PATIENT INFORMATION		REFERRAL INFORMATION	
NAME **John Doe**		CLINIC NAME **Clinic1**	
Gender **Male**		CLINIC ID **123456789**	
DATE OF BIRTH **DD-MM-YYYY**		REFERRING CLINICIAN **Clinician1**	
TEST INDICATIONS **Colorectal cancer**		CLINIC EMAIL **Clinician1@clinic1.com**	
SAMPLE INFORMATION			
ORDER NUMBER **XXXXXX**	LAB NUMBER **XXXXXX**	DATE OF COLLECTION **DD-MM-YYYY**	DATE RECEIVED **DD-MM-YYYY**

CANCER TEST SELECTED

☐ NeoThetis NSCLC ☒ NeoThetis Colorectal

TEST RESULTS

POSITIVE
At least 1 clinically significant variant identified

RESULT SUMMARY

BIOMARKER FINDINGS

MSI-H
☒ **DETECTED** ☐ **NOT-DETECTED**

GENOMIC FINDINGS

Gene	Variant Detected	Allele Fraction	FDA/EMA Approved Therapies (In patient's indication)*	FDA/EMA Approved Therapies (In other indications)**	Clinical Trials
KRAS	c.35G>A (p.G12D)	25%	✗ NCCN Panitumumab ✗ NCCN Cetuximab	None	56
TP53	c.646G>A (p.V216M)	15%	None	Venetoclax	5

✓ Approved in indication ✗ Contra-indication NCCN Included in NCCN guidelines

*List of FDA and/or EMA approved drugs in the patient's cancer type

**List of FDA and/or EMA approved drugs in other tumor types. Therapies that are included in the NCCN guidelines for the patient's cancer type are clearly indicated above.

Note: Clinical trials listed in this report are retrieved from clinicaltrials.gov[1] and only include not yet recruiting and recruiting trials for the indicated cancer type and gene. The list of therapies and clinical trials included in this report may not be complete and/or exhaustive. Therapies contained in this report are FDA/EMA approved, however information on drug approvals for different indications is updated regularly, based on new evidence, and may not reflect the current status at any time. This report should not be used as the sole basis when making treatment decisions, instead it should be regarded as a supplementary source of information for guiding therapy decisions. All treatment decisions remain the full and final responsibility of the treating clinician.

INTERPRETATIONS

MSI-H: Detected
Microsatellite instability has been detected in the patient's sample and the tumor has been classified as MSI-H.

Overview
The diagnostic hallmark of microsatellite instability (MSI) is defined as the gain or loss of nucleotides from repetitive DNA sequences, known as microsatellite tracts, resulting in novel alleles of varying lengths[1]. This genomic hypermutability can arise from a defective mismatch repair system (dMMR), limiting the identification and correction of spontaneous mutations in microsatellite tracts[2,3,4].

Clinical significance
MSI is most commonly observed in colorectal, endometrial, and gastric cancers, but has also been reported in other solid tumors albeit at lower rates[2,5,6]. MSI testing has been recommended by both the NCCN and ESMO guidelines in multiple cancer types, including colorectal cancer[6,7]. MSI has been shown to offer a good prognostic value, with MSI-H Stage II colorectal patients having a better prognosis and not requiring adjuvant chemotherapy[8]. Importantly, MSI can predict response to immune checkpoint inhibitors, as indicated by FDA/EMA approvals, most notably the FDA site-agnostic approval of pembrolizumab for MSI-H tumours[9].

Approved therapies and clinical trials
The FDA and EMA have approved **pembrolizumab** as first line treatment for metastatic MSI-H colorectal cancer. Additionally, the FDA has approved **nivolumab** (+/- ipilimumab) for metastatic MSI-H colorectal cancer.

KRAS c.35G>A (p.Gly12Asp)

Variant details
The KRAS c.35G>A (p.Gly12Asp) is classified as a Tier 1 variant with strong clinical significance in colorectal cancer[10]. This variant is a missense substitution on exon 2 of the KRAS gene (NM_ 033360.4). This nucleotide substitution results in an amino acid change from glycine to aspartic acid at position 12 of the protein sequence. The KRAS Gly12Asp is an activating mutation previously described in various cancer types including colorectal, pancreatic, lung, bile duct and ovarian cancer and submitted in the COSMIC database (COSV55497369)[11] and the ICGC database (MU37643)[12].

Gene information and significance
The KRAS gene encodes for the K-RAS protein, a member of the RAS protein family and is part of the RAS/MAPK pathway. The K-RAS protein is a GTPase that converts GTP onto GDP. The activating GTP-bound form of the KRAS protein relays the signal to the cell's nucleus to instruct proliferation or differentiation[13]. Activating KRAS mutations result in unregulated signaling through the MAP/ERK pathway. The KRAS gene is an oncogene that is one of the most frequently mutated in colorectal cancer with 34% of cases studies (23948 mutated/70961 total colorectal adenocarcinoma cases submitted in the COSMIC database)[11].

While highly recurrent in various cancer types, attempts to target KRAS mutations with inhibitors have not yet been successful or used in clinical practice. Mutations in the KRAS gene may indicate poor prognosis and poor drug response with therapies targeted to EGFR and resistance to cetuximab and panitumumab EGFR antagonist therapies[14]. The NCCN guidelines for colorectal cancer contain recommendations that the targeted therapies cetuximab and panitumumab should only be used in the context of wild type KRAS[15].

Approved therapies and Clinical trials available
Several clinical trials are currently recruiting patients with colorectal cancer related to KRAS status. A phase 1/2 clinical trial is currently investigating the administration of blood lymphocytes with a murine T-cell receptor recognizing the G12V mutation in RAS positive patients (NCT03190941). A different phase 1 study is investigating a mutated pooled KRAS-targeted long peptide in combination with nivolumab and Ipilimumab for patients with resected MMR positive colorectal and pancreatic cancer (NCT04117087). For more information on the abovementioned and other clinical trials regarding KRAS mutated colon cancer please visit clinicaltrials.gov[1].

TP53 c.646G>A (p.Val216Met)

Variant details

The TP53 c.646G>A variant is classified as a Tier2 variant of potential clinical significance in colorectal cancer[14]. This variant is a missense substitution on exon 6 of the TP53 gene (ENST00000269305.4) which results in a valine to methionine substitution at position 216 of the amino acid sequence. This variant occurs in the DNA binding region of TP53 in a region that also plays a critical role in the interaction with other proteins including HIPK1, CCAR2, ZNF385A and AXIN1[16]. This variant mutation has been previously identified in multiple cancer types including breast, acute myeloid leukemia and colorectal cancer and submitted in the COSMIC database (COSV52671096), the ICGC database (MU12153)[12] and the IARC TP53 database[11,17].

Gene information and significance

The TP53 gene encodes for the TP53 protein, a tumor suppressor protein that is essential for regulating DNA repair and cell division. TP53 protein binds directly to DNA. When the DNA is damaged, TP53 plays a critical role in determining whether the DNA will be repaired, or the damaged cell will undergo apoptosis. If the DNA damage can be repaired, TP53 activates DNA repair proteins to correct the DNA damage. If the DNA damage cannot be repaired, then TP53 prevents the cell from undergoing cell division and induces programmed cell death (apoptosis) eventually preventing the development of tumors[13]. Mutations in the TP53 gene occur in 46% of colorectal cancer cases (8537 mutated/18387 total colorectal adenocarcinoma cases submitted in the COSMIC database)[18].

Approved therapies and clinical trials

Currently there are no approved therapies targeting TP53 in colorectal cancer tumors. However, TP53 mutations may be a potential prognostic and predictive biomarker in some tumor types as well as targets for pharmacological intervention in some clinical settings. A preclinical study showed that MDM2 Inhibitor Nutlin-3a induced senescence in presence of functional TP53 in murine primary fibroblasts, oncogenically transformed fibroblasts, and fibrosarcoma cell lines. TP53 mutant cells lacking functional TP53 were completely insensitive to the drug[19]. A phase I study of the vascular endothelial growth factor inhibitor pazopanib and the histone deacetylase inhibitor vorinostat, known to target factors activated by TP53 mutations and facilitate p53 degradation, evaluated the safety and efficacy of these inhibitors in patients' solid tumors. In patients with detected hotspot TP53 mutation advanced solid tumors the treatment led to 45% rate of stable disease (SD) ≥6 months/partial response(PR) (1PR and 3 SD≥6 months), median progression free survival (PFS) of 3.5 months and median overall survival (OS) of 12.7 months compared favourably with the results for patients with undetected hotspot TP53 mutations (16% (1 PR and 3 SD ≥6 months, P = 0.096), 2.0 months (P = 0.042), and 7.4 months (P = 0.1), respectively). These preliminary results support a further evaluation of the combination in cancer patients with TP53 mutations (NCT01339871)[20]. For information on clinical trials please visit clinicaltrials.gov.

VARIANTS OF UNKNOWN SIGNIFICANCE

TP53 (c.37delA)

ERBB2 (c.2725+31_2725insGGGGG)

ERBB3 (c.614-51C>T)

Note**:** One or more variants of unknown significance (VUS) have been detected in this patient's tumor sample. These variants are known as VUS due to their limited characterisation and clinical evidence in the scientific literature at the time of writing of this report, making their significance unclear. However, we do include them here for reference in case they become clinically important in the future.

METHODOLOGY

NeoThetisis a Laboratory Developed Test (LTD) from NIPD Genetics Public Company Ltd for tumor molecular profiling. Cell free DNA (cfDNA) is extracted from blood samples using a standardized methodology, followed by DNA library preparation. DNA enrichment for the genomic regions of interest is carried out using a solution-based hybridization method followed by next generation sequencing (NGS). Sequence data is aligned to a reference genome and variants are identified using proprietary bioinformatics pipelines. NeoThetis can be used for the identification of selected single nucleotide variants (SNVs), small insertions and deletions (Indels, ≤30bp), rearrangements and copy number variations (CNAs) depending on the test ordered. Tumor-related actionable and clinically relevant alterations are reported. Analysis and Interpretation is performed using but not limited to Varsome Clinical CE-IVD platform (ISO 13485) according to published knowledge at the time of testing. Genetic counselling for the clinical interpretation and significance of the results is recommended. The NeoThetis test development and performance evaluation was carried out by NIPD Genetics Public Company Limited, which is regulated under the Clinical Laboratory Improvement Act of 1998 (CLIA) as qualified to perform high-complexity testing. NeoThetis is intended for clinical purposes and should not be regarded as investigational or for research. The test has not been cleared or approved by the U.S.Food and Drug Administration (FDA), which does not require this test to go through premarket FDA review.

TECHNICAL SPECIFICATIONS AND LIMITATIONS

NIPD Genetics NeoThetis therapy selection test is designed to detect selected targeted variants, including SNVs (Single Nucleotide Variants), Indels (Insertions and/or deletions), CNAs (Copy Number Alterations), Structural Rearrangements and MSI (Microsatellite Instability), associated with cancer development. NIPD Genetics NeoThetis therapy selection test targets exonic and other hotspot regions in selected genes (listed above). Variants on the flanks or outside of the targeted regions are not intended to be detected by this assay. The analytical sensitivity for detecting sequence specific alterations such as SNVs and Indels depends on the true variant allele frequency (VAF) of the mutation and it is estimated at (i) 92% (81-97% at 0.05 sign. level) when the true VAF lies between 0.1% and 0.5%, and (ii) 100% (92-100% at 0.05 sign. level) when the true VAF is greater than 0.5%. The test cannot detect variants with VAF<0.1%. The estimated analytical specificity is >99.99%. SVs are reported when VAF exceeds 0.5% with an estimated sensitivity and specificity of 100% (66-100% at 0.05 sign. level) and 100% (93-100% at 0.05 sign. level), respectively. The test cannot detect SVs with VAF<0.5%. The limit of detection for detection of selected gene level CNAs is 2.8 copies (average copy number state of the tumor/normal admixture in cfDNA sample) with an estimated sensitivity and specificity of 100% (48-100% at 0.05 sign. level) and 100% (93-100% at 0.05 sign. level), respectively. MSI status is reported when VAF of insertions and/or deletions at selected microsatellite regions is greater than or equal to 0.25% with an estimated sensitivity and specificity of 100% (98.8-100% at 0.05 sign. level) and 100% (92.9-100% at 0.05 sign. level), respectively. Variants are classified according to the criteria set by the American College of Medical Genetics and Genomics[21]. Classification and interpretation of variants is performed using the Varsome Clinical platform and is according to published knowledge at the time of testing. Variants which are classified as variants of strong clinical significance (Tier I) or variants of potential clinical significance (Tier II) are reported. VUS (Tier III) will only be reported in cases of potential pathogenicity, as synonymous and intronic VUS are not reported. Variants which have been detected and are classified as benign or likely benign (Tier IV) are not reported. Genetic counselling for the clinical interpretation and significance of the results is recommended. A 'no clinically significant variant' result reduces the chance of presence but does not guarantee the absence of a somatic variant in the patient's tumor. Genomic findings from cfDNA may originate from circulating tumor DNA (ctDNA), germline alterations, or non-tumor somatic alterations, namely clonal hematopoiesis of indeterminate potential (CHIP). Mutations in genes covered by the test that may be derived from CHIP include, but are not limited to ATM, JAK2, and TP53.

ADDITIONAL INFORMATION / DISCLOSURE

Test performance is valid only for the presence or absence of the tested cancer-associated variants in the genes included in the test. Therefore, a negative result indicates the absence of a cancer variant out of all the targeted variants included in the test and does not eliminate the possibility of a variant in a genomic position not tested by this assay. A positive result indicates the presence of a clinically relevant alteration. The results are interpreted based on information provided on the sample information form. Misinterpretation of results may occur if insufficient or inaccurate information is provided. A positive finding does not guarantee association with a certain treatment or drug. Drugs or treatments mentioned in this report may not necessarily be suitable for the patient. Decisions on medical management must be based on the clinician's judgment taking into

consideration all available information such as the patient's medical history, family history and other medical tests and examinations performed.

Although this test is highly accurate, there is still a small possibility for false positive or false negative results. This may be caused by technical and/or biological limitations, including but not limited to poor sample quality, bone marrow transplants or other rare molecular events. Other reasons for false positive or false negative results include, but are not limited to: mislabeled samples, inaccurate reporting of clinical/medical information and rare technical errors.

Genetic testing is an important part of the diagnostic process. However, genetic tests may not always give a definitive answer. In some cases, testing may not identify a genetic variant even though one exists. This may be due to limitations in current medical knowledge or testing technology. Clinical correlation with other clinical data and tests is recommended. Results should always be considered in the context of other clinical criteria. The analysis is specific only for the test ordered. The referral clinician is responsible for counselling before and after the test; including the provision of advice regarding the need for additional genetic testing. Other diagnostic tests may still be necessary.

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Date of report (DD/MM/YYYY):

NeoThetis COLORECTAL Gene List

AKT1 (NM_001014432.1) Exon 4 ▲	**APC** (NM_000038.6) *Selected intronic regions covered* ▲	**ATM** (NM_000051.3) *Selected intronic regions covered* ▲	**BRAF** (NM_004333.6) Exon 9-12, 15 ▲	**BRCA1** (NM_007294.4) Exons 2-23 *Selected intronic regions covered* ▲	**BRCA2** (NM_000059.3) *Selected intronic regions covered* ▲
CTNNB1 (NM_001904.4) Exon 3 ▲	**EGFR** (NM_005228.5) Exon 2-5, 8-10, 12, 14-15, 17-24, 27-28 *Selected intronic regions covered* ▲ ●	**ERBB2** (NM_004448.3) Exons 7-8, 11-12, 14, 23-28, 31 *Selected intronic regions covered* ▲ ●	**FBXW7** (NM_033632.2) Exons 6-7, 9-14 ▲	**FGFR1** (NM_001174067.2) Exons 3, 6-10, 13-14, 17, 19 *Selected intronic regions covered* ●	**FGFR2** (NM_000141.4) Exons 2-9, 11, 13-18 *Selected intronic regions covered* ●
FGFR3 (NM_000142.4) Exons 4, 7-8, 11-12, 16-18 *Selected intronic regions covered* ●	**GNAS** (NM_000516.6) Exons 8 ▲	**KRAS** (NM_033360.3) Exons 2-5 *Selected intronic regions covered* ▲ ●	**MET** (NM_000245.4) Exons 2-6, 8-21 *Selected intronic regions covered* ●	**MLH1** (NM_000249.3) *Selected intronic regions covered* ▲	**MSH2** (NM_000251.3) *Selected intronic regions covered* ▲
MSH6 (NM_000179.2) *Selected intronic regions covered* ▲	**MTOR** (NM_004958.4) Exons 43, 47, 53, 56 ▲	**NRAS** (NM_002524.5) Exons 2-4 ▲	**NTRK1** (NM_002529.3) Exons 7-15 ▲ ■	**NTRK2** (NM_006180.4) Exons 12-13, 15-16 *Selected intronic regions covered* ■	**NTRK3** (NM_002530.4) Exons 14-15 *Selected intronic regions covered* ■
PALB2 (NM_024675.4) *Selected intronic regions covered* ▲	**PDGFRA** (NM_006206.6) Exons 18 ▲	**PIK3CA** (NM_006218.4) Exons 2- 6, 8, 10, 15-17, 19-21 *Selected intronic regions covered* ▲	**PIK3CB** (NM_006219.3) Exons 13, 15, 24 ▲	**PMS2** (NM_000535.7) Exons 6-8, 10 *Selected intronic regions covered* ▲	**POLE** (NM_006231.4) Exons 2-49 *Selected intronic regions covered* ▲
PTEN (NM_000314.8) *Selected intronic regions covered* ▲	**RAF1** (NM_002880.3) Exons 7 ▲	**SMAD4** (NM_005359.6) *Selected intronic regions covered* ▲	**TP53** (NM_000546.6) *Selected intronic regions covered* ▲		

Full exonic coverage, unless otherwise stated.
Selected intronic regions which are covered by the test are indicated above.

▲ Single Nucleotide Variant / Indels

● Copy Number Alterations

■ Rearrangements

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