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DRAFT

Roche

Case ID **XB-1074**	Patient sex **Male**	Diagnosis **Colorectal cancer**	Tumor purity **53%**	Ordering physician **Dr. Catherine Hockney**
Patient MRN not specified	Patient DOB **09/19/1959**	Sample type **Tissue**	Sample collection date **09/29/2020**	Ordering institution **Midlands Oncology Centre**
Patient name not specified	Patient ethnicity not specified	Sample site **Colon**	Sample receipt date **10/05/2020**	Ordering institution ID not specified

Report summary

5 clinically significant variants & combinations
1 other biomarker
0 relevant therapies
17 clinical trials

present in combination **NRAS** p.G13D | variant **TP53** p.R306* | variant **TP53** p.S269R | variant **CTNNB1** p.D32Y | present in combination **MSI** High

variant present in combination

NRAS p.G13D VAF **52%** RD **1,174** Tier I-A

Two monoclonal anti-EGFR antibodies are contraindicated and not recommended for CRC harboring NRAS hotspot mutations (FDA, EMA, Health Canada, Swissmedic, TGA, NCCN, ESMO, NICE, eviQ). In a phase II clinical trial NRAS-mutant CRC patients did not benefit from treatment with a multikinase inhibitor. In a phase II clinical trial, 2.1% (1/47) of NRAS-mutant solid tumor patients responded to a MEK inhibitor, and NRAS codon 61 mutations were associated with better prognosis than NRAS codon 12 or 13 mutations. In a phase I trial, KRAS- and NRAS-mutant CRC patients did not respond to a RAF inhibitor. Patients with NRAS-mutant solid tumors match inclusion criteria for additional clinical trials, such as trials with inhibitors of ERK, BRAF, and MEK, alone or in combination with HDM2, PI3K, PI3K/MTOR, and CDK4/6 inhibitors. Patients with NRAS hotspot mutations may match inclusion criteria for additional trials with BCL2 and MEK inhibitors and therapies targeting mutant RAS.

Therapies approved/guidelines-recommended in: **Colorectal cancer**

× cetuximab resistance | × panitumumab resistance

2

combination

NRAS p.G13D, **Microsatellite Instability (MSI) - High** Tier II-C

Patients with microsatellite instability-high (MSI-H) colorectal cancer harboring NRAS mutations match inclusion criteria for clinical trials, such as a trial with PD-1 antibody therapy.

no approved therapies

variant
TP53 p.R306* VAF **13%** RD **1,728** Tier II-C

Patients with solid tumors harboring TP53 inactivating mutations match inclusion criteria for clinical trials, including trials with a PD-1 antibody, a CHK1 inhibitor, a PARP inhibitor in combination with a WEE1 inhibitor, and a PARP inhibitor in combination with a VEGFR inhibitor. Clinical studies suggest CRC patients harboring TP53 mutations may be sensitive to a VEGFR/PDGFR inhibitor in combination with a HDAC inhibitor, while TP53 status may not affect response to an anti-VEGF antibody or anti-EGFR antibody. TP53 inactivating mutations are associated with resistance to various chemotherapy agents. Additionally, TP53 germline mutations are associated with the hereditary cancer predisposition syndrome Li-Fraumeni Syndrome.

no approved therapies

variant
TP53 p.S269R VAF **19%** RD **1,727** Tier II-C

Patients with solid tumors harboring TP53 inactivating mutations match inclusion criteria for clinical trials, including trials with a PD-1 antibody, a CHK1 inhibitor, a PARP inhibitor in combination with a WEE1 inhibitor, and a PARP inhibitor in combination with a VEGFR inhibitor. Clinical studies suggest CRC patients harboring TP53 mutations may be sensitive to a VEGFR/PDGFR inhibitor in combination with a HDAC inhibitor, while TP53 status may not affect response to an anti-VEGF antibody or anti-EGFR antibody. TP53 inactivating mutations are associated with resistance to various chemotherapy agents. Additionally, TP53 germline mutations are associated with the hereditary cancer predisposition syndrome Li-Fraumeni Syndrome.

no approved therapies

variant
CTNNB1 p.D32Y VAF **50%** RD **1,746** Tier II-D

Activating mutations in CTNNB1 result in increased β-catenin-dependent transcription. Small molecule WNT/β-catenin inhibitors are under clinical investigation in patients with solid tumors, including β-catenin/TCF interaction inhibitors or downstream transcriptional coactivator antagonists.

no approved therapies

variant present in combination
Microsatellite Instability (MSI) - High Tier I-B

One PD-1 antibody (FDA, Health Canada, TGA, TFDA, NCCN), another PD-1 antibody (FDA, Swissmedic, TFDA, NCCN), and a PD-1 antibody in combination with a CTLA-4 antibody (FDA, Swissmedic, TFDA, NCCN) are approved and recommended for certain patients with microsatellite instability-high (MSI-H) or mismatch repair deficient (dMMR) colorectal cancer (CRC). MSI-H status may be associated with good prognosis and lack of benefit from certain antimetabolite chemotherapy regimens (NCCN, ESMO). In phase I/II/III clinical studies, patients with MSI-H or dMMR CRC experienced clinical benefit on PD-1 antibodies, PD-L1 antibodies, PD-1 or PD-L1 antibodies in combination with VEGF antibodies, or PD-1 antibodies in combination with CTLA-4 antibodies, RTK inhibitors, EGFR antibodies, or COX inhibitors. In retrospective analyses, patients with MSI-H CRC experienced longer OS compared to MSS CRC patients on triple EGFR antibody/VEGF antibody/chemotherapy. In a retrospective analysis, patients with MSI-H colorectal cancer had longer OS on a VEGF antibody compared to an EGFR antibody in combination with chemotherapy. In additional clinical studies, MSI status was not associated with clinical benefit in patients with CRC treated with PARP inhibitor, EGFR antibody, VEGF antibody, or RTK inhibitor therapy.

Therapies associated with: **Colorectal cancer**

- ● **ipilimumab + nivolumab**
- ● **nivolumab**
- ● **pembrolizumab**
- × 5-fluorouracil resistance

Selected trials recruiting: **Male, age ≥ 18** within **United Kingdom**

NCT02633098 Phase **2**

CTNNB1

A Safety and Effectiveness Study of Pre-operative Artesunate in Stage II/III Colorectal Cancer

NCT04008030 Phase **3**

Microsatellite instability

A Study of Nivolumab, Nivolumab Plus Ipilimumab, or Investigator's Choice Chemotherapy for the Treatment of Participants With Deficient Mismatch Repair (dMMR)/Microsatellite Instability High (MSI-H) Metastatic Colorectal Cancer (mCRC)

NCT02563002 Phase **3**

Microsatellite instability

Study of Pembrolizumab (MK-3475) vs Standard Therapy in Participants With Microsatellite Instability-High (MSI-H) or Mismatch Repair Deficient (dMMR) Stage IV Colorectal Carcinoma (MK-3475-177/KEYNOTE-177)

EUDRACT2017-000370-10 Phase **3**

Microsatellite instability

Avelumab plus fluoropyrimidine-based chemotherapy as adjuvant treatment for stage III dMMR or POLE exonuclease domain mutant colon cancer: A phase III randomised study.

NCT03656718 Phase **1, 2**

Microsatellite instability

A Study of Subcutaneous Nivolumab Monotherapy With or Without Recombinant Human Hyaluronidase PH20 (rHuPH20)

NCT03767348 Phase **1, 2**

Microsatellite instability

Study of RP1 Monotherapy and RP1 in Combination With Nivolumab

NCT03386721 Phase **2**

Microsatellite instability

Basket Study to Evaluate the Therapeutic Activity of RO6874281 as a Combination Therapy in Participants With Advanced and/or Metastatic Solid Tumors

NCT02554812 Phase **2**

Microsatellite instability

A Study Of Avelumab In Combination With Other Cancer Immunotherapies In Advanced Malignancies (JAVELIN Medley)

EUDRACT2016-004548-12 Phase **1, 2**

Microsatellite instability

Phase 1/2 Study of RP1 +/- other therapies in solid tumours

NCT03742895 Phase **2**

Microsatellite instability

Efficacy and Safety of Olaparib (MK-7339) in Participants With Previously Treated, Homologous Recombination Repair Mutation (HRRm) or Homologous Recombination Deficiency (HRD) Positive Advanced Cancer (MK-7339-002 / LYNK-002)

NCT03907969 Phase **1, 2**

Microsatellite instability

A Clinical Trial to Evaluate AZD7648 Alone and in Combination With Other Anti-cancer Agents in Patients With Advanced Cancers

NCT03829501 Phase **1, 2**

Microsatellite instability

Safety and Efficacy of KY1044 and Atezolizumab in Advanced Cancer

NCT03459222 Phase **1, 2**

Microsatellite instability

An Investigational Study of Immunotherapy Combinations in Participants With Solid Cancers That Are Advanced or Have Spread

NCT03150810 Phase **1, 2**

Microsatellite instability

Study to Assess Safety, Tolerability and Clinical Activity of BGB-290 in Combination With Temozolomide (TMZ) in Participants With Locally Advanced or Metastatic Solid Tumors

NCT02758587 Phase **1, 2**

Microsatellite instability

Study of FAK (Defactinib) and PD-1 (Pembrolizumab) Inhibition in Advanced Solid Malignancies (FAK-PD1)

NCT02264678 Phase **1, 2**

Microsatellite instability

Ascending Doses of Ceralasertib in Combination With Chemotherapy and/or Novel Anti Cancer Agents

NCT01968109 Phase **1, 2**

Microsatellite instability

An Investigational Immuno-therapy Study to Assess the Safety, Tolerability and Effectiveness of Anti-LAG-3 With and Without Anti-PD-1 in the Treatment of Solid Tumors

End of findings section

NRAS p.G13D missense variant

Tier I-A

Variant Group	Variant Oncogenicity	Position (GRCh38)	HGVS	Transcript
NRAS codon 13 mutation NRAS hotspot mutation	Gain of function	Chr:1 Pos:114716123 Change:C>T	c.38G>A p.Gly13Asp	ENST00000369535.4

Gene clinical summary
Oncogenic NRAS missense mutations are found in cancers, including melanoma, colorectal, and thyroid cancer (PMID: 28666118). Hotspot mutations result in loss of GTPase function and net downstream activation (PMID: 26815308). Germline mutations in NRAS are associated with RASopathies, which confer increased cancer risk (PMID: 28957739). Currently, no effective RAS inhibitors have been developed, however emerging drugs target codon 12 and the hypervariable domain, as well as downstream effectors of RAS and regulators of RAS membrane association and activity (PMID: 25878363). Additionally, studies have reported that NRAS activation leads to resistance to EGFR tyrosine kinase inhibitors (PMID: 27279914) and anti-EGFR antibodies (PMID: 24758538).

Variant group clinical summary
Cetuximab and panitumumab are contraindicated and not recommended for patients with colorectal cancer (CRC) harboring NRAS hotspot mutations (FDA, EMA, Health Canada, Swissmedic, TGA, NCCN, ESMO, NICE, eviQ), which is supported by clinical studies (PMID: 20619739)(PMID: 23041588)(PMID: 25605843)(PMID: 26989027)(PMID: 26341920).

In a phase II clinical trial, regorafenib treatment resulted in 0% (0/15) 6-month progression free survival (PFS), a 2.2-month median PFS, and a median overall survival of 3.3 months in metastatic colorectal cancer patients with KRAS (n=9), NRAS (n=3) or BRAF (n=2) mutations who failed first line therapy; however, the trial was terminated early due to poor accrual (PMID: 30120161).

In a phase II clinical trial, 2.1% of patients (1/47) with NRAS-mutant solid tumors, excluding melanoma, responded to binimetinib treatment. In this study, NRAS codon 61 mutations were associated with better prognosis compared to NRAS codon 12 and 13 mutations (AACR annual meeting 2020, abstr. CT061). In a phase I trial, 12 of 20 NRAS- and KRAS-mutant CRC patients had stable disease with a RAF inhibitor (PMID: 32182156). Patients with NRAS-mutant solid tumors match inclusion criteria for additional clinical trials, such as trials with inhibitors of ERK (NCT03698994), BRAF (NCT01225536), and MEK, alone (NCT03213691) or in combination with HDM2 (NCT03714958), PI3K (NCT01449058), PI3K/MTOR (NCT01337765), and CDK4/6 inhibitors (NCT02065063). Patients with NRAS hotspot mutations may also match inclusion criteria for trials with BCL2 and MEK inhibitors [(NCT02079740)]((https://clinicaltrials.gov/ct2/show/NCT02079740) and therapies targeting mutant RAS (NCT03190941)(NCT03745326).

Gene biological summary
NRAS is a member of the RAS GTPase superfamily and is a key regulator of the RAS/RAF/MEK/ERK pathway and the downstream PI3K/AKT/MTOR pathway, regulating cell survival, proliferation and migration. RAS proteins cycle between an inactive GDP-bound and an active GTP-bound state in response to receptor tyrosine kinase activation. Downstream effectors are recruited to the membrane and activated by RAS (PMID: 28666118). Important domains in NRAS include the catalytic domain (residues 1-166) and the hypervariable domain (residues 167-189) (PMID: 28597297).

Variant functional summary
NRAS G13D lies within a GTP-binding region of the NRAS protein (UniProt.org). This mutation results in decreased NRAS GTPase activity, leading to activation of NRAS downstream pathway signaling in cell culture (PMID: 17517660).

Variant group functional summary
NRAS codon 13 lies within a GTP-binding region of the NRAS protein (UniProt.org). Missense mutations in codon 13 result in decreased NRAS GTPase activity, leading to activation of NRAS downstream pathway signaling in cell culture (PMID: 17517660).

NRAS hotspot mutation refers to a mutation in codons 12, 13, 59, 61, 117, or 146. Many of these mutations are known to be activating (PMID: 27664710)(PMID: 6092966).

NRAS p.G13D, **MSI** High

Tier II-C

Variant combination clinical summary
Patients with microsatellite instability-high (MSI-H) colorectal cancer harboring NRAS mutations match inclusion criteria for clinical trials, such as a trial with PD-1 antibody (NCT03519412).

Variant combination functional summary
NRAS hotspot mutation refers to a mutation in codons 12, 13, 59, 61, 117, or 146. Many of these mutations are known to be activating (PMID: 27664710)(PMID: 6092966).

MSI high indicates a high level of microsatellite instability (MSI). Microsatellites are repetitive stretches of DNA that are susceptible to mutation, and MSI is defined as a change in the size of these microsatellites compared to the normal genome. MSI results from a deficient DNA mismatch repair pathway (dMMR) caused by mutations or epigenetic inactivation of genes in the MMR pathway, including MSH2, MSH6, MLH1 and PMS2. MSI been identified in many solid tumor types, and high MSI can be associated with distinct prognosis and therapeutic response, including sensitivity to checkpoint inhibitor immunotherapy (PMID: 2974385)(PMID: 26031544).

TP53 p.R306* stop gained variant

Tier II-C

Variant Group	Variant Oncogenicity	Position (GRCh38)	HGVS	Transcript
TP53 inactivating mutation TP53 truncating mutation	Loss of function (predicted)	Chr:17 Pos:7673704 Change:G>A	c.916C>T p.Arg306*	ENST00000359597.8

Gene clinical summary
Alterations in TP53 are found in most cancers, including lung, breast, pancreatic, and colorectal cancer (PMID: 27815305) (PMID: 28452926). Tumor suppressor mutations may cause partial, complete, or dominant-negative loss of function, and oncogenic mutations cause gain of function through

interactions with new partners (PMID: 28948977). Germline TP53 mutations cause Li-Fraumeni syndrome, which confers increased cancer risk (PMID: 28270529). Therapies under preclinical and clinical investigation include drugs that reactivate mutant TP53, block interactions with TP53 inhibitors, and promote mutant TP53 degradation (PMID: 28927521).

Variant group clinical summary
Patients with solid tumors harboring TP53 inactivating mutations match inclusion criteria for clinical trials, including trials with a PD-1 antibody (NCT02432963), a CHK1 inhibitor (NCT02797964), a PARP inhibitor in combination with a WEE1 inhibitor (NCT02576444), and a PARP inhibitor in combination with a VEGFR inhibitor (NCT03645200).

In a phase I trial, the combination of the VEGFR/PDGFR inhibitor pazopanib and the histone deacetylase inhibitor vorinostat resulted in improved progression-free survival and overall survival in metastatic sarcoma and colorectal cancer patients harboring TP53 hotspot mutations, and resulted in a stable disease rate of 83% (5/6), compared to a stable disease rate of 9% (1/11) in patients without detected TP53 mutations (PMID: 25669829). Presence of TP53 mutations in colorectal cancer did not affect response to the anti-VEGF monoclonal antibody bevacizumab in combination with chemotherapy in a phase III trial (PMID: 17145525) or response to anti-EGFR monoclonal antibody cetuximab in a clinical study (PMID: 26989027).

Mutations leading to a loss of or a reduction in normal TP53 function have been associated with resistance to various chemotherapy agents (PMID: 27888811), (PMID: 25201193).

Additionally, TP53 germline mutations are associated with the hereditary cancer predisposition syndrome Li-Fraumeni Syndrome (PMID: 26014290), (PMID: 24706533).

Gene biological summary
TP53 is a homotetrameric transcription factor that responds to metabolic and stress signals to direct many functions including cell survival or death. In addition to transcription-level regulation, TP53 interacts with proteins in the cytoplasm to regulate metabolism and apoptosis (PMID: 26122615). Important domains in TP53 include the N-terminal transactivation domain (residues 1-92), the DNA binding domain (residues 101-306), the oligomerization domain (residues 307-355), and the C-terminal regulatory domain (residues 356-393) (PMID: 22713868).

Variant functional summary
TP53 R306* results in the premature truncation of the TP53 protein at amino acid 306 of 393, prior to the TP53 tetramerization domain (PMID: 22713868). Due to the loss of this functional domain (UniProt), this mutation is predicted to lead to a loss of TP53 function.

Variant group functional summary
TP53 inactivating mutation indicates that variants in this group result in a loss of function of the TP53 protein, which has been verified via biochemical, in vitro, or in vivo assays. Additionally, this group includes variants that are predicted to have a loss of function due to a truncation leading to the disruption or deletion of key functional domains (PMID: 20182602) (PMID: 11401317) (PMID: 27759562).

TP53 truncating mutation indicates any nonsense or frameshift mutation resulting in a premature termination codon. This class of TP53 mutation leads to a loss TP53 tumor suppressive function due to the deletion of key functional domains, and has been reported to promote tumorigenesis and increased activation of downstream target genes (PMID: 27759562) (PMID: 11401317).

TP53 p.S269R missense variant

Tier II-C

Variant Group	Variant Oncogenicity	Position (GRCh38)	HGVS	Transcript
TP53 inactivating mutation TP53 missense inactivatin…	Loss of function	Chr:17 Pos:7673813 Change:G>T	c.807C>A p.Ser269Arg	ENST00000359597.8

Gene clinical summary
Alterations in TP53 are found in most cancers, including lung, breast, pancreatic, and colorectal cancer (PMID: 27815305) (PMID: 28452926). Tumor suppressor mutations may cause partial, complete, or dominant-negative loss of function, and oncogenic mutations cause gain of function through interactions with new partners (PMID: 28948977). Germline TP53 mutations cause Li-Fraumeni syndrome, which confers increased cancer risk (PMID: 28270529). Therapies under preclinical and clinical investigation include drugs that reactivate mutant TP53, block interactions with TP53 inhibitors, and promote mutant TP53 degradation (PMID: 28927521).

Variant group clinical summary
Patients with solid tumors harboring TP53 inactivating mutations match inclusion criteria for clinical trials, including trials with a PD-1 antibody (NCT02432963), a CHK1 inhibitor (NCT02797964), a PARP inhibitor in combination with a WEE1 inhibitor (NCT02576444), and a PARP inhibitor in combination with a VEGFR inhibitor (NCT03645200).

In a phase I trial, the combination of the VEGFR/PDGFR inhibitor pazopanib and the histone deacetylase inhibitor vorinostat resulted in improved progression-free survival and overall survival in metastatic sarcoma and colorectal cancer patients harboring TP53 hotspot mutations, and resulted in a stable disease rate of 83% (5/6), compared to a stable disease rate of 9% (1/11) in patients without detected TP53 mutations (PMID: 25669829). Presence of TP53 mutations in colorectal cancer did not affect response to the anti-VEGF monoclonal antibody bevacizumab in combination with chemotherapy in a phase III trial (PMID: 17145525) or response to anti-EGFR monoclonal antibody cetuximab in a clinical study (PMID: 26989027).

Mutations leading to a loss of or a reduction in normal TP53 function have been associated with resistance to various chemotherapy agents (PMID: 27888811), (PMID: 25201193).

Additionally, TP53 germline mutations are associated with the hereditary cancer predisposition syndrome Li-Fraumeni Syndrome (PMID: 26014290), (PMID: 24706533).

Gene biological summary
TP53 is a homotetrameric transcription factor that responds to metabolic and stress signals to direct many functions including cell survival or death. In addition to transcription-level regulation, TP53 interacts with proteins in the cytoplasm to regulate metabolism and apoptosis (PMID: 26122615). Important domains in TP53 include the N-terminal transactivation domain (residues 1-92), the DNA binding domain (residues 101-306), the oligomerization domain (residues 307-355), and the C-terminal regulatory domain (residues 356-393) (PMID: 22713868).

Variant functional summary

TP53 S269R lies within the DNA-binding domain and HIPK1, ZNF385A, AXIN1 and E4F1-interacting region of the TP53 protein (UniProt.org). This mutation confers a loss of function to the TP53 protein as demonstrated by impaired promoter-specific transcriptional activities in yeast functional assays (PMID: 12826609).

Variant group functional summary
TP53 inactivating mutation indicates that variants in this group result in a loss of function of the TP53 protein, which has been verified via biochemical, in vitro, or in vivo assays. Additionally, this group includes variants that are predicted to have a loss of function due to a truncation leading to the disruption or deletion of key functional domains (PMID: 20182602) (PMID: 11401317) (PMID: 27759562).

TP53 missense mutations are the most frequent cause of loss of TP53 function and occur predominantly in the DNA-binding domain. This class of mutations is reported to typically lead to decreased transactivation of downstream TP53 gene targets (PMID: 20182602) (PMID: 11401317).

CTNNB1 p.D32Y missense variant

Tier II-D

Variant Group	Variant Oncogenicity	Position (GRCh38)	HGVS	Transcript
CTNNB1 activating mutati…	Gain of function	Chr:3 Pos:41224606 Change:G>T	c.94G>T p.Asp32Tyr	ENST00000349496.9

Gene clinical summary
Alterations in CTNNB1 are found in cancers, including colorectal, breast, uterine cancer (PMID: 28731148). Activating missense mutations and small deletions in the N-terminal domain (PMID: 28927523) result in WNT-independent activation of the WNT/β-catenin signaling pathway and constitutive activation of genes promoting cell growth and division. Emerging drugs under preclinical and clinical investigation inhibit β-catenin protein-protein interactions. WNT signaling inhibitors are currently in clinical trials and drugs targeting the nuclear β-catenin complex and downstream pathways are under preclinical investigation (PMID: 28731148).

Variant group clinical summary
Activating mutations in CTNNB1 result in increased β-catenin-dependent transcription. Small molecule WNT/β-catenin inhibitors are under clinical investigation in patients with solid tumors, including β-catenin/TCF interaction inhibitors or downstream transcriptional coactivator antagonists (PMID: 28474989)(PMID: 28731148)(NCT01302405).

Gene biological summary
CTNNB1 or β-catenin, is a component of cadherin-based adherens junctions and a transcriptional co-activator in the WNT/β-catenin signaling pathway (PMID: 28731148). Activation of WNT signaling results in translocation of cellular β-catenin to the nucleus and activation of genes regulating cell proliferation, migration, differentiation and survival (PMID: 28752891). Important domains in CTNNB1 include the N-terminal domain (residues 1-151), the C-terminal domain (residues 664-781), and a central armadillo repeat domain (residues 151-664) (PMID: 28927523).

Variant functional summary
CTNNB1 D32Y lies within the ubiquitination recognition motif of the β-catenin protein (PMID: 15064718). CTNNB1 D32Y confers a gain of function to the β-catenin protein as demonstrated by decreased ubiquitination and increased β-catenin-dependent transcription (PMID: 15064718), (PMID: 10987273).

Variant group functional summary
CTNNB1 activating mutations result in a gain of function in the β-catenin protein, leading to increased β-catenin-dependent transcription (PMID: 28731148).

MSI High

Tier I-B

Variant clinical summary
Pembrolizumab (FDA, Health Canada, TGA, TFDA, NCCN), nivolumab (FDA, Swissmedic, TFDA, NCCN), and nivolumab in combination with ipilimumab (FDA, Swissmedic, TFDA, NCCN) are approved and recommended for certain patients with microsatellite instability-high (MSI-H) or mismatch repair deficient (dMMR) colorectal cancer (CRC). MSI-H status may be associated with good prognosis and lack of benefit from 5-fluorouracil chemotherapy regimens (NCCN, ESMO) (PMID: 12867608) (PMID: 27432916).

In phase II and III clinical studies, patients with MSI-H or dMMR CRC experienced clinical benefit on pembrolizumab, nivolumab, or the combination or nivolumab and ipilimumab (PMID: 31725351)(NCT02460198)(PMID: 28734759)(PMID: 31147488)(NCT02060188)(PMID: 32251400)(NCT03026140) (J Clin Oncol 38: 2020 (abstr LBA4))(NCT02563002).

In a phase II trials, patients with MSI-H/dMMR CRC (n=22) demonstrated clinical benefit on combined PD-1 antibody (BAT 1306, pembrolizumab or nivolumab) and COX inhibitor (celecoxib or aspirin) therapy, with an overall response rate (ORR) of 83.3% (5 complete responses), a median PFS of 80.7%, and an OS of 91.3% (J Clin Oncol 38(4): 111). In a phase I/II study, patients with MSI-H or dMMR colorectal cancer had an ORR of 8% (2/26) on cetrelimab (J Clin Oncol 37(8): 31)(NCT02908906). In a phase II study, patients with MSI-H colorectal cancer treated with durvalumab achieved an ORR of 27% (3/11) (J Clin Oncol 37(4): 670)(NCT02227667).

In a phase I study, patients with MSI-H or dMMR colorectal cancer had an ORR of 43.3% (13/30) on dostarlimab (J Clin Oncol 38 (4): 218) (NCT02715284). In a phase Ib clinical study, patients with MSI-H colorectal cancer (n=10) treated with the combination of bevacizumab and atezolizumab had a DCR of 90% and an ORR of 30% (3 partial responses) respectively (J Clin Oncol 35(4): 673)(NCT01633970).

In additional phase I/II/III studies, patients with MSI-H colorectal cancer responded to atezolizumab (PMID: 30918950), atezolizumab and cobimetinib (PMID: 31003911), TAS-102 and bevacizumab (PMID: 28760399), regorafenib and nivolumab (J Clin Oncol 38(4): 135)(NCT03406871), durvalumab and pexidartinib (J Clin Oncol 37(15): 2579)(NCT02777710), durvalumab and tremelimumab (Cancer Immunol Res 2019;7(2 Suppl):Abstract nr A006)(NCT01975831), or avelumab and cetuximab (J Clin Oncol 38(4): 96). In a retrospective analysis, patients with MSI-H colorectal cancer treated with chemotherapy and bevacizumab (n=21) experienced longer OS (30.0 vs 11.9 months, p<0.001) compared to patients treated with chemotherapy and cetuximab (n=16) (PMID: 30865548). In a clinical study, among patients with MSI-H rectal cancer treated with chemoradiotherapy followed by nivolumab and radical surgery, a pathologic complete response rate of 60% (3/5) was observed (J Clin Oncol 38: 2020 (abstr 4100)).

In retrospective analyses, patients with MSI-H CRC experienced longer OS on triple irinotecan, bevacizumab, and cetuximab/panitumumab therapy and shorter OS and PFS on cetuximab or bevacizumab, compared to patients with MSS CRC (PMID: 31868905)(PMID: 27622042). In additional clinical studies, MSI status was not associated with clinical benefit in patients with CRC treated with olaparib, cetuximab, bevacizumab, or regorafenib (PMID: 28928870)(Annals of Oncology (2017) 28 (5): v158-v208)(PMID: 29805705)(PMID: 26786262).

Variant functional summary
MSI high indicates a high level of microsatellite instability (MSI). Microsatellites are repetitive stretches of DNA that are susceptible to mutation, and MSI is defined as a change in the size of these microsatellites compared to the normal genome. MSI results from a deficient DNA mismatch repair pathway (dMMR) caused by mutations or epigenetic inactivation of genes in the MMR pathway, including MSH2, MSH6, MLH1 and PMS2. MSI been identified in many solid tumor types, and high MSI can be associated with distinct prognosis and therapeutic response, including sensitivity to checkpoint inhibitor immunotherapy (PMID: 2974385)(PMID: 26031544).

Variants of Unknown Significance

The variants listed here are not sufficiently characterized in the current literature and variant databases, and are therefore, currently, of uncertain or unknown clinical significance. They are reported here for future reference in the event they become clinically significant in light of additional supporting evidence.

NOTCH3 p.A1802fs **PIK3R1** p.R557_K561delinsQ

Appendix

Genomic Regions Tested

We sequence all coding exons for each given transcript, plus approximately 10 basepairs of flanking non-coding DNA in each intron-exon junction. Unless specifically indicated, test results contain no information about other regions of the gene, such as regulatory domains or deep intronic regions. The genes on the panel: ABL1, AKT1, AKT2, AKT3, ALK, APC, AR, ATM, BAP1, BRAF, BRCA1, BRCA2, CCND1, CCNE1, CDH1, CDK12, CDK4, CDK6, CDKN1B, CDKN2A, CHEK2, CREBBP, CSF1R, CTNNB1, DDR2, EGFR, ERBB2, ERBB3, ERBB4, ESR1, EZH2, FANCA, FBXW7, FGFR1, FGFR2, FGFR3, FGFR4, FLT1, FLT3, FLT4, GNA11, GNAQ, GNAS, HNF1A, HRAS, IDH1, IDH2, JAK1, JAK2, JAK3, KDR, KEAP1, KIT, KRAS, MAP2K1, MAP2K2, MDM2, MDM4, MET, MLH1, MPL, MSH2, MSH6, MTOR, MYC, MYCN, NF1, NF2, NFE2L2, NOTCH1, NOTCH2, NPM1, NRAS, NTRK1, NTRK2, PALB2, PDGFRA, PDGFRB, PIK3CA, PIK3R1, PMS2, POLE, PPP2R1A, PTCH1, PTEN, PTPN11, RAF1, RB1, RET, ROS1, SMAD4, SMARCB1, SMO, SRC, STK11, TERT (including 454 bases upstream of translation start site), TP53, TSC1, TSC2, UGTA1A1, VHL.

LIMIT OF DETECTION: 5 percent variant allele fraction for single nucleotide variants (SNVs), small to medium sized multi-nucleotide variants (MNV) (less than 50bp).

Methodology

Genomic DNA is isolated from microscopically-guided dissection of tumor tissue and then enriched for the targeted regions of the tested genes. The variant status of the targeted genes is determined by massively parallel sequencing (next generation sequencing). The hg38 (GRCh38) reference sequence is used as a reference for identifying genetic variants.

Limitations

This test will not detect variants in areas outside the targeted genomic regions or below the assay's limit of detection. More complex structural variants, such as complex rearrangements, will not be detected. This test evaluates for variants in solid tumor tissue only. It cannot distinguish between somatic and germline variants.
For variants of potential germline origin, germline testing may be warranted. Consider seeking genetic counseling prior to such testing. In some cases, variants may not be identified due to technical limitations, especially when in the presence of known pseudogenes, homologous regions or regions of low mappability. Larger insertions or deletions (>50 basepairs) may not be detected.

Clinical Disclaimers

Interpretation of the test results is limited by the information that is currently available at the time. Treatment decisions are the responsibility of the physician. Results of this test must always be interpreted within the clinical context, such as the patient's conditions, patient and family history, physical examinations, information from other diagnostic tests and patient preferences.

The Inov Onco Panel was developed and its performance characteristics determined by Inov Labs.

NAVIFY® Mutation Profiler disclaimer

The information available in this report is obtained from third party sources (such as biomedical literature, medical guidelines, and publicly available data such as drug labels and clinical trials) and is subject to change over time based on future findings (including scientific and medical research).

NAVIFY® Mutation Profiler is not able to differentiate between germline and somatic variants. In general, variant interpretations are provided assuming the variants are of somatic origin.

3rd party attributions

A portion of the somatic gene variant annotations and related content have been provided by The Jackson Laboratory Clinical Knowledgebase (JAX-CKB™)

Clinical trial matching based on reported biomarkers are provided by MolecularMatch.

Tier definitions

Tier I-A: Approved therapy. Included in professional guidelines.

Tier I-B: Well-powered studies with consensus from experts in the field.

Tier II-C: Approved therapies for different tumor types or investigational therapies. Multiple small published studies with some consensus. Inclusion criteria for clinical trials.

Tier II-D: Limited clinical or preclinical studies.

Tier III (VUS): Variants of Unknown Clinical Significance.

Tier IV: Benign or likely benign variants (not included in the report, except for other biomarkers).

Software and content version numbers

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dbNSFP Version 4.0, Release date: 05/03/2019

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Mitelman Version 15-apr-2020, Release date: 04/15/2020

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