyzed for mutation detection analysis using Ion Proton sequencer. cfDNA extracted from the plasma of submitted specimen was subjected to target enrichment by multiplex PCR amplification using panel targeting 409 Oncogenes and Tumor suppressor genes. (see gene list in the 'Biomarkers analysed section'). Enriched DNA sequences were ligated with platform specific adaptor molecules and was sequenced on using semiconductor P1 chip. The minimum average depth was 1000x for gene panel analyzed. High quality sequencing data (proportion Q20 bases ≥75%) was analyzed using a customized in-house pipeline DCGL NGS Bioinformatics Pipeline v 7.4 designed to accurately detect the rare somatic variants.

Cell free nucleic acids (cfDNA) were analyzed for mutation detection using Ion Proton sequencer. cfDNA extracted from the plasma of submitted specimen was subjected to target enrichment by multiplex PCR amplification using panel of genes (see gene list in the 'Biomarkers analysed section'). Enriched DNA sequences were ligated with platform specific adaptor molecules and was sequenced on using semiconductor P1 chip. The minimum average depth was 10000x for gene panel analyzed. High quality sequencing data (proportion Q20 bases ≥75%) was analyzed using a customized in-house pipeline DCGL NGS Bioinformatics Pipeline v11.2 designed to accurately detect the rare somatic variants.

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 93.75% and specificity is 97.14% for SNV, CNV and Fusion.

Pathogenic/likely pathogenic mutation > 40% allele frequency if detected in the sample is confirmed by Sanger sequencing platform and SeqScape® Software ver 3.0.

Shot gun sequencing of cell free nucleic acid (cfDNA) was performed and analyzed for Copy Number Variation using semiconductor based Next Generation Sequencing technology. cfDNA was extracted from the submitted specimen and subjected to target enrichment by multiplex PCR amplification using fragment library panel. Enriched DNA sequences were ligated with platform specific adaptor molecules and was sequenced on using semiconductor P1 chip. High quality sequencing data (proportion Q20 bases ≥75%) was analyzed using Ion Reporter™ Software V5.10 by inbuilt Low-pass whole-genome aneuploidy workflow. For Input data, a BAM file was generated on torrent server was uploaded to Ion Reporter server for Low-pass whole-genome aneuploidy workflow which detects aneuploidies and large chromosome abnormalities from a single whole-genome sample with low coverage (minimum 0.01x). Normalization is done using an informatics baseline generated from multiple normal samples.

Blood was analyzed for mRNA expression detection using semiconductor based Next Generation Sequencing method. High quality Exosomal RNA was extracted from the submitted specimens was subjected to mRNA library preparation using a targeted panel. RNA sequencing was performed to achieve at least 4 million mappable high-quality reads for the paired analysis. Sequence reads were aligned to the hg19 transcriptome reference sequence in Torrent Suite Software using the Ion Torrent Mapping Alignment Program. Differential Gene Expression analysis was performed using a customized in-house pipeline DCGL NGS Bioinformatics Pipeline v 5.4 designed to detect the Significantly expressed genes.

Blood was analyzed for miRNA expression detection. The OpenArray® system (ABI, USA) was used for profiling of >700 human miRNA and data analyzed by Expression Suite Software (v1.0.4).

EDTA blood was analysed for mutation detection using semiconductor based Next Generation Sequencing technology. High quality genomic DNA was extracted from the submitted specimen and subjected to target enrichment by high multiplex PCR amplification using panel targeting mutation of genes mentioned above. Enriched DNA sequences were ligated with platform specific adaptor molecules and was sequenced on using semiconductor chip. The minimum average wide depth of coverage of panel covering above listed genes was 1000X. High quality sequencing data (proportion of Q20 bases≥75%) using DCGL NGS Bioinformatics Pipeline v 2.5.

Pathogenic/likely pathogenic mutation if detected in the sample is confirmed by gold standard Sanger Sequencing method. Sanger sequencing data is analyzed using SeqScape® Software ver 3.0.

Blood was analyzed for genotyping using Ion Proton semiconductor sequencer (Life Technology, USA). High quality genomic DNA was extracted from the submitted specimen and subjected to target enrichment by high multiplex PCR amplification using panel targeting variants of genes. Enriched DNA sequences were ligated with platform specific adaptor molecules and was sequenced on using semiconductor P1 chip. The minimum average depth was 500x for gene panel analyzed. High quality sequencing data (proportion of Q20 bases ≥75%) was analyzed using DCGL NGS Bioinformatics Pipeline v14.2. This test does not detect polymorphisms other than those listed. Drug metabolism may be affected by non-genetic factors. DNA testing does not replace the need for clinical and therapeutic drug monitoring.

The performance of the assay specific reagents used in this assay has been established and its performance characteristics defined by Datar Cancer Genetics Limited. This test may not detect all variants in non-coding regions that could affect copy number changes encompassing all or a large portion of the gene. Tumor mutation analysis panel testing is limited in detecting the following types of mutations (this might not be exhaustive): large rearrangements and deletion/ duplications, epigenetic factors, mutations in repetitive or high GC rich regions and mutations in gene with corresponding pseudo genes or other highly homologous sequences. Presence of PCR inhibitors in the sample may prevent DNA amplification for mutation analysis. Rare and novel mutations may be clinically uncharacterized.

Also note that the current knowledge on the genetic of the disease or pathogenic disorder or on the inheritance of the genes may be incomplete. If the test identifies the genetic cause of the disorder, it is possible that this knowledge may or may not help with the prognosis and management of the disease.

A negative test result does not exclude the possibility of mutations not being present in the test sample probably due to the representation of reads representing minor allele fraction is below the detectable limit of the assay.

The clinical sensitivity of most assays for detection of mutant cfDNA is limited as compared with tumor tissue-based testing. This may result from a high ratio of normal to tumor DNA or excess degradation of cfDNA or may simply reflect the biologic heterogeneity of solid tumors, some of which may shed abundant nucleic acid into the circulation and others that may not. Tumor type, size, disease stage, sites of metastasis, histologic grade, or other features may also affect levels, however, much remains to be elucidated.

Analytical validation of this test for 452 gene panel has shown sensitivity of 96.43% and specificity of 100%.

For CTCs detection:

CTCs from the submitted peripheral blood were labelled with EPCAM, Cytokeratin and CD45 antibodies and analyzed by Fluorescent microscopy. The performance of the assay specific reagents in this assay has been established by Datar Cancer Genetics Ltd.

CTCs were isolated from submitted peripheral blood sample. The live cancer cells were tested against multiple chemotherapy agents or a combination of chemotherapy agents. The number of drugs selected for testing, either singly or in combination, depending on the number of CTCs detected/cultured.

A defined number of cells were incubated with different drugs, singly as well as in combination with respective drug concentrations and cell death events were measured. The extent of cell death was determined either using Varioskan LUX platform or by fluorescence-based staining of live/dead cells. The Kinetic Units (KU Value) or percent cell death was calculated respectively to evaluate the response level of the drug. Appropriate positive and negative controls were tested and evaluated in a similar manner simultaneously with the test sample.

This test was developed, and its performance characteristics determined by Datar Cancer Genetics Limited. It has not been cleared or approved by the US FDA.

The Patient Analysis raw data may be shared on written request by the individual patient.

GENES ANALYZED

ABL1, ABL2, ACVR2A, ADAMTS20, AFF1, AFF3, AKAP9, AKT1, AKT2, AKT3, ALK, APC, AR, ARAF, ARID1A, ARID2, ARNT, ASXL1, ATF1, ATM, ATR, ATRX, AURKA, AURKB, AURKC, AXL, BAI3, BAP1, BCL10, BCL11A, BCL11B, BCL2, BCL2L1, BCL2L2, BCL3, BCL6, BCL9, BCR, BIRC2, BIRC3, BIRC5, BLM, BLNK, BMPR1A, BRAF, BRD3, BRIP1, BTK, BUB1B, CARD11, CASC5, CBL, CCND1, CCND2, CCND3, CCNE1, CD79A, CD79B, CDC73, CDH1, CDH11, CDH2, CDH20, CDH5, CDK12, CDK4, CDK6, CDK8, CDKN2A, CDKN2B, CDKN2C, CEBPA, CHEK1, CHEK2, CIC, CKS1B, CMPK1, COL1A1, CRBN, CREB1, CREBBP, CRKL, CRTC1, CSF1R, CSMD3, CTNNA1, CTNNB1, CYLD, CYP2C19, CYP2D6, DAXX, DCC, DDB2, DDIT3, DDR2, DEK, DICER1, DNMT3A, DPYD, DST, EGFR, EML4, EP300, EP400, EPHA3, EPHA7, EPHB1, EPHB4, EPHB6, ERBB2, ERBB3, ERBB4, ERCC1, ERCC2, ERCC3, ERCC4, ERCC5, ERG, ESR1, ETS1, ETV1, ETV4, EXT1, EXT2, EZH2, FAM123B, FANCA, FANCC, FANCD2, FANCF, FANCG, FAS, FBXW7, FGFR1, FGFR2, FGFR3, FGFR4, FH, FLCN, FLI1, FLT1, FLT3, FLT4, FN1, FOXL2, FOXO1, FOXO3, FOXP1, FOXP4, FZR1, G6PD, GATA1, GATA2, GATA3, GDNF, GNA11, GNAQ, GNAS, GPR124, GRM8, GUCY1A2, HCAR1, HIF1A, HLF, HNF1A, HOOK3, HRAS, HSP90AA1, HSP90AB1, ICK, IDH1, IDH2, IGF1R, IGF2, IGF2R, IKBKB, IKBKE, IKZF1, IL2, IL21R, IL6ST, IL7R, ING4, IRF4, IRS2, ITGA10, ITGA9, ITGB2, ITGB3, JAK1, JAK2, JAK3, JUN, KAT6A, KAT6B, KDM5C, KDM6A, KDR, KEAP1, KIT, KLF6, KRAS, LAMP1, LCK, LIFR, LPHN3, LPP, LRP1B, LTF, LTK, MAF, MAFB, MAGEA1, MAGI1, MALT1, MAML2, MAP2K1, MAP2K2, MAP2K4, MAP3K7, MAPK1, MAPK8, MARK1, MARK4, MBD1, MCL1, MDM2, MDM4, MEN1, MET, MITF, MLH1, MLL, MLL2, MLL3, MLLT10, MMP2, MN1, MPL, MRE11A, MSH2, MSH6, MTOR, MTR, MTRR, MUC1, MUTYH, MYB, MYC, MYCL1, MYCN, MYD88, MYH11, MYH9, NBN, NCOA1, NCOA2, NCOA4, NF1, NF2, NFE2L2, NFKB1, NFKB2, NIN, NKX2-1, NLRP1, NOTCH1, NOTCH2, NOTCH4, NPM1, NRAS, NSD1, NTRK1, NTRK3, NUMA1, NUP214, NUP98, PAK3, PALB2, PARP1, PAX3, PAX5, PAX7, PAX8, PBRM1, PBX1, PDE4DIP, PDGFB, PDGFRA, PDGFRB, PER1, PGAP3, PHOX2B, PIK3C2B, PIK3CA, PIK3CB, PIK3CD, PIK3CG, PIK3R1, PIK3R2, PIM1, PKHD1, PLAG1, PLCG1, PLEKHG5, PML, PMS1, PMS2, POT1, POU5F1, PPARG, PPP2R1A, PRDM1, PRKAR1A, PRKDC, PSIP1, PTCH1, PTEN, PTGS2, PTPN11, PTPRD, PTPRT, RAD50, RAF1, RALGDS, RARA, RB1, RECQL4, REL, RET, RHOH, RNASEL, RNF2, RNF213, ROS1, RPS6KA2, RRM1, RUNX1, RUNX1T1, SAMD9, SBDS, SDHA, SDHB, SDHC, SDHD, SEPT9, SETD2, SF3B1, SGK1, SH2D1A, SMAD2, SMAD4, SMARCA4, SMARCB1, SMO, SMUG1, SOCS1, SOX11, SOX2, SRC, SSX1, STK11, STK36, SUFU, SYK, SYNE1, TAF1, TAF1L, TAL1, TBX22, TCF12, TCF3, TCF7L1, TCF7L2, TCL1A, TET1, TET2, TFE3, TGFBR2, TGM7, THBS1, TIMP3, TLR4, TLX1, TNFAIP3, TNFRSF14, TNK2, TOP1, TP53, TPR, TRIM24, TRIM33, TRIP11, TRRAP, TSC1, TSC2, TSHR, UBR5, UGT1A1, USP9X, VHL, WAS, WHSC1, WRN, WT1, XPA, XPC, XPO1, XRCC2, ZNF384, ZNF521.

GENES ANALYZED FOR CELL FREE NUCLEIC ACIDS ANALYSIS

SNV Genes: AKT1, ALK, APC, AR, ARAF, BRAF, CHEK2, CTNNB1, DDR2, EGFR, ERBB2, ERBB3, ESR1, FBXW7, FGFR1, FGFR2, FGFR3, FGFR4, FLT3, GNA11, NAQ, GNAS, HRAS, IDH1, IDH2, KIT, KRAS, MAP2K1, MAP2K2, MET, MTOR, NRAS, NTRK1, NTRK3, PDGFRA, PIK3CA, PTEN, RAF1, RET, ROS1, SF3B1, SMAD4, SMO, TP53.
Fusion Genes: ALK, BRAF, ERG, ETV1, FGFR1, FGFR2, FGFR3, MET, NTRK1, NTRK3, RET, ROS1.
CNA Genes: CCND1, CCND2, CCND3, CDK4, CDK6, EGFR, ERBB2, FGFR1, FGFR2, FGFR3, MET, MYC.

EXOSOMAL GENE EXPRESSION ANALYSIS

Exosomal RNA: 20805 mRNA
756 miRNA

BIOMARKERS ANALYZED FOR MICROSATELLITE INSTABILITY (MSI)

MLH1, MSH2, MSH6, PMS1 and PMS2

GENES ANALYZED FOR PHARMACOGENETICS

GENES	VARIANTS ANALYZED
ABCB1	c.3435T>C
CEP72	n.366+1469G>A
CYP2D6	*2,*3,*4,*5,*6,*7,*8,*9,*10,*11,*12,*15,*17,*19,*20,*29,*35,*38,*41,*42,*44,*56 and XN
DPYD	*2A,*2B,*3,*4,*5,*6,*7,*8,*9A,*9B,*10,*11,*12,*13
ERCC1	c.354T>C,1516C>A
G6PD	c.95A>G, c.103_105delATC, c.131C>G,c.143T>C, c.172G>A, c.202G>A, c.208T>C, c.209A>G, c.241C>T, c.242G>A, c.317C>G, c.337G>A, c.376A>G, c.383T>G/C, c.466G>A, c.493A>G, c.517T>C, c.542A>T, c.544C>T, c.563C>T, c.592C>T, c.593G>A/C, c.634A>G, c.637G>T, c.680G>A/T, c.703C>T, c.806G>A, c.844G>T/C, c.871G>A, c.949G>A, c.964T>C, c.968T>C, c.1003G>A, c.1024C>T, c.1037A>T, c.1048G>C, c.1057C>T, c.1082C>T, c.1089C>G/A, c.1132G>A, c.1153T>C, c.1156A>G, c.1159C>T, c.1160G>A, c.1178G>A, c.1180G>C, c.1192G>A, c.1228G>T, c.1229G>A/C, c.1291G>A, c.1311C>T, c.1316G>C, c.929G>A
MTHFR	c.665C>T, 1286A>C
NT5C2	c.175+1178A>G
NUDT15	*2,*3,*4,*5,*6
TPMT	*2, *3A, *3B, *3C, *4, *5, *6, *7, *8, *9, *10, *11, *12, *13, *14, *15, *16, *20, *21, *23, *24, *25, *26, *29, *31, *32, *33, *34, *37
UGT1A1	*28
XPC	c.2815C>A

DRUGS TESTED IN CORREKT CHEMO ANALYSIS

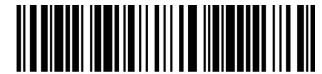
5-Fluorouracil, Carboplatin, Cisplatin, Cyclophosphamide, Dacarbazine, Decitabine, Docetaxel, Doxorubicin, Epirubicin, Etoposide, Gemcitabine, Irinotecan, Methotrexate, Mitomycin, Paclitaxel, Pemetrexed, Temozolomide,, Topotecan, Vinblastine, Vinorelbine.

DISCLAIMER

This report documents the genetic alterations detected in the submitted sample material. Information in this report is provided for information purpose only and should only be considered in conjunction with all other relevant information regarding a particular patient, before the patient's treating physician recommends a course of treatment.

Decisions on patient care and treatment must be based on the independent medical judgment of the treating physicians, taking into consideration all applicable information concerning the patient's condition, such as personal and family history, physician's examination, information from other diagnostic test and patient references, in accordance with the standard of care in a given community. A treating physician's decisions should not be based on a single test or on the information contained in this report.

This information in this report does not constitute a treatment recommendation by Datar Cancer Genetics Limited, either to use or not to use any specific therapeutic agent, and should not be interpreted as treatment advice. Decisions on patient care and treatment rest solely within the discretion of the patient's treating physician.



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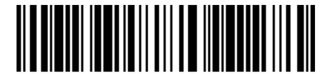

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****End of Report****

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