Nordic Laboratories

PATIENT: **Sample Report**			TEST REF: ##########
TEST NUMBER: ##########	COLLECTED: #########		PRACTITIONER: **Nordic Laboratories ApS**
GENDER: ####	RECEIVED: #########		
AGE: ##	TESTED: #########		

TEST NAME: Cancertrack™ Monitoring (ctDNA and CTC)

(No relevant clinical trials)

Report Highlights

Indications	USFDA Approved/ NCCN Recommended	Off Label Therapy
No variants detected		

CTC: Circulating Tumor Cells; NCCN: National Comprehensive Cancer Network - Esophageal and Esophagogastric Junction Cancers.

Longitudinal Monitoring Biomarkers

Biomarkers	Result	Biomarkers	Result
Highest mutant allele frequency (HMAF)	0%	Number of CTCs detected	2 CTCs /ml

Disease Relevant Findings

Biomarkers	Result	Biomarkers	Result
BRAF	No mutations detected	ERBB2/HER2	No alterations detected
RET	No fusions detected	NTRK1/3	No fusions detected

Summary of other Genomic Alterations

Gene	Alteration Type (SNAs / Indels / CNAs/ Fusion)	Variant Classification	Therapeutic / Clinical Significance
None			

SNA: Single Nucleotide Alteration; CNA: Copy Number Alteration; INDELS: Insertion / Deletion

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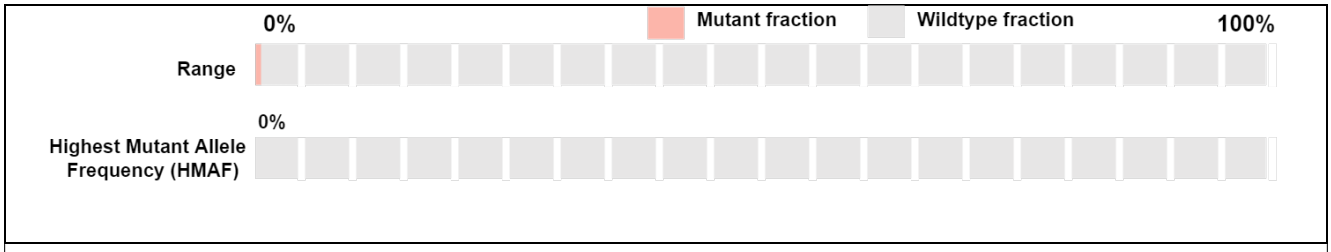
TEST NAME: Cancertrack™ Monitoring (ctDNA and CTC)

Cell Free Nucleic Acids : Somatic Genome Alterations

Genomic Findings

Highest Mutant Allele Frequency



No mutations were detected in the cell free nucleic acids isolated from patient's plasma.

Genomic Findings

Single Nucleotide Alterations / Indels / Copy Number Alterations / Fusion

Markers (Transcript ID)	Variant	Category
No variants detected		

Circulating Tumor Cells Enumeration

CTCs

Circulating tumor cells (CTCs): **DETECTED**

No. of CTCs: **2 CTCs / ml peripheral blood**

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

Interpretation

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

Recommendation

Circulating tumor cell enumeration may be performed every 8 to 12 weeks to monitor disease status in consultation with the treating physician.

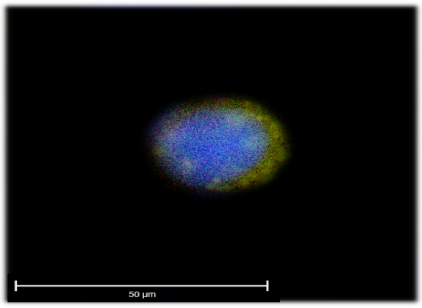


Fig 1: Fluorescent microscopic image of CTC

Cell Free Nucleic Acids Analysis

Variant Allele Fraction And Coverage

Variant (Transcript ID)	Genomic co-ordinates	Allele fraction	Coverage (X)
None			

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

Criteria For Classification of Somatic Variants

Analysis 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).

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- **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.

Genes Analyzed

Gene List

SNV Genes:

AKT1	ALK	APC	AR	ARAF	BRAF	CHEK2	CTNNB1	DDR2	EGFR
ERBB2	ERBB3	ESR1	FBXW7	FGFR1	FGFR2	FGFR3	FGFR4	FLT3	GNA11
GNAQ	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								

CNV Genes:

CCND1	CCND2	CCND3	CDK4	CDK6	EGFR	ERBB2	FGFR1	FGFR2	FGFR3
MET	MYC								

Methods and Limitations

Methods

Cell free nucleic acids analysis:

Cell free nucleic acids were analyzed for mutation and fusion detection using semiconductor based Next Generation Sequencing technology. Cell free nucleic acids 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 'Genes analysed section'). Enriched DNA sequences were ligated with platform specific adaptor molecules and were sequenced on using semiconductor chip. The minimum average depth was 17000x for gene panel analyzed. High quality sequencing data (proportion Q20 bases ≥ 75%) was analyzed using a customized in-house pipeline DCGL NGS Bioinformatics Pipeline vS11.12 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 97.06% and specificity is 100% for SNV, CNV and Fusion.

A negative test result does not exclude the possibility of mutations being present in the test sample probably due to the reads representing minor allele fraction is below the detectable limit of the assay or other limiting technical / analytical factors.

The clinical sensitivity of most assays for detection of mutant cell free nucleic acids 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 cell free nucleic acids 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.

CTCs enumeration:

Enriched CTCs from the submitted peripheral blood were labelled with EPCAM, Cytokeratin and CD45 antibodies and analyzed by High content imaging platform.
Analytical Validation of this assay shown sensitivity of 99.99% and specificity 99.99%

About CTCs

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TEST NAME: Cancertrack™ Monitoring (ctDNA and CTC)

CTC detection is a promising prognostic tool in both primary and metastatic setting.

CTCs are rare cells in a background of 10^6 -10^7 nucleated blood cells.

Evaluation of CTCs at any time during the course of therapy allows assessment of patient prognosis and is predictive of progression-free survival and overall survival. Circulating tumor cells (CTCs) in the blood stream play a critical role in establishing metastasis.

As an adjunct to standard monitoring methods, monitoring patients with the circulating tumor cell test can help to assess patient's status based on real-time prediction. Enumeration of the number of circulating tumor cells (CTCs) before and during treatment helps predicting response to chemotherapy. Throughout therapy, CTC testing can be used to monitor a patient's status to understand response to the given therapy is favorable or unfavorable at any given time.

Circulating tumor cell test results should be used in conjunction with a clinical information derived from other diagnostic tests, physical examination and complete medical history, in consultation with treating oncologist.

This test does not detect variants in gene other than tested. Cancertrack is limited in detecting the epigenetic factors, mutations in repetitive or high GC rich regions. Rare and novel mutations may be clinically uncharacterized.

Important Information for Patients

This test is a Laboratory Developed Test, and its performance characteristics were determined by Datar Cancer Genetics UK Private Limited, United Kingdom. It has not been cleared or approved by the U.S. Food and Drug Administration.
This facility is certified by the College of American Pathologists (CAP) and under the Clinical Laboratory Improvement Amendments (CLIA)-USA as qualified to perform high complexity clinical laboratory testing.

Disclaimer

The aberrant / absent/ downregulated expression of cell surface or intracellular markers used for CTCs detection can give rise to ambiguous test results. Cells with EPCAM/Cytokeratin down regulation or absent expression will not be detected with this test.

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 patients 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.

The information in this report does not constitute a treatment recommendation by Datar Cancer Genetics, 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.

References

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4 Ried K, Eng P, Sali A. Screening for circulating tumour cells allows early detection of cancer and monitoring of treatment effectiveness: an observational study. Asian Pacific journal of cancer prevention: APJCP. 2017;18(8):2275.

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End of Report

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